EFFECTS OF PLANT GROWTH REGULATORS ON VEGETATIVE DEVELOPMENT AND SEED PRODUCTION OF BIRDSFOOT TREFOIL (*Lotus corniculatus. L*)

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ABSTRACT

Effects of chemical manipulation of a crop of birdsfoot trefoil (*Lotus corniculatus* L.) grown for seed in two consecutive years were investigated in this study. In the first year, treatments included Cultar (paclobutrazol) at 0.5 or 1.0 kg a.i./ha, Cycocel (chlormequat chloride) at 1.5 or 3.0 kg a.i./ha and Alar (daminozide) at 2.0 or 4.0 kg a.i./ha applied twice at either the late vegetative stage (October) or at the early flowering stage (November). None of these treatments affected seed yield (average 549 kg/ha),or umbel components (pods per umbel, seeds per pod and thousand seed weight).

In the second year, at the same stages of plant development, Cultar and Cycocel were applied at the same rates as previously, with an additional treatment added using RSW-0411 (triapenthenol) at 0.5 or 1.0 kg a.i./ha. Again, no seed yield improvement was obtained by any chemical treatment, but average seed yield being increased 27% from 769 kg/ha by 6 days delay in harvesting from 41 to 47 DAPF. Shoot length was reduced by chemical applications, especially at the time of rapid growth, and Cultar had the strongest and longest retarding capability. However, plant branching was not improved by any treatment. Although early flowering pattern was increased by October Cultar application at the higher rate and peak flowering pattern by November Cultar application at either rate, total reproductive structures at harvest in treated plants were similar to those in untreated plants due to flower abortion. Cultar applications in the first year had no carry-over effects on seed production in the second year, but delayed early plant growth in terms of ground cover. Plant growth regulators had no effect on the quality of the subsequently harvested seeds.

Effects of Cultar, Cycocel and RSW-0411 applied at higher rates in October on reproductive abortion were examined in flowers produced during the flowering season in the second year. Chemical treatments increased flower abortion by 20%, especially in the early flowers. However, there was no effect on abortion of pods in an umbel, on abortion of ovules or seeds in a pod, or on seed weight. Time of flowering also modified flower abortion rate (late flowers having up to 48% greater flower survival than early flowers), and seed development rate (being slower in early season flowers), had no effect on pod abortion and seed abortion (average 44% and 70%, respectively). Flower abortion was first found as early as 10 DAOF. Pod abortion occurred consistently after flower opening, and ovule or seed abortion occurred particularly in the early stages of seed development.

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GENERAL INTRODUCTION

Birdsfoot trefoil (*Lotus corniculatus L.*) is a perennial herbage legume introduced into New Zealand about one hundred years ago. It grows and persists on most soil types but has special value in marginal soils. Its superiority in herbage production and persistency compared to other conventional legumes, such as white clover, red clover and lucerne, has been recorded when the species was grown under infertile, acid, dry or poorly drained soils (Chevrette *et.al.*, 1960; Hunt and Wagner,1963; Charlton *et.al.*, 1978; Morton, 1981; Scott and Charlton, 1983; Chariva, 1986; Widdup, *et.al.*, 1987; Enright and Floate, 1988; Fraser, *et.al.*, 1988; Chapman *et.al.*, 1990a). Nutritive value of this species also compares favourably with other commonly used legumes (MacDonald, 1946; Smith, 1964; John and Lancashire, 1981) and declines less rapidly with advancing maturity (Mays and Washco, 1960; Taylor *et.al.*, 1973; Collins, 1982), and therefore it can be used for summer grazing to provide a more even availability of forage throughout the grazing season. The non-bloating characteristic of this species, makes it a good substitute or alternative for lucerne in improving pasture productivity (Marten and Jordan, 1979; Marten *et.al.*, 1987).

Because of these merits, birdsfoot trefoil will likely become an important species in current development of low input sustainable agriculture. Uncertain wool and meat market conditions in recent years has resulted in a dramatic decline in fertilizer use in New Zealand (Nicholls *et.al.*, 1990). This situation is confounded by the need to develop pasture on marginal soils. It could therefore be expected that this species will become a better alternative to more conventional species which need high soil fertility. A similar trend has occurred in the USA (Knight, 1985). In addition, favourable climatic conditions for seed production offer promise to New Zealand farmers by allowing them to benefit from birdsfoot trefoil seed production for selfsustainability and for export in the future (Hampton *et.al.*, 1990)

The widespread use of *Lotus corniculatus* in pastoral systems, however, has been reportedly slow, and inconsistent. Unreliable seed production has been considered to be the major limiting factor. Commercial seed yields reportedly range from 50 to 400 kg/ha with 100 kg/ha considered average and 400 kg/ha excellent (McGraw and Beuselink, 1983). The best seed yields obtained, however, are still considerably lower than the crop's seed yield potential of 675-1125 kg/ha estimated by Seaney and Henson (1970) from crops grown under favourable environmental conditions.

A number of aspects have been identified as major factors which may be responsible for poor seed production in birdsfoot trefoil (McGraw and Beuselink, 1983). In particular, the protracted flowering period of the plant causes difficulty in defining the proper harvest time. Also, low assimilate distribution to reproductive parts combined with the pod dehiscence nature of the plant can contribute to great losses of seed during harvesting.

Successful seed production in birdsfoot trefoil depends on seed yields which are governed by the number of shoots and subsequently the number of inflorescences produced during the period of peak flowering (Li and Hill, 1988). Management practices for improving seed yields can probably be directed through manipulating plant structure, particularly through maximizing the number of potentially fertile shoots. Mechanical manipulation through cutting or grazing the plant at any time during spring has been found to have detrimental effects on seed production (Anderson and Metcalfe, 1957; Bader and Anderson, 1962a), since cutting simply removes older shoots and new crown-shoots grow and replace them (Li, 1989).

Chemical manipulation by the use of plant growth regulators has been proven to increase seed yields in several plants. Cultar (paclobutrazol) has been successfully used in *Lotus uliginosus* (Clifford and Hare, 1987); RSW-0411 (triapenthenol) in oil seed rape (Child, 1987);Cycocel (chlormequat chloride) in wheat and barley (Kust, 1986); and Alar (daminozide) in red clover (Jakesova and Svetlik, 1986). The effects of these chemicals have occurred particularly through increasing and synchronizing branching or tillering, and by shortening shoot growth and hence reducing lodging. The present study examined the value of plant growth regulators as a shoot manipulator for improving seed yield in *Lotus corniculatus*. Their effects on reproductive structure and abortion was also investigated during the flowering season of the plant.

CHAPTER ONE
LITERATURE REVIEW

Birdsfoot trefoil (*Lotus corniculatus L.*) is a perennial legume widely used as a forage. The species has been the subject of extensive reviews (MacDonald, 1946; Seaney and Henson, 1970; Turkington and Franko, 1980; Jones and Turkington, 1986; Li, 1989). This chapter considers some aspects of *L. corniculatus*, its use in agriculture, growth and development as affected by environmental factors, and seed production. The use of plant growth chemicals will also be reviewed since it is related to the experiment.

1.1. Terminology

Several terms have been used in relation to *Lotus corniculatus*, so brief clarification is needed to avoid confusion. Lotus, birdsfoot trefoil, broadleafed birdsfoot trefoil, common birdsfoot trefoil, and trefoil are all synonyms of the species *L. corniculatus* and are used to distinguish from other species in the genus *Lotus*. Although the term 'birdsfoot trefoil' is mostly used in the literatures to refer to *L. corniculatus*, it is also used for describing other *Lotus* species which are important for pastures, including *L. uliginosus* Schk. syn. *L. pedunculatus* Cav., *L. angustissimus* L., and *L. hispidus* Desf. (MacDonald, 1946). In this review birdsfoot trefoil is used to describe *L. corniculatus*, and trefoil to describe the genus *Lotus*.

1.2. Origin and Distribution

The genus *Lotus* contains a diverse group of annuals and perennials with up to 200 species (Seaney and Henson, 1970). Since the greatest diversity is found in the Mediterranean basin (temperate Europe and Asia), Seaney and Henson (1970) suggested this region as the centre of origin for the species.

MacDonald (1946) presents an interesting account of the history of *L. corniculatus* and states that the species was first described as *Lagopus primus* in the mid 16th century in Buck's Kreuterbuch, and was first described in English in 1597 in Gerald's Herbal. The early agriculture of *L.corniculatus* , as reviewed by Robinson (1934), indicates that the value of the plant as a forage crop has been recognised for more than 200 years, as shown by Ellis (1774) cited by MacDonald (1946) by its use for cattle feeding in England. Since then, birdsfoot trefoil has been widely found throughout Europe (except in England), Russia, Africa, India, and greater Asia (Robinson, 1934), and Australia, North and South America, and New Zealand (MacDonald, 1946). In tropical areas, however, its use is limited only to highland regions (Quiros, 1946, cited by Seaney and Henson, 1970).

Although *L. corniculatus* has a long history, worldwide distribution and is of considerable agricultural interest, there was apparently no real expansion in its culture until 1900 or later (Seaney and Henson, 1970). The great difficulty of harvesting substantial quantities of seed, as reported by Anderson (1777) cited by Seaney and Henson (1970), undoubtedly caused the failure of greater use of the species in its early history.

1.3. Agronomic Aspects

L. corniculatus has been used in agriculture long before its recognition and culture as a valuable forage legume. It is believed that the comprehensive discussion of birdsfoot trefoil, including its description, adaptation, culture, production, nutritive value and use,presented by Stebler and Schloeter in 1889(cited by MacDonald, 1946), served as a turning point in interest and studies in the species which lead to the real expansion of its culture early in this century.

1.3.1. Forage Quality

The agricultural value of *L. corniculatus* has been known by many pioneer agriculturalists since the 17^{th} century (MacDonald, 1946). Ellis (1774) in MacDonald (1946) give an interesting account of its agricultural value and states that "the Tyne grass (wild vetch) and the Lady finger grass (birdsfoot trefoil) are the two best kinds of natural grasses, whether for grass or hay, for feeding and fattening animals, including conies, deer, race-horses and cattle." Later reports, as cited by MacDonald (1946), emphasised its comparative palatability and nutritional quality compared with other legumes, such as clovers. Parallel with the development of analytical techniques, the high quality of *L. corniculatus* as a pasture legume was confirmed by more accurate measurements.

The feeding value of birdsfoot trefoil, in terms of *in vitro* digestible dry matter, crude protein, readily fermentable carbohydrates, and various mineral elements, has been found to be similar or better than that of high quality pasture legumes commonly used, such as clovers, lucerne, and sainfoin (MacDonald, 1946; Suckling, 1960; Smith, 1964, 1970; van Soest, 1975; John and Lancashire, 1981). Marten and Jordan (1979) reported substitution of *L. corniculatus* for lucerne as one-third of seasonal pasture increased legume composition, *in vitro* digestibility, crude protein concentration, and apparent intake potential, and resulted a mean of 17-18% increase in lamb product/ha. In addition, birdsfoot trefoil declines in quality less rapidly with advancing maturity than other legumes (MacDonald, 1946; Suckling, 1960; Mays and Wasco, 1960; Taylor *et.al.*, 1973; Collins, 1982), and hence it can be used for summer grazing to provide a more uniform availability of forage.

The condensed tannin property (non bloat inducement character) of *L. corniculatus* suggests its use as a good alternative to conventional bloat inducing legumes, such as red and white clovers and lucerne. Condensed tannins are recently known to prevent bloat, and are also present in some other pasture legumes, including big trefoil (*Lotus uliginosus*), sainfoin (*Onobrychis viciifolia*), haresfoot trefoil (*Trifolium arvense* L.), cicer milkvetch (*Astragalus cicer*L.), and the common weeds crown vetch (*Coronilla varia*L.) and dock (*Rumex obtusifolius*) (Jones and Lyttleton, 1971; Jones, *et.al*, 1976; Marten, *at.al.*, 1987). Condensed tannins have also recently been found to be related to nutritive value of herbage for ruminants. High nutritive value of *Lotus* and sainfoin cannot be attributed to plant digestibility or the level of intake (Egan and Ulyatt, 1980; Waghorn *et.al.*, 1987; Marten *et.al.*, 1987; Mangan, 1988). However, it is related to the fact that condensed tannins in these crops increase the availability and absorbtion of essential amino acids in the intestine of livestock (Waghorn *et.al.*, 1990). Waghorn *et.al.* (1990) suggested that an increase of 10-15% ruminant production of milk, meat and wool could be expected if grazed pasture containing 2-3% condensed tannins (*L. corniculatus* cv. Maitland contains 1.5-3.5 % condensed tannins (John and Lancashire, 1981)). Moreover, Marten *et.al.* (1987) reported that birdsfoot trefoil is more promising as an alternative to lucerne than cicer milkvetch and sainfoin.

Birdsfoot trefoil is also a palatable legume for livestock. Henson and Schoth (1962) reported that animals found birdsfoot trefoil less palatable than other common legumes, but they readily accepted it once they become accustomed to it. High lignin content has been suggested as the drawback of this species as a forage crop (John and Lancashire, 1981). Buxton *et.al.* (1984) found that although lignin content in birdsfoot trefoil is higher than in red clover, it is lower than in lucerne. Moreover, a photographic technique recently used by Hunt and Hay (1990) to measure relative palatability in forage grasses and legumes showed birdsfoot trefoil to be the second most preferred legume by deer and calves.

1.3.2. Likely Role in Temperate Agriculture

1.3.2.1. Environmental Requirements

Soil fertility, water, and light requirements of birdsfoot trefoil for optimal growth are not greatly different to those of other temperate legumes. Birdsfoot trefoil stands are generally longer-lived and more productive on clay soils than on sandy soils (Seaney and Henson, 1970), with ample water (Fould,1978), and enough nutrients(Ruselle and McGraw, 1986) at a pH range of 6.2-6.5 (McKee, 1961). The plant favours cool summer temperatures (about 27/13 ^{o}C day/night)(Long *et.al.*, 1989), high light intensity (Cooper, 1966) and longer photopheriods for its satisfactory growth.

Under optimum conditions as mentioned above, however, some other pasture legumes, such as white and red clover, and lucerne are more productive than birdsfoot trefoil (Scott and Charlton, 1983). The merits of birdsfoot trefoil mainly lies in its attributes such as good productivity on marginal soils (Seaney and Henson, 1970), and its high quality. Moreover, it is worth noting Scott's finding in 1985 that after 5 years birdsfoot trefoil was the most persistent legume among various forage legumes tested in the New Zealand high country.

1.3.2.2. Likely Role in New Zealand Pastures

In New Zealand there has been a growing interest in *Lotus corniculatus* as a potential herbage legume for some pastoral situations. Screening of about 200 introductions from many overseas sources has been conducted during recent years (Charlton *et.al.*, 1978; Widdup *et.al.*, 1987). Scott and Charlton, in 1983, concluded that birdsfoot trefoil has a potential as a productive and persistent legume for moderate soil fertility conditions in drier hill and high country, probably as late summer or autumn feed. Parallel with the dramatic decline in fertilizer use in New Zealand (Nicholls *et.al.*, 1990), it could be expected that this species will become a better alternative to some conventional species which need high soil fertility, similar to those occur in U.S.A. (Knight, 1985).

Lucerne (*Medicago sativa*) has proven to be the most productive forage legume on droughty soils which are relatively fertile (Douglas, 1986), but birdsfoot trefoil can be used in less fertile and more acidic areas. Although some reports (Seaney, 1975; Fould, 1978) suggest that lucerne is more drought tolerant, in New Zealand, Chapman *et.al.* (1990a) found that some birdsfoot trefoil lines showed superior tolerance to both drought and frost, and were more productive on drought prone, low fertile soils with acid subsoils which occupy much of the Upper Waitaki (c.260 000 ha of tussock grassland below 900 m altitude). In screening trials at six New Zealand sites with various environmental conditions, ranging from a warm dry fertile Alexandra site to a cold, wet infertile Alisa Craig in the South Island tussock country, Widdup *et.al.* (1987) reported that only at the Alexandra site did lucerne outyield birdsfoot trefoil. Birdsfoot trefoil is also more resistant to flooding stress (Heinrich, 1970; Chariva, 1986). Thus it may be superior in poorly drained soils. In pastures in areas with good lucerne growth, birdsfoot trefoil is considered to be a good substitute for lucerne-grass pasture under rotational grazing (Marten and Jordan, 1979; Collins, 1982) because of its high condensed tannin content and its high quality during the late season.

Maku lotus (*Lotus uliginosus)* has been recommended for use on wet, moderately fertile soils where pH and phosphate levels are insufficient for white clover (Amstrong, 1974), such as the Otago upland (Sheath, 1981). However, later work (Widdup *et.al.*, 1987; Fraser *et.al.*,1988) revealed that some *Lotus corniculatus* lines were more productive than Maku lotus in Waipori, Castle Dent and Alisa Craig, showing the potential of this species on moist, acid and infertile tussock grasslands. In droughty outwash soils at the Mackenzie high country, Chapman *et.al.* (1990a) reported that Maku lotus outyielded birdsfoot trefoil in the first year, but not in the following year. Moreover, birdsfoot trefoil is more tolerant to frost and drought stress (Chapman *et.al.*, 1990a), and shows superior establishment from selfseeding than Maku lotus (Scott and Charlton, 1983).

Red clover (*Trifolium pratense*) consistently produced high yields in the MacKenzie (Tara Hills) and Upland Otago sites (Waipori and Castle Dent) in the initial years (Widdup *et.al.*, 1987; Enright and Floate, 1988; Chapman *et.al.*, 1990a) because of the slow establishment of birdsfoot trefoil and the need to allow crown development. After three or four years when soil fertility had depleted, red clover stands drastically reduced, and birdsfoot trefoil become superior in dry matter yield. In addition, birdsfoot trefoil is more drought and flooding tolerant than red clover (Chariva, 1986; Chapman *et.al.*, 1990a). Thus it may serve as a good alternative to red clover in such pasture situations.

Alsike clover (*Trifolium hybridum*), which is similar to red clover, was initially superior to birdsfoot trefoil in Tara Hills and Waipori (Widdup *et.al.*, 1987, Enright and Floate, 1988; Chapman *et.al.*, 1990a). In contrast, its production dropped more drastically in the second year than red clover, and it did not survive in the third year (Chapman *et.al.*, 1990a), apparently due to a high incidence of crown and root rots (Widdup *et.al.*, 1987) or to drought sensitivity (Chapman *et.al.*, 1990a). Birdsfoot trefoil was found to be the most productive and persistent legume after five years (Scott, 1985). In addition, since birdsfoot trefoil is more tolerant to flooding than alsike clover (Chariva, 1986), better herbage production could be expected in poorly drained soils.

White clover (*Trifolum repens*), the 'backbone' legume of New Zealand farming, is advocated for growth on fertile soils with adequate moisture (Scott *et.al.*, 1985). In all sites tested by Widdup *et.al.*, 1987; Enright and Floate, 1988; Fraser *et.al.*, 1988, and Chapman *et.al.*, 1990a, however,white clover showed consistently low herbage production. This demonstrates that white clover is not well adapted to dry or infertile soil conditions. Several reports have shown that birdsfoot trefoil can grow satisfactorily in poor drained soils (Robinson, 1934; Heinrich, 1970), such as those found by Morton (1980) in Pahiki coastal soils where birdsfoot trefoil performed better than white clover.

1.3.3. Establishment

Difficulty in gaining good establishment is one of the major limitations to the productivity of birdsfoot trefoil. This is because of poor seedling vigor and the poor competitive nature of this species compared with conventional forage legumes such as lucerne and red clover (Seaney, 1975; Cooper, 1977; McKersie and Tomes, 1982; Matches, 1989; Chapman *et.al.*, 1990b). Consequently, correct establishment techniques should be considered to ensure good forage productivity or seed yield.

Establishment failures are often attributable to sowing too deep, particularly after uneven paddock consolidation. A fine, well compacted tilth with the seed drilled no more than 13 mm deep has been recommended (Stickler and Wassom, 1963; Seaney and Henson, 1970; Scott and Charlton, 1983; Chapman *et.al.*, 1990b). When hardseededness is likely to be a problem, autumn sowing is advisable to allow hardseed to break and germinate in the following spring (Seaney and Henson, 1970). Increased herbage yield following the use of high seeding rates (more than 7.5 kg/ha), was reported unlikely (Wakefield and Skaland, 1965), but seeding rate is mainly related to seed viability or vigour and soil conditions. In New Zealand, seeding rate of 5 -10 kg/ha (Scott *et.al.*, 1985), and the application of lime to acidic soils (pH <5.2) Chapman *et.al.*,1990b) are advocated. There is a special emphasis on seed inoculation in New Zealand, because effective strains of rhizobia are not present naturally (Greenwood and Pankhurst, 1977; Charlton *et.al.*, 1981; Lowther *et.al.*, 1987, 1989). Chapman *et.al.* (1990b) suggested the need to inoculate the seed at 5 times the manufacturer's stipulated rate with the incorporation of 10% gum arabic and seed sown within one day of inoculation. On acid soils, he recommends the coating of the seed with lime.

Competition by weeds and companion grasses is another factor limiting establishment success in birdsfoot trefoil. Cultivation and herbicide application is crucial since under most environments, birdsfoot trefoil can not effectively compete with fast growing weed species (Wakefield and Skaland, 1965; Chapman *et.al.*, 1990b). The use of selective herbicides offers a good method for controlling weed competition (Seaney and Henson, 1970), but strategic grazing may serve as a good alternative in some environments (Chapman *et.al.*, 1990b). Mixture of birdsfoot trefoil with perennial grasses has been extensively investigated to reduce weed invasion (MacDonald, 1946; Anderson and Metcalve, 1957; Chevrette *et.al.*, 1960; Scholl and

Brunk, 1962; Wakefield and Skaland, 1965; Sheaffer *et.al.*, 1984). In general, these reports show that mixtures with high yielding competitive grasses have resulted in the least weed invasion, but are often detrimental to birdsfoot trefoil seedling growth and persistence. Mixture with less competitive companion grasses is practically recommended for better forage quality or for higher seed yields.

1.3.4. Grazing Management

Stand productivity and persistence, and forage quality are major considerations in managing birdsfoot trefoil crops. Stand persistency is affected by interactions between growth habit and management practices (Smith and Nelson, 1967). For better persistence, it is well established that the erect types of birdsfoot trefoil (e.g. cv. Viking) are more suitable for hay, and the prostrate types (e.g. cv. Empire) for pasture (Van Kauren and Davis, 1968). Lax grazing or rotational grazing systems with rest periods longer than three weeks is advocated since the regrowth of birdsfoot trefoil is slower than that of lucerne and red clover (Parson and Davis, 1961,1964; Smith and Nelson, 1967; van Kauren *et.al.*, 1969). Since energy for regrowth in birdsfoot trefoil roots remains low during the growing season, even without cutting, (Smith, 1962;Heichel *et.al.*, 1985; Allison and Hoveland, 1989a,b), leaving a tall stubble (8 cm or more) is necessary not only for providing an energy reserve, but also for providing more sites for vigorous regrowth (Nelson and Smith, 1968a,b, 1969; Greub and Wedin, 1971a,b). Smith and Nelson (1967) and Allison and Hoveland (1989a) suggested a survival advantage in prostrate over erect types under conditions of close cutting since better carbohydrate reserves and more photosynthetically active tissue remain after harvest.

Forage quality of birdsfoot trefoil declines less rapidly with advancing maturity (see 1.3.1). It is worth noting the use of good quality summer forage of birdsfoot trefoil, even in pastures where lucerne grows well, to provide more even forage availability (Mays and Washco, 1960;Taylor *et.al.*, 1973; Smith and Soberalske, 1975; Marten and Jordan, 1979; Collins, 1982). Taylor *et.al.* (1973) found that delayed harvesting (stockpiling) increases stand persistency and productivity because of natural reseeding. When seedlings from reseeding were eliminated, Beuselink *et.al.* (1984) reported lower herbage yields in stockpiled and unharvested management systems due to a high incidence of foliar blight caused by *Rhizoctonia solani*.

1.4. Description of the plant

Birdsfoot trefoil is the most variable species in the genus Lotus, and the genetic basis for many characters has been discussed by Jones and Turkington (1986). The developmental anatomy of the plant has also been described by Hansen (1953), ad will not be accounted here. This section reviews the morphological description of *Lotus corniculatus* with some emphasis on the variation and the development of plant morphology.

1.4.1. Vegetative Structure

Birdsfoot trefoil has a deep woody tap root, up to 1 m long, with numerous lateral roots which can spread up to 0.75 m in width. These lateral roots often form a dense fibrous root system, especially in the upper 30-60 cm of the soil (MacDonald, 1946; Seaney and Henson, 1970). Although in most common conditions, birdsfoot trefoil is not rhizomatous, unlike *L. pedunculatus* (Sheath, 1981), old woody stems, particularly in prostrate types, sometimes form roots (Wassom and Barnett, 1971; Jones and Turkington, 1986).

Shoots of birdsfoot trefoil are erect, decumbent or prostrate, and are capable of reaching 170 cm in length under favourable conditions (Jones and Turkington, 1986). Shoot growth habit, however, is modified by photoperiod. For example, plants of prostrate types, when grown under short days, will become more erect under long days (Mckee, 1962). Aerial stems are commonly solid, slender, terete at the base, and square in the upper regions, and are glabrous to pubescent. The internodes of the stem are typically short, but become longer towards the stem apex, especially during the season of most rapid growth (Seaney and Henson, 1970; Jones and Turkington, 1986)

The aerial system is composed mainly of shoots emerging from the crown; with few or many lateral branches, much smaller in size, arising from leaf axils of these crown shoots (MacDonald, 1946; Hughes and Heath, 1949; Li, 1989). Li (1989) observed that the shoot system displays a 'continuous replacement' habit, *i.e.* old shoots tend to die soon after new shoots emerge and grow vigorously. In contrast, Stephenson (1984) reported that the shoot system is only formed during spring, and no new shoots emerge once flower buds begin to form, unless the plant is grazed or mown. According to Smith (1962) and Nelson and Smith (1968a), when basal shoots growing in the early spring are cut, regrowth comes largely from the upper axilary buds on the residual stubble, and a few buds developing on the crown become shoots during the summer.

The leaves of birdsfoot trefoil are attached alternately on opposite sides along the stem. Each leaf consists of three distal and two basal leaflets. Distal leaflets are obovate, obtuse or apiculate, while basal pairs are ovate to lanceolate, entire to minutely serate (MacDonald, 1946; Seaney and Henson, 1970; Jones and Turkington, 1986). The basal leaflets are almost sessile, and resemble stipules as small projections at the base of the leaf (Heyn, 1976).

1.4.2. Reproductive Structure

How shoots produce flowers has been studied by Li (1989) and Li and Hill (1989). Each apical meristem of a growing shoot tends to switch to reproductive growth only under favourable conditions for flowering. As a result older shoots bear flowers at higher node numbers whilst younger shoots produce flowers at lower node numbers. Flowers are produced continuously along the shoot without leaving any barren or sterile nodes. However, flower number per shoot does not vary greatly, usually 3-5 flowers, even among different shoot ages. Since the node of first flower emergence and the final node number on a shoot are almost identical, Li and Hill (1989) suggested that a shoot cease vegetative growth when it flowers. Since each shoot only flowers for less than one month, they also suggested that the prolonged flowering period in birdsfoot trefoil (sometimes up to 3 months, particularly depending on daylength) is caused by the continuous shoot replacement behaviour of the plant.

The inflorescence of birdsfoot trefoil is born in the leaf axils of the upper leaves. Flower heads have been well differentiated when the entire buds are still less than 2 mm in length (Hansen, 1953). Flowers emerge first at lower nodes or lower lateral branches, and then expand up the stem or branches as they are elongating. The flower is a typical umbel consisting of 4 to 8 florets attached by short pedicles to a long peduncle. Each floret consists of a calyx with 5 united sepals and a typical legume corolla with 5 petals. The reproductive part of the flower consists of 10 stamens forming a tube with the upper one free and a simple pistil. (MacDonald, 1946; Seaney and Henson, 1970; Jones and Turkington, 1986). There are variations in the colour of the petals (Buzzel and Wilsie, 1963; Jones and Crawford, 1977; Abbot, 1981; Crawford and Jones, 1986).

Pods are attached at almost right angles to the top of the peduncle, resembling a bird's foot (hence the common name birdsfoot trefoil). Mature pods are cylindrical, 15-30 mm long, 2-3 mm wide, and brown to almost black (Jones and Turkington, 1986). During pod dehiscence, seeds which are attached to the central suture of the pod are shattered. Buckovic (1952) cited by Metcalfe *et.al.* (1957) suggested a morphological explanation of pod dehiscence. The differential moisture loss between exocarp and mesocarp tissue increases tension between the fibre layers, and subsequently causes separation and twisting of the two valves of the pod.

The seed of birdsfoot trefoil is small, irregularly rounded, and somewhat flattened, about 1.5 x 1.3 x 1.0 mm in dimension. Seed colour at maturity varies from olive green to brownish to almost black. (Jones and Turkington, 1986). Seed weight also varies considerably from 0.92 mg to 1.67 mg (MacDonald, 1946; Robinson, 1934; Albrechtsen *et.al.*, 1966; Grime *et.al.*, 1981; Beuselink and McGraw, 1988). The position of seeds within the pod also influences the extend of seed development, heavier seeds being at the distal end (Long *et.al.*,1989).

1.4.3. Dry Matter Distribution

Assimilate partitioning in birdsfoot trefoil has been reported by Heichel *et.al.* (1985) and McGraw and co-workers (1983,1986). These reports show that only about 10% of assimilate is distributed in the root system during the growing season, and increases only slightly during autumn. Stems are the major sink prior to reproductive growth commencing, but stem weight does not decrease with the advance of reproductive development. Reproductive development apparently only depends on current photosynthesis and a small reallocation of assimilate from the leaves. All reproductive parts comprise only about 30% of the above ground parts, with about

30% of them allocated to the seeds. Thus, only 4% to 10% of assimilate has been distributed to the seed at harvest time.

1.5. Seed Production (both potential and actual): Effect of Environment.

Satisfactory vegetative growth is a prerequisite for high seed production of birdsfoot trefoil. The importance of a great number of shoots available during profuse flowering has been noted by Li, 1989 and Li and Hill, 1988. Dragmoir (1982) found positive correlations between seed yield and plant weight, and between plant weight and shoot number per plant, shoot erectness, the percentage of leaves on dry matter and the degree of branching. Apart from genetic variability, environmental factors greatly affect vegetative and reproductive development of *Lotus corniculatus*, thus also seed production.

1.5.1. The influence of environment on vegetative growth

Light. Light strongly limits birdsfoot trefoil growth. The rate of growth is highly sensitive to shade or low light intensity (Cooper, 1966, 1967). Under these conditions, both shoot and root growth are inhibited (Gist and Mott, 1957); leaf/stem ratio (Rhykerd *et.al.*, 1959a,b), and leaf area per plant (McKee, 1962) also being reduced. Photoperiod studies conducted by Joffe (1958) and McKee (1963) showed that a gradual increase in daylength from 9 to 24 hours increases internode length, plant height, and plant weight, and results in alteration of plant form from prostrate to more erect.

Temperature. Vegetative growth of birdsfoot trefoil is favoured by cool weather. Germination is best at about 20^{O}C, and is delayed or reduced by lower or higher temperatures with the calculated minimum temperature being 4.7°C (Hur and Nelson, 1982). Seedling growth, however, is reduced when exposed to increasing temperatures of 15.5 to 32°C, with root growth being affected more than shoot growth (Gist and Mott, 1957). Plant growth decreases markedly with increasing altitude from 460 m to 1040 m in the South Island, New Zealand (Enright and Floate, 1988, Fraser *et.al.*, 1988, Floate *et.al.*, 1989), an effect they suggested was due to differences in temperature.

Soil moisture and nutrients. Although *L. corniculatus* is known to be a drought resistant and poor land plant (Seaney and Henson, 1970; Scott and Charlton, 1983), water stress affects germination, and seedling and mature plant growth. Field emergence is delayed under soil moisture stress of 4.5 atm and completely inhibited at 11 atm (Woods and MacDonald, 1971). Its response to water stress in terms of growth, reproduction and mortality is better than red clover, but not as good as lucerne (Gist and Mott, 1957; Fould, 1978) but some birdsfoot trefoil lines may have better growth performance than lucerne, especially when soil fertility is low (Chapman *et.al.*, 1990a). Regardless of its persistence on poor land, birdsfoot trefoil responds well to improved soil fertility (McKee, 1961; Floate *et.al.*, 1989). Ruselle and McGraw (1986) reported the effects of single nutrient stress (deficiency or toxicity) on plants grown hydroponically. Dry mass of shoots, shoot branching and leaf area are typically lower in stressed plants than in plants grown under complete nutrient solution, and leaf mass per cm^2 is generally greater in stressed plants.

Weeds. Slow growth of seedlings and slow regrowth after cutting make birdsfoot trefoil crop susceptible to weed invasion and other crop competition (Seaney and Henson, 1970). Weeds compete with the birdsfoot trefoil crop for light, nutrients and space, and subsequently may cause a decrease in crop growth, an increase in mortality, and a reduction in seed yield. Leep and Schaltz (1980) found that totally controlling quackgrass in an established stand of birdsfoot trefoil with pronamide resulted in a three-fold increase in seed yield. Reports on the use of herbicides demonstrate that improved seed yields may not always be obtained, especially in controlling broad-leaved weeds since the herbicides used are often toxic to the crop (Dimitrova, 1984; MacLean and Grant, 1986; Amadeo, 1987; Wyse and McGraw, 1987). However their use is still of advantage, since seed produced consistently has a higher analytical purity.

Insects and Diseases. Jones and Turkington (1986), in their review, listed a number of phytophagus insects in birdsfoot trefoil. These include 9 families with 137 species, in which 29 species are apparently destructive. Plant parts which are attacked include roots (e.g. *Bambecia spp.*), buds and stems (e.g. mirids), leaves (larvae or caterpillar of *Zigaea spp.* and *Polyomnatus spp.*), flower and young pods (e.g. *Aphis spp.*), pollen (e.g.*Meligethes orythropus*), and seeds (e.g. *Cydia compositella*). However their effect on seed production is not well known (see 1.5.3.). Fungal diseases appear to impose the most important limitation to birdsfoot trefoil adaptation and persistence. Beuselink (1988) in his review noted that perennial birdsfoot trefoil appears to become more biennial in the southern range of North America and suggested a disease complex commonly referred to as root and crown rots as the main cause. Rarely can disease complexes be attributed to a single pathogen. A number of non-pathogenic fungi, pathogenic fungi (particularly *Fusarium*), bacteria and nematodes are usually observed in diseased tissue. A number of environmental factors including soil moisture, drainage, air and soil temperature, nutrients, stand density, crop rotation, frequency and height of cutting, insect injury and previous invasion by viruses and nematodes can all have an effect on the expression of disease complexes and plant susceptibility (Beuselink, 1988).

1.5.2. The influence of environment on reproductive growth

Flowering. Birdsfoot trefoil is a quantitative long day plant, both initiation and further development of inflorescence being strongly affected by the intensity and total quantity of light received as well as by photoperiod (Joffe, 1958; McKee, 1963). However, cultivars may show different responses to photoperiod with regard to their growth and flowering behaviour. These characters have been successfully used for varietal purity identification by Nittler and Kenny (1964, 1965). In general, the critical photoperiod for inflorescence initiation is 14 to 14.5 hours. At a photoperiod of 16 hours or more, profuse flowering is initiated, being more rapid at higher light intensity. However occasionally aborted buds appear under optimum photoperiod, and abortion is more frequent in plants grown under shorter days and/or lower light intensity. Other factors such as high night temperature, low nutrient levels, and insects and diseases may also restrict the development of floral primordia and cause abortion of flower buds (Joffe, 1958; Seaney and Henson, 1970).

Plant sensitivity to photoperiod is affected by temperature and nutrient availability. Growing birdsfoot trefoil until first flowering at four temperature regimes, *i.e.* 32/27^{o}C, 27/21^{o}C, 21/15^{o}C and 15/10^{o}C day/night combination, Smith (1970) found that the earliest flowering occurred in the 27/21^{o}C. Flowering was delayed with deceasing temperature up to 35 days, and in the highest temperature regime 20 days. Ruselle and McGraw (1986) reported that sulphur deficiency also delays flowering in this species. However, the relevance of delayed flowering on inflorescence profuseness and subsequent seed yield has not been clear. Stephenson (1984) stated that birdsfoot trefoil regulates floret numbers per inflorescence and inflorescences per crown shoot under different resource conditions.

Pollination and Fertilization. The number of seeds set per floret in a legume is actually dependent upon the number of ovules per carpel, efficiency of pollination, fertility of pollens and ovules, and effectiveness of fertilization and seed development (Thomas, 1987). Little is known about the influence of environment on these aspects and on seed set in birdsfoot trefoil.

Lotus corniculatus is generally considered to be a self-incompatible plant although some individuals are partially self compatible (Silow, 1931; Tome and Johnson, 1945; Giles, 1949; Bradenburg, 1961; Seaney, 1962, 1964; Seaney and Henson, 1964; Miri and Bubar, 1965; Dobrofsky and Grant, 1980a,b). Thus cross pollination is fundamental to the success of the reproductive process. According to Knuth (1908), Hymenopthera, particularly the large bumble bees, are 'the only effective pollinators'. *Lotus corniculatus* was found to be the main source of pollen gathered by *Bombus monticola* Smith and by *B. lucorum* L. in England (Yalden, 1982,1983). Under commercial seed production areas, however, honey bees are the considered to be the most important pollinating agents (Morse, 1958; Free, 1970), since this species is easily managed. Morse (1958) found that the number of seeds set increases with the number of bee visits to the floret and the time spent by each bee at the flower. He also reported that 12-20 visits are required for maximum seed set. Visiting rate of honey bees is high, being 3-6 flowers pollinated per minute (Kobisova-Kopralova and Nedbalova (1978), and a population of 11.000 bees/ha has been reported to be efficient for all flowers to be pollinated (Morse, 1958). In practice, however, placing honey bee hives in birdsfoot trefoil crops at a density of 2 hives per ha (bee forager number about twice as high as those reported by Morse) is the present recommendation in New Zealand (Bryant and Vardy, 1986).

Some environmental factors have been known to influence honey bee foraging behaviour in *Lotus corniculatus*. In general, the foraging behaviour of bees is influenced by weather conditions (Free, 1970). Bees favour warm dry weather for foraging, and during unfavorable weather (wet, cool, windy) bees tend to forage poorly or remain in their hive. Moreover, there is some evidence that honeybees prefer to forage other nearby crops, such as clovers (Lancashire and Gomez, 1980; Marten, 1985), especially during the cooler part of the day. Within a birdsfoot trefoil crop, as studied by DeGrandi-Hoffman and Collison (1982), honeybee activity is closely associated with the progression of flowering throughout the season and the magnitude of flower display among cultivars. Activity was heaviest during peak flowering periods. The largest number of foragers was found in cultivars that produced the most florets. They also found that bee activity was not related to nectar secretion characteristics of the cultivars studied. This is in accordance with Bader and Anderson's report (1962b) that pollen-collecting honey bees are more efficient than nectar-collectors.

Duration of ovule availability for fertilization has been reported by Bubar (1958), to be 2-3 days, but ovaries contain fertilizable ovules for 8-10 days. Li (1989) and Li and Hill (1989a) also reported that the duration of flowers with at least one good open floret was 4-8 days. The differences between these reports is apparently because Morse's observation was started at the flower bud stage. Fertilization usually takes place within 24-48 hours after cross pollination (Seaney and Henson, 1970), and thus the time of pollination is generally also referred to as the time of fertilization.

Seed Development. Seed development in birdsfoot trefoil has been studied in detail by many workers, including Anderson (1955), Wiggans *et.al.* (1956), Winch (1958), Long *et.al.* (1989 and Li (1989). After pollination, pods develop rapidly, reaching their maximum length in about three weeks, and then change colour from green to brown and finally black. According to Anderson (1955), morphological maturity of seeds, as indicated by maximum dry weight, is obtained slightly before or at the time pods turn light brown. Weather conditions influence the rate of seed development and so the time for seed to attain maturity. Seasonal variation of the time for seed to mature has been reported in Iowa to be between 24-47 days (Anderson, 1955) and in New York between 26-38 days(Winch, 1958), being longer for pods produced early in the season. In a study on the effects of temperature, Long *et.al.* (1989) found that less time (6-8 days) is needed for a seed to reach maximum size when the plants are grown under warm (day/night 31/21^{o}C) conditions than under cool temperature (27/13^{o}C) regimes.

Pod and Seed Abortion. The low percentage of seeds set in birdsfoot trefoil has been reported by Seaney and Henson (1970), Stephenson (1984) and Stephenson and Winsor (1985) and to occur as a result of flower and seed abortion. Only 40 percent of the 20-70 ovules in an ovary develop mature seeds (Seaney and Henson, 1970). Moreover, Stephenson (1984) reported that only one of every three florets produces a mature pod, and three of every five initiated pods aborted. In later studies, Stephenson and co-workers (1986,1988) reported that each inflorescence commonly aborts about half of its immature fruits. Compared to the random pattern of fruit abortion (conducted by hand-thinning fruits on some inflorescences prior to any abortion), natural patterns of fruit abortion produce mature fruit that contains significantly more seeds. In addition, progeny from flowers which abort their fruits naturally are more likely to germinate, to produce more vigorous seedlings, and then produce more reproductive output when mature. These results indicate that birdsfoot trefoil selectively aborts those fruit with fewest seeds and subsequently increases the average quality of its offspring (Stephenson et.al., 1986,1988).

One of the main causes of this abortion situation has been identified, as being due to pollination and fertilization failure (Seaney, 1964; Dobrofsky and Grant, 1980a).

Negri (1984) in his pollination study in birdsfoot trefoil found that with the presence of insect pollinators 38% of ovules and 83% of flowers set seed. On the contrary, only 1% of ovules and 1.4% of flowers set seed when the flowers were bagged. A lack of assimilate supply is also suggested as another main reason for floret or seed abortion (Stephenson, 1984).

1.5.3. The Influence of Environment on Seed Yield and Seed Yield Components

Seed yield in a plant consists of a series of seed yield components which in turn are affected by genetic and environmental factors. In birdsfoot trefoil yield components are usually expressed as the number of umbels per plant or per unit area, pod numbers per umbel, seed numbers per pod and individual seed weight. Growing *Lotus corniculatus* in three different places in U.S.A., McGraw, Beuselink and Smith (1986) observed better seed yield and yield components in plants grown in greater latitude locations (43^{o}N and 48^{o}N) than at a lower latitude (38^{o}N). They suggested that this may occur because of the comparatively longer photoperiod suitable for profuse flowering and cooler temperature promoting seed set and filling in the greater latitude regions. There is also year to year inconsistency in seed yields, particularly due to variations in yearly weather conditions (Li, 1989).

Light and Temperature. Seed yield components vary according to the time of their production during the flowering season. Several workers found that umbels set early in the season produced more pods, more seeds per pod, and had a greater seed mass than those produced later in the season (Anderson, 1955; Beuselink and McGraw, 1988). This variation is partly attributable to the influence of temperature on reproductive development. Long *et.al.* (1989) reported that birdsfoot trefoil grown under a cool alternating temperature 27/13^{o}C day/night produced greater vegetative dry mass, more umbels per plant and seed mass compared to those grown under high alternating temperature 31/21^{o}C. In cool alternating temperatures, the plant generally also produced better vegetative growth and seed yield components than in warm constant (26^{o}C) or in cool constant (20^{o}C) temperature regimes. Since cool alternating temperatures produced greater growth than cool constant temperatures for every traits observed (excluding peduncle length), Long *et.al.* (1989) suggested birdsfoot trefoil is a thermoperiodic plant.

Soil moisture and Nutrient. Seed production of *Lotus corniculatus* is consistently lower under dry than under moist soils, primarily due to reduced flower production (Fould, 1978). Fould found that when mature plants were exposed to drought conditions, photosynthetic products were used in developing a strong root system. He further suggested that this was as a survival strategy by bidrsfoot trefoil. The effect of nutrient stress has been examined by Ruselle and McGraw (1986). They found that seed production and seed yield components were less frequently affected by nutrient stress than were root and shoot dry mass. Low seed yield per plant was associated with reduced umbel numbers alone; however it was not clear whether this was because fewer meristematic regions were present to produce umbels or because relatively fewer meristematic regions produced reproductive tissue. There was apparent compensation of higher seed weight due to lower umbel number in Mg-deficient plants. Although lack of Cu and Fe resulted in more seeds per pod, total seed yield and other yield components were not changed. (Ruselle and McGraw, 1986).

Insects. The parts of the birdsfoot trefoil plant which are attacked by harmful insects have been mentioned earlier (see 1.5.1.). Increased seed yield through control of injurious insects has been reported (MacCollom, 1958; Rigdway and Gyrisco, 1959;

Bader and Anderson, 1962a). However, the possibility that insect invasion may be beneficial, as reported in *Lotus uliginosus* (Clifford *et.al.*, 1983), has not been explored in birdsfoot trefoil. Clifford *et.al.* (1983) found that mirid control at anthesis alone increased seed yield in *Lotus uliginosus* by 40 percent through increasing flowerhead numbers per stem. Mirid control prior to anthesis reduced mirid population , but flower head numbers per stem were also reduced. They observed that mirid attack prior to anthesis initiated a high level of primary stem branching which supported the development of high numbers of lateral formed flower heads in a more concentrated span. This led to evenly mature umbels and reduced pod shattering losses.

Plant population density. Plant population density influences microclimate condition in a crop and in turn affects seed yield and seed yield components. McGraw, Beuselink and Ingram (1986) reported that seed yields per unit area of birdsfoot trefoil respond to plant density asymptotically. This was surprising because in a number of crops in which reproductive parts are harvested, including maize, soybean, ryegrass and subterranean clover, as indicated in Holliday's review (1960), the response pattern was a flat-topped parabola. An asymptotic pattern is a typical response of crops in which vegetative parts are harvested (Holliday, 1960). However, later reports (McGraw and Beuselink, 1987; Gataric and Alibegovic-grbic, 1989) in studies on seeding rates clarified that birdsfoot trefoil responds in the same manner to other crops, such as soybean. The asymptotic response reported by McGraw *et.al.* (1986) occurred because the plant population density used was only up to 60 plants per m^2, and maximum seed yield had been achieved at about 19 plants per m^2. Seed yield responded to increasing plant population density by reducing all yield components. The response sensitivity of seed yield components of birdsfoot trefoil to plant population density showed a decline in order of importance, with umbel number the greatest, pod number and seed number intermediate, and seed weight the least sensitive component. Regardless of plant population density, it is important to note that better seed yield is usually achieved in birdsfoot trefoil grown in wide rows (40-60 cm) than in narrower rows (Pankiw *et.el.*, 1977; Gataric and Alibegovic-grbic. 1989).

1.5.4. The Influence of Environment on Seed Harvest

Seed yields in birdsfoot trefoil are reduced significantly by losses at harvest because of harvesting immature seeds and loss due to dehisced mature pods. Seed losses due to dehiscence of ripe pods either prior to harvest or in mowed and curing herbage are high if relative humidity drops to approximately 40% or less (Anderson, 1955). Low relative humidity promotes rapid reduction in pod moisture content. Buckovic (1952), cited by Metcalfe *et.al.* (1957), found that pods dried at a rapid rate had a high rate of dehiscence even though they had lost as much water. As pods dry more slowly, moisture loss difference between exocarp and mesocarp tissue is reduced and so is the tension between the fibre layers. Subsequently separation and twisting of the two valves of the pod is reduced.

Higher temperature is associated with lower relative humidity and this enhances pod dehiscence. During the day, temperature within and at the surface of mature pods varies as much as 5.5^{o}C above the air temperature depending on cloud cover (Metcalfe *et.al.*, 1957). A rapid rise in pod temperature in full sunlight may cause reduced relative humidity at the pod surface, thus accounting for the higher incidence of pod dehiscence under such conditions than on partly cloudy days.

The conditions above suggest that the optimum time to harvest birdsfoot trefoil seed crop is often difficult to assess, particularly during hot dry weather (Kelly, 1988). Anderson (1955) advised it would be wiser to save the seed from umbels produced during the main flowering period by harvesting when maximum pods are light green to light brown, and to ignore those formed subsequently. MacDonald (1946) suggested that the correct harvest time is when 70-80 percent of pods turn light brown.

1.6. Problems in Seed Production

The widespread use of *Lotus corniculatus* is mainly hindered by the inconsistent and unreliable seed yields commonly encountered. A continuous increase in average yields from the middle of this century can be traced in the USA. Anderson and Metcalfe, in 1957, reported that very low seed yields (below 50 kg/ha) were commonly obtained. Later, in 1970, Seaney and Henson reported 50-175 kg/ha as the regular harvested seed yield in commercial production. More recently, McGraw and Beuselink (1983) reported that commercial seed yields in USA ranged from 50 to 400 kg/ha with 100 kg/ha considered average and 400 kg/ha excellent. This increase is apparently due to the application of more suitable management techniques and more understanding of plant growth. However, these yields are still below those reported under experimental conditions which vary from 280 to 920 kg/ha (McGraw and Beuselink, 1983). Moreover, actual yields are often lower than potential seed yields particularly due to problems of indeterminate flowering habit and pod dehiscence. Under favourable environmental conditions, potential seed yields in birdsfoot trefoil of 675-1125 kg/ha has been estimated by Seaney and Henson (1970) and Jones and Turkington (1980)

In New Zealand, Li (1989) reported commercial seed yields of 200-350 kg/ha. However, yield may be as low as 50 kg/ha because of problems in deciding correct harvest timing and due to major losses of seed or poor recovery during harvesting (Personal communication, M.J. Hill). In research trials where Li (1989) applied various shoot manipulation management techniques, including cutting time and the use of plant growth chemicals applied either pre- or post-peak flowering, seed yields of 400-650 kg seed per ha were obtained.

1.6.1. Three Major Obstacles in Seed Production

Seed production in birdsfoot trefoil is extremely limited by three major barriers which have been highlighted by McGraw and Beuselink (1983) and Li (1989). Firstly, The rate of assimilate partitioning to the seed is very low. Even when the plant is grown in a good environment for seed production, only about 12 percent of av ilable assimilate is translocated to the seed (McGraw and Beuselink, 1983; McGraw, Ruselle and Grava, 1986). This low partitioning to seed is caused by low partitioning to reproductive growth and small seed size in relation to other reproductive organs. As a result, a bulk of vegetative mass, often more than 10 metric tonnes, has to be harvested to recover around 500 kg seeds per ha. This brings about extraordinary difficulties in separating the seeds from the vegetative mass. Secondly, this species has an indeterminate flowering habit, in which the plant flowers over a long period (McGraw and Beuselink, 1983). In Palmerston North flowering can continue for up to three months (Li, 1989). This results from a continuous shoot succession and the capability of shoots to switch to reproductive growth as soon as conditions are suitable for flower induction (Li, 1989; Li and Hill, 1988). During the season, two or three flowering peaks sometimes occur (Degrandi-Hoffman and Collison, 1982; Li, 1989). Also, flower buds, blooming flowers, immature pods, mature pods and pods ready to shatter may all be present in the simultaneously. These conditions create great difficulty in determining correct harvest time of the crop to gain maximum seed yield. The third obstacle is the pod dehiscent nature of the plant (see also 1.5.4.). This worsens harvesting problems, particularly in hot and dry environments (Metcalfe *et.al.*, 1957). A certain portion of seed loss, either due to immature or dehisced pods being harvested, is inevitable. In Minnesota, USA, McGraw and Beuselink (1983) found that harvesting at maximum pod maturity, yielded approximately 63% mature umbels, 25% immature, and 12% dehisced. Although a somatic hybrid between birdsfoot trefoil and *Lotus conimbricensis* has been successfully bred which produces non-dehiscent pods (apparently due to a reduced level of mesocarp lignification transferred from *L. conimbricensis*), the pods are infertile (Yang *et.al.*, 1990). These drawbacks combine to ensure it is impossible to recover all potential seed yield in birdsfoot trefoil.

Another problem is related to management practices, such as the dual purpose use of birdsfoot trefoil for animal feeding and seed production in the same year. It has been shown that cutting the crop at any time during spring and early summer results in the removal of early growth and subsequently in decreased seed yields (Anderson and Metcalfe, 1957; Bader and Anderson, 1962a). In addition, management for maximum herbage yield and for maximum seed yield is often different, e.g. in the case of plant population density (McGraw, *et.al.*, 1986).

Lodging may become a problem in birdsfoot trefoil seed production, especially in pure stands. MacDonald (1946) found that lodging before or during flowering caused significant reduction in seed yield. Mixture of birdsfoot trefoil and less competitive grasses, such as Kentucky blue grass, has been used effectively to reduce lodging, and subsequent increases in seed yields have been obtained (Anderson and Metcafe, 1957). However such a practice is questionable because pure stands of birdsfoot trefoil are more potent for higher seed yields than mixture crops (Seaney and Henson, 1970). Application of plant growth retardants may substitute this practice.

Further problems in birdsfoot trefoil seed production relating to pollination, and insects and diseases have been discussed in section 1.5.

1.7. Plant Growth Regulators

Manipulation of growth, shape and form, and yield of crop plants can be achieved by breeding new genotypes, by modification of environment or by the use of plant growth regulating chemicals. Breeding for a reduction in vegetative growth and better allocation of assimilate to reproductive parts of the plant has been successfully achieved especially in cereal grains with semi-dwarf varieties. However this is unlikely for herbage seeds in which vegetative parts of the plant are used for forage. For this reason, plant growth retardants, a group of plant growth regulating chemicals which primarily inhibit sub-apical cell division and elongation and hence reduce the growth of aerial parts of the plants (Dick, 1979), may provide alternatives for increasing seed yield.

Physiological and biochemical effects of plant growth retardants have been studied extensively, and reviewed by Dicks (1979), Davis *et.al.* (1988) and Rademacker (1989). The primary effects of plant growth retardants is that the chemical influence endogenous plant hormone levels. The inhibitory effects on shoot growth of a number of growth retardants, such as the triazole group, ancymidol and tetcyclacis, occur by inhibiting GA biosynthesis, although the sites of interference may be different with different materials (Dicks, 1979; Rademacker, 1989). Some growth retardants also interfere with the levels of other endogenous hormones. In general, increased levels of ABA, cytokinin and sterol formation, but not auxin, may be obtained in treated plants (Grossman *et.al.*, 1987; Izumi *et.al.*, 1988; Rademacker, 1989). Secondary effects on physiological alteration can be expected from hormonal changes. Early plant growth retardants have been found capable of promoting photosynthetic enzymes, altering respiration, and delaying leaf and plant senescence (Thomas, 1979). Better resistance to drought stress and to fungal attack have also been reported following the application of plant growth retardants (Rademacker, 1989).

1.7.1. Paclobutrazol

Paclobutrazol [(2RS)-1-(4chlorophenyl)-4,4-dimethyl-2-1,2,4-triazole-1-yl pentan-3-ol], also known as PP 333, Cultar, Parlay, Bonzi or Clipper, is a member of the triazole group which has generated much research interest, especially in horticultural plants. The applied chemical reaches the target site, the active sub-apical meristem, by transport in the xylem from the roots following soil application or through the young sub-apical shoot tissue following foliage spraying (Lever, 1986). In studies using ^{14}C, Quinland and Richardson (1986) found no phloem mobility of the chemical occurred. Paclobutrazol absorbed by leaves is not exported to adjacent shoots or leaves, even at the end of the season immediately before leaf fall. Wang *et.el.* (1986) also reported that foliarly applied paclobutrazol was not transported to stems or roots.

There is some interest in the use of paclobutrazol in herbage legume seed production. In white clover, Marshall and Hides (1986,1987,1989), and Bambang Budhianto (personal communication, 1989) found that the chemical did not affect the total number of nodes per stolon, the number of reproductive nodes per stolon or the intermittent pattern of flowering along the stolon. However, stolon length was reduced and the proportion of axillary buds per stolon was increased which may create more sites and space for inflorescence production. Paclobutrazol also suppressed petiole growth in such a way that inflorescences were elevated above the leaf canopy. This may improve pollination and ease of harvesting particularly in areas with wet weather during flowering conducive to increased vegetative growth. Seed yield potential was enhanced, mainly through increased total inflorescences, and ripe inflorescences at harvest. Little effect was found in other yield components. However, seed yields were not always significantly increased, probably because of harvesting difficulties. In general, middle or late spring application results in better seed yields, with an application rate of 1 kg a.i./ha superior to 0.5 kg a.i./ha. Application of 1 l/ha at the first flowering period has also been found to be beneficial in improving red clover seed production (Niemelainen, 1987). Although plant height was not reduced, red clover inflorescence numbers were increased in treated plants.

Lotus seed production has been improved through paclobutrazol application. Similar results have been obtained between *Lotus corniculatus* and *L. uliginosus* (Clifford and Hare, 1987; Hampton *et.al.*, 1989; Li, 1989; Li and Hill, 1989b). Seed yield increases of more than double have been often obtained with November spraying (early flowering stage) although with October (pre-bud stage) application significant increases are also occasionally found. Both September and December sprays seemed to not benefit seed production. Increased inflorescence numbers are the crucial contributor to increased seed yields. In birdsfoot trefoil, Li (1989) and Li and Hill (1989) found that improved seed yields with the application of paclobutrazol were mainly due to increased branching, since the chemical has no beneficial effect on general plant growth and assimilate allocation for promoting reproductive growth. In sward situations, seed yield increases were due to increases in the number of lateral branches with a subsequent increase in available sites for flowering. In spaced planting conditions, the promotive effect of paclobutrazol occured because of increased crown shoots formed on treated plants (Li, 1989; Li and Hill, 1989). In *L. uliginosus*, reports are conflicting. R.S. Tabora (1990, personal communication) found that in both sward and spaced plant situations increased seed yield potential was due to branching promotion, main shoot numbers being often reduced in treated plants of *L. uliginosus*, whilst Clifford and Hare (1987) reported that the effect on the species was because of increased stem numbers. These differences may possibly have been because of differences in the shoot criteria used.

1.7.2. Triapenthenol

Triapenthenol [-(cyclohexyl methylene)- -(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol] or RSW 0411 is a new triazole plant growth regulator. It has been reported that RSW 0411, when applied as a foliar spray to apple trees, was as effective as paclobutrazol, but that growth control was short lived (Curry *et.al.*, 1987). Spraying RSW 0411 and paclobutrazol at 1 g/L on two-year-old trees of Granny Smith variety, Irving and Pallesen (1989) found that RSW 0411 significantly reduced growth below that of the control between 27 and 40 days after application, compared with effects with paclobutrazol between 27 and 82 days after application. This may result in more controllable shoot growth. The effects of the chemical in reducing plant height and increasing seed yield have been found to be consistent in oil seed rape, rice, field beans and broad beans (Hack and Lembrick, 1985). In oil seed rape, Child *et.al.* (1987) found treatments at the beginning of lateral bud extension resulted in shorter stems and increased lateral bud outgrowth which led to greater axillary shoot leaf area and more flowers, and subsequently increased branching and canopy density during pod development. Treatment at the end of stem extension reduced branch length and petal size and color. The canopy was more open and the pod layer shallower. Measurement of vertical light profiles indicated that light penetration to the lower parts of the treated crop was increased. Oilseed rape yields were increased in treated plants regardless of application time when the incidence of light leaf spot (*Pyrenopeziza brassicae*) was prevalent. When disease incidence was small, yields were only increased by treatments given at the end of stem extension (Child *et.al.*, 1987). In the forage legume red clover, Niemelainen (1987) reported little advantage from spraying 1 kg a.i. RSW 0411/ha at the first flowering period on plant growth reduction, flower production or seed yield.

1.7.3. Cycocel

Cycocel [N-trimethyl (-chloroethyl)-ammonium chloride] or Chlormequat chloride, chlorocholine chloride or CCC is primarily used in cereal crops such as wheat and oat. It is also used in perennial ryegrass seed crops (Anonymous, 1989). The chemical shortens plant stems by about 20%, prevents stem break and lodging, and can increase grain yields by both increasing the number of fertile tillers and improving the synchrony of tiller growth and development within plants (Kust, 1986; Anonymous, 1989). No detailed study has been reported on the effect of Cycocel on plant structure of herbage legumes. However, in a study in white clover seed production Marshall and Hides (1984) found no significant effects on peduncle and petiole height following early summer Cycocel spraying at up to 2.56 kg a.i./ha. Cycocel (1.28 kg a.i./ha) significantly increased total flower number and the number of ripe heads at harvest, but flower size (florets per head) was also significantly reduced. The chemical did not affect the percentage of ripe heads at harvest, seed set or seed weight. Significant increases in flower numbers were not reflected in a higher potential seed yield or higher harvested seed yield. Similar negative results have been found in red clover when Cycocel (2.5 l/ha) was applied in summer (Niemelainen, 1987) or when 2 l/ha of chemical was applied in the start of flowering (Ryback and Walczak, 1988).

1.7.4. Daminozide

Daminozide [butaneodioic acid mono-(2,2-dimethyl hydrazide)] also known as Alar has been widely used in horticultural plants. In fruit crops, such as apples, cherries, kiwifruit and peaches, it has been recommended for use to inhibit shoot growth, induce flower bud initiation, increase fruit set and prevent pre-harvest drop (Anonymous, 1989). In some ornamental plants such as chrysanthenums the chemical can promote more compact flowering. In forage legumes, some studies have reported advantages following the use of daminozide, e.g. in red clover seed production, with seed yield increases of up to 200% (White *et.al.*, 1987). Alar has also been shown to increase seed yield in tetraploid red clover cultivars (by an average of 115%), but lower seed yield increases may be expected in diploid red clover (Christie and Choo, 1990). More advantage may be obtained when the chemical is applied in combination with the trace element boron (Jakesova and Svetlik, 1986). Spraying four red clover cultivars with daminozide (12 kg/ha) at 5-15% bloom in two consecutive seasons over three years, Christie and Choo (1990) only recorded seed yield increases in two cultivars and then only in the second season. Alar reduced plant height in three cultivars, but shortened corolla tube length of all cultivars studied. They noted that although a reduction in corolla tube length did not always result in an increase in seed yield, seed yields in excess of 100 kg/ha were associated with significantly shorter corolla tubes.

CHAPTER TWO
MATERIALS AND METHODS

2.1. Site and Treatments

The *Lotus corniculatus* seed lot used in these experiments was an unreleased selection (code number S2078); obtained from the Grasslands Division of the Department of Scientific and Industrial Research (DSIR), Palmerston North, New Zealand. The experiments were carried out over two consecutive growing seasons, from March 1988 to March 1990 at Massey University Frewin's Block Experimental Area, Palmerston North, New Zealand (40^{o}23'S, 175^{o}27'E). Weather data at Palmerston North during the cropping months is presented in Appendix 1. These experiments involved a crop grown at a row spacing of 60 cm with 71 plants and 62 plants per metre row in the first and the second years respectively. No irrigation, fertilizer application or pollinator introduction was applied. Management and treatment details are presented in table 2.1. Plant growth regulators paclobutrazol (Cultar^{R}), daminozide (Alar^{R}), and chlormequat chloride (Cycocel^{R}) were applied at two rates in the 1988/1989 growing season. Based on seed yield results, Cultar and Cycocel were again applied at the same rate in the second year 1989/1990, but Alar was replaced with RSW-0411 (a triazole plant growth retardant considered to be less persistent than Cultar (Curry *et.al.*, 1987)). In addition, residual Cultar effects from the 1988 treatments were also studied in the second year. The two application times in both years, *i.e.* 9 October and 18 November in 1988 and 19 October and 15 November in 1989, represented pre-flowering bud and onset of flowering stages.

Table 1. Cultural management and experimental details

Management	First year seed crop (1988/1989)	Second year seed crop (1989/1990)
Site	Massey University (40°23'S, 175°37'E)	
Soil type	Tokomaru Clay soil	
Soil analysis	pH-5.8, Olsen P-13, Exch.K-0.32,SO4-5.8	
Sowing rate ; Row spacing	1.5 kg/ha ; 60 cm	
Sowing date	23 March 1988	second year crop
Age of crop	One year	Two years
No. of plants/m row	71 (13 October 1988)	64 (13 October 1989)
Fertilizer	None	None
Last grazing	15 August 1988	16 August 1989
Interrow cultivation	27 September 1988	22 September 1989
Weed control	2.4-DB at 3 l a.i./ha & Kerb at 1.5 kg a.i./ha on 10 Oct. 1988	2,4-DB at 5 l a.i./ha, Kerb at 2 kg a.i./ha, & MCPB at 3 kg a.i./ha on 7 October 1989
Insect control	Mavrick at 30 ml/100 l of water on 30 Dec.1988	Mavrick at 30 ml/100 l of water on 5&24 Dec. 1989, 3&16 Jan. 1990
Treatment:		
-PGR (kg or l a.i./ha)	Cultar (0.5 or 1.0) Cycocel (1.5 or 3.0) Alar (2.0 or 4.0)	Cultar (0.5 or 1.0) Cycocel (1.5 or 3.0) RSW (0.5 or 1.0) Residual PP333 1988
-time	9 October 1988 18 November 1988	19 October 1989 15 November 1989
Harvest time	2 February 1989 (36 DAPF)	21 February 1990 (41 DAPF) 27 February 1990 (47 DAPF)

A Randomized Complete Block Design (RCBD) was used with three replications in the experiments.

2.2. Vegetative development

Vegetative development was measured in the 1989/1990 growing season monthly, from 13 September 1989 (one month after last grazing) to 13 January 1990 (2 days after peak flowering). Measurements included the number of plants; the number and weight of main shoots, lateral shoots and secondary lateral shoots; and the length and the number of nodes of the longest main shoots. These were done only on untreated (control) and high-rate chemical treated plants, using samples obtained by removing all plants emerging within a randomly selected 25 cm section of row and carefully separating them from those emerging outside the sampled row. The number of plants was counted, and the shoots then cut approximately 5 cm above the crowns to assist in separation of plant parts. The number of cut shoots were counted (main shoots). Shoots emerging directly from main shoots were then counted and separated (lateral shoots). Secondary lateral shoots (shoots borne on lateral shoots) were only analyzed at the last measurement. The ten-longest main shoots were separated for shoot length and node number measurement before all shoot categories were oven dried at $80^{o}C$ for two days for dry weight determination.

2.3. Inflorescence Production

Inflorescence production was recorded in the second growing season. Patterns of flowering during the flowering season were measured regularly at 5-6 day intervals, according to the rate of flower development at that specific time, by counting flowers within a 0.25 metre square permanent quadrant. Only inflorescences which had at least one open floret and no withered florets was counted. Double counting was minimized by tagging 10 inflorescences with one open floret at the day of each counting to determine the next counting day. Although the flowering season extended from the middle of November to late February in the following year, flowering records were only recorded from 9 December 1989 to 14 February 1990.

Inflorescence distribution among shoot categories was assessed at two days after peak flowering (13 January 1990). Flower buds, open flowers, and umbels were counted in each shoot catergory before the shoots were put in the oven. Inflorescence classification was as follows :

flower bud : visible inflorescence with florets not yet open,
open flower : inflorescence with at least one open floret,
pod umbel : inflorescence with all florets withered or pods set.

Total reproductive structures were also measured from 0.25 metre square quadrants in all treatments at 8 days after peak flowering (19 January 1990).

2.4. Reproductive Abortion

In 1989/1990, reproductive abortion was studied in control plants and in plants which had been treated with the higher rates of Cultar, RSW-0411, and Cycocel applied on October (three treatments). Assessments were made of levels of floret bud abortion, flower abortion, floret or pod abortion, and ovule or seed abortion of open inflorescences produced at four different times during the flowering season, *i.e.* 16, 31 December 1989, 15 and 30 January 1990 (referred as very early, early, peak and late flowers). The data were analyzed by using a split-plot design model, with time as the main plot and plant growth regulator treatment as the sub-plot, as described by Steel and Torrey (1980).

Plate 2.1. *Lotus corniculatus* 1989/1990 seed crop showing tagged inflorescences with different colored of plastic tags, each representing a different monthly tagging time, and a bamboo stick as a position marker for locating the tagged flowers in the field.

1. orange tags = tagging at 16 December 1989 (early flowers)
2. blue tags = tagging at 31 December 1989 (middle flowers)
3. white tags = tagging at 15 January 1990 (peak flowers)
4. red tags = tagging at 30 January 1990 (late flowers)

with time as the main plot and plant growth regulator treatment as the sub-plot, as described by Steel and Torrey (1980).

Floret bud abortion (FBA) was assessed from a sample of 30 inflorescences by counting the number of floret buds per inflorescence at the visible inflorescence stage (VB, 15 days before open flower stage) and the number of florets per inflorescence at the open flower stage (OF) 16, 31 December 1989, 15 and 30 January 1990. FBA was equal to VB minus by OF.

Flower abortion, floret or pod abortion, and ovule or seed abortion were assessed from tagged inflorescences during the flowering season. On the same days when representing a different tagging time. Therefore, 720 inflorescences (60x4x3) were tagged at any one tagging time (2880 inflorescences in the whole flowering period). Ten inflorescences showing the same stage of development were taken at tagging date (referred as OF). Ten tagged inflorescences were taken randomly at 5, 10, and 20 days after tagging (referred as days after open flower, DAOF). At 30 DAOF, twenty tagged inflorescences were randomly removed and the number of live inflorescences (umbels) counted. The number of florets or pods per inflorescence were measured from inflorescences directly after sampling.

The number of ovules per ovary or seeds per pod was counted from a sample of ten ovaries or pods taken randomly from the inflorescence samples. Since these were not counted immediately, the samples were preserved in formalin-acetic acid (FAA) solution. This solution consisted of 90% ethyl alcohol 70%, 5% glacial acetic acid, and 5% formalin 40%. The number of ovules per ovary was counted from inflorescences at the OF stage by dissecting the ovary and counting the ovules under a dissecting microscope. The number of seeds set per pod at 5, 10 and 20 DAOF in very early, early and peak flower groups were counted by the same method. However, the number of seeds set at 20 DAOF in late flowers and at 30 DAOF were determined by using X-ray radiography at 25 KVP for about 2 minutes.

In addition to this abortion study, ovary and pod lengths were measured from the sampled ovaries or pods. Seed weight was also measured for 30 DAOF samples by weighing 50 seeds from the most mature pods after oven-drying at $80^{o}C$ for 2 days. Only seeds positioned in the middle two-thirds region of the pods were used to reduce variation in seed weight (Long, *et.al.*, 1989).

Weather data during the flowering period and abortion study are presented in Appendix 2.

Weather data during the flowering period and abortion study are presented in Appendix 2.

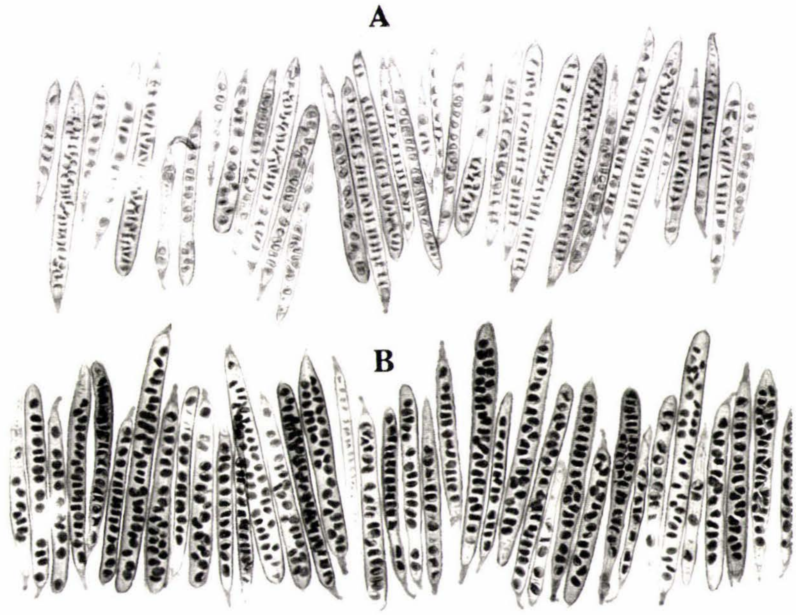

Plate 2.2. An example of an X-ray photograph of pods used to count the number of seeds per pod in late flowers, showing (A) seeds at 20 DAOF and (B) seeds at 30 DAOF.

2.5. Seed Yield, Seed Yield Components and Harvest Index

In the first season, seed yields were determined by cutting and removing all plants within 1 m^2 permanent quadrants on 2 February 1989. In the second season, seed yields were assessed twice, on 21 February 1990 (41 days after peak flowering, DAPF) from plants sampled within 1 m^2 permanent quadrants and on 27 February 1990 (47 DAPF) from 0.5 m^2 permanent quadrants. The plants were air dried, and threshed using a laboratory thresher "Westrup" type LAH. The seeds were cleaned by using sieve no. 14 on a "Clipper" seed cleaner and a mini blower. All straw was and oven dried at 80^{o}C for two days for harvest index assessment. Harvest index (HI) was counted as seed yield (SY) at 0 percent moisture content divided by sum total dry weight (TDW) of the collected straw and seed yield. For seed yield components, number of pods per umbel were determined from 20 umbels taken randomly at harvest date in the first season, and 50 umbels at first harvest for the second growing season. Number of seeds per pod was determined from a sample of 30 pods photographed by using a Faxitron X-ray machine set at 25 KVP for about 2 minutes. Thousand seed weight was determined from 4 X 100 seed weighings.

2.6. Dry Matter Distribution

Dry matter distribution of above ground parts was determined from the first harvest material in the second year crop. Before threshing, total number of umbels (TUN) was counted. A sample of 100 unshattered umbels was randomly taken from the field on the harvest day by cutting the umbels at the base of their peduncles. The seeds were extracted from the reproductive straw by hand rubbing dried umbels inside a cloth bag. Both seed dry weight (SWTs) and reproductive straw dry weight (RSWT) were assessed by putting the seeds and the reproductive straw in an oven at 80^{o}C for 2 days and then weighing them. Total weight of harvested material (TWT), used in harvest index determination (see 2.4.), was also used in this assimilate partitioning calculation. Seed weight (SWT), reproductive weight (RWT) and vegetative weight (VWT) were calculated by using following formulae:

SWT = TUN : 100 x SWTs

RWT = TUN : 100 x (RSWT + SWTs)

VWT = TWT - RWT

2.7. Seed Quality

In 1990, the machine-harvested seeds from control and high-rate treated plants were tested for seed quality. Seed quality was assessed using the germination test prescriptions in the ISTA Rules (ISTA, 1985) with 50 seeds and two replicates on 20 May 1990.

2.8. Statistical Analysis

Statistical analysis was carried out by using the SAS system (SAS, 1987). Least significant difference with probability 5 percent ($LSD_{0.05}$) was used to differentiate data where analysis of variance (ANOVA) or general linear model (GLM) was sigificant at 0.05 , 0.01 or 0.001 levels. Graphs were made by using a Microsoft Chart.

CHAPTER THREE

RESULTS

3.1. Crop Growth and Development

Considerable variation in growth between the plants within 25 cm sampled rows was observed. Plate 3.1. illustrates this variation during the early growing season in the second year. Vegetative growth comparisons of two consecutive years' seed crops used in these experiments also showed a considerable difference in growth recovery after grazing. Both crops were last grazed on approximately the same date in both years (15 August 1988 and 16 August 1989, Table 2.1.). By 10 November 1988 (87 days after last grazing) average shoot length in the untreated control was 31.6 cm, while in the second year (13 November 1989, 89 days after last grazing) average shoot length had reached 81.2 cm. Since no further vegetative measurement were conducted in the first year seed crop, these results only report crop growth and development in the second year. In addition, only crops sprayed with plant growth regulators at high rates, *i.e.* 1.0 kg a.i./ha of Cultar or RSW-0411, and 3.0 l a.i./ha of Cycocel, were measured for this purpose.

Noticeable differences between control and plant growth regulator treated crops occurred only in Cultar treatments. Plants to which Cultar had been applied looked greener (Plate 3.2.), although chlorophyll content in the leaves were not determined. Plant shoots were also shorter (Appendix 3.). However, measurements of longest

Plate 3.1. Variation in *Lotus corniculatus* plants sampled from 25 cm row at 13 October 1989 (58 days after last grazing).

Plate 3.2. *Lotus corniculatus* crop at 23 days after application (11 November 1989) showing some October Cultar treated plots which look greener and more prostrate compared to other plots. (B) control plot, (A) & (C) 0.5 kg a.i. Cultar/ha, plants almost recovering from the retarding effect of Cultar, (D) 1.0 kg a.i. Cultar/ha, more prostrate than (a).

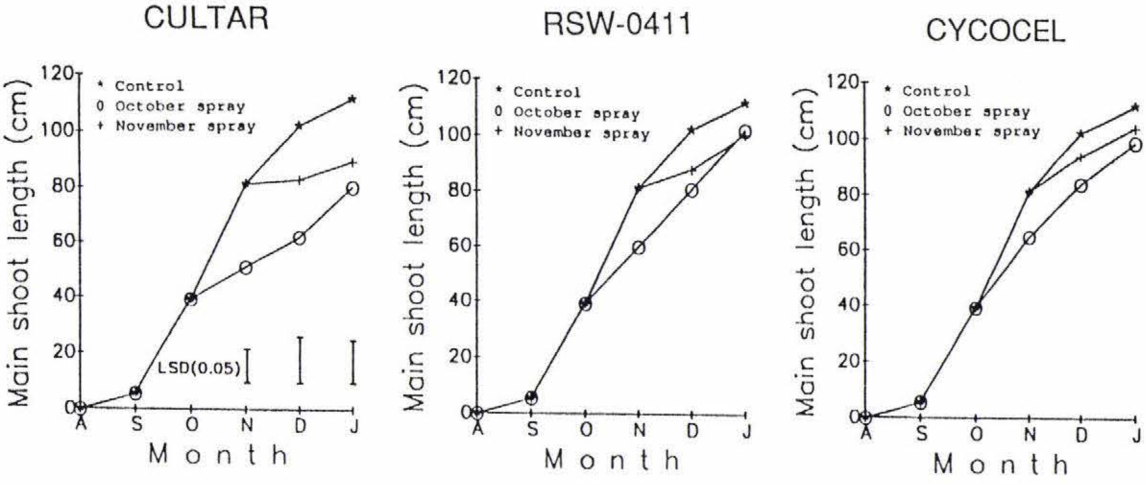

Figure 3.1. Effect of plant growth regulators applied at higher rates on the longest main shoot length of the second year seed crop (1989/1990). LSD(0.05) values presented the in Cultar figure also represent LSD(0.05) values in RSW-0411 and Cycocel.

shoot length in Figure 3.1. also shows the effect of RSW-0411 and Cycocel. Following October application, (the time of rapid shoot growth), all plant growth regulators significantly shortened shoot length ($P_{0.01}$), the reduction being 37% with Cultar, 32% with RSW-0411 and 20% with cycocel at 24 days after application. By peak flowering (85 days after application), Cultar treated plants showed significant shoot shortening (72%, $P_{0.05}$) compared with 116 cm in the control. However the other plant growth regulators did not significantly reduce shoot length at that time. After November application, when shoot growth had slowed down, only Cultar significantly shortened shoot length ($P_{0.05}$) at peak flowering. It was also found that none of the plant growth regulators affected node numbers on the longest main shoot (Appendix 3)

Crown shoot (main shoot) number, as shown in Figure 3.2. and Appendix 4., increased rapidly during the first month after last grazing in the early spring (August), before slowing down until a maximum number was reached (approximately 300 per

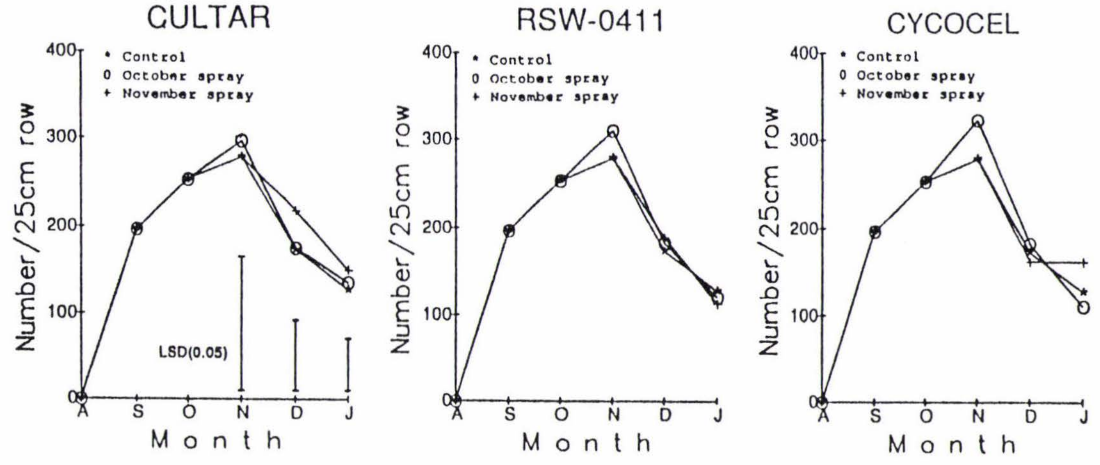

Figure 3.2. Effect of plant growth regulators applied at higher rates on the development of main shoot numbers of the second year *Lotus corniculatus* seed crop (1989/1990). LSD(0.05) values presented in the Cultar figure are also represent LSD(0.05) values in RSW-0411 and Cycocel.

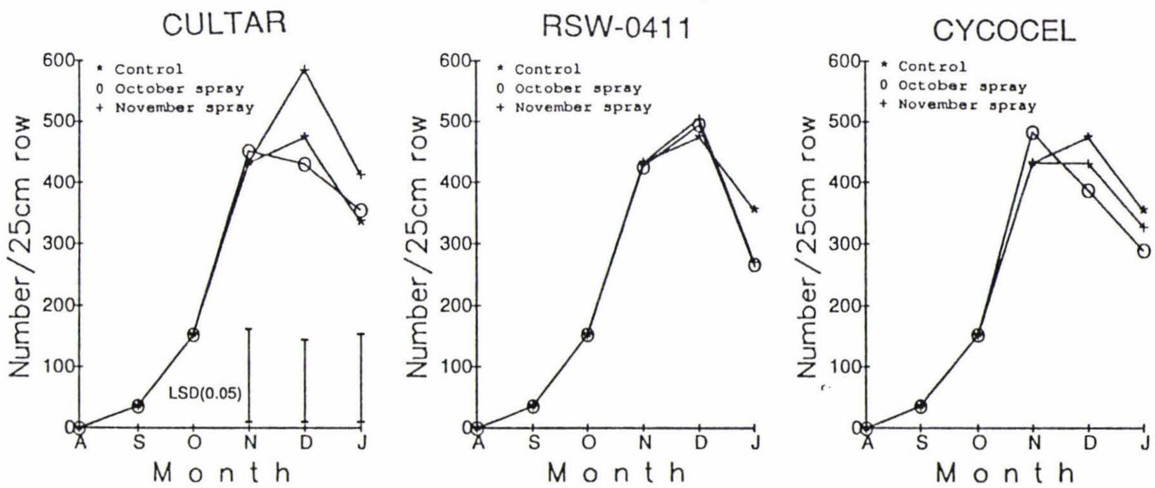

Figure 3.3. Effect of plant growth regulators applied at higher rates on the development of lateral shoot numbers of the second year *Lotus corniculatus* seed crop (1989/1990). LSD(0.05) values presented in the Cultar figure are also represent LSD(0.05) values in RSW-0411 and Cycocel.

25 cm row) in November. A great number of main shoots then died, only 48% surviving by the time of peak flowering. None of the tested plant growth regulators significantly modified this pattern of main shoot production. Although it appeared that October applied plant growth promoted slight increases in main shoot number, these increases were not significant. The rapid increases in lateral shoot numbers during September-November (Figure 3.3), coincide with the slowing down of main shoot production, and the time of complete ground coverage. Peak numbers of lateral shoots occurred on December, with the exception of Cultar and Cycocel applied in October, which peaked in November. No significant differences were found although the November-Cultar sprayed crop appeared to produce more lateral shoots (Figure 3.3. and Appendix 4.).

Patterns of general growth, in terms of dry weight, are presented in Figure 3.4. and Appendix 5. In the untreated crop, dry weight of main shoot and lateral shoot increased rapidly, beginning one month following rapid increases in main shoot number and lateral shoot number, respectively. In December or January, however, both main shoot weight and total lateral shoot weight contributed similarly to total dry weight. During these two months, dead material contributed 10-20% of total dry weight. A total above ground dry matter production of 13 metric tons was present by the time of peak flowering. Application of plant growth regulators at pre-flowering (October) and the onset of flowering time (November), although resulting in some variation, did not produce significant effects on the weight of main shoots, total lateral shoots, dead matter, and total dry matter (Appendix 5.). When total lateral shoots were separated into lateral shoots and secondary lateral shoots, secondary lateral shoot number was found to be significantly reduced (P0.05) by up to 32% by October application of RSW-0411, and by up to 35% following November application of Cycocel (Appendix 4). However this effect was not reflected in

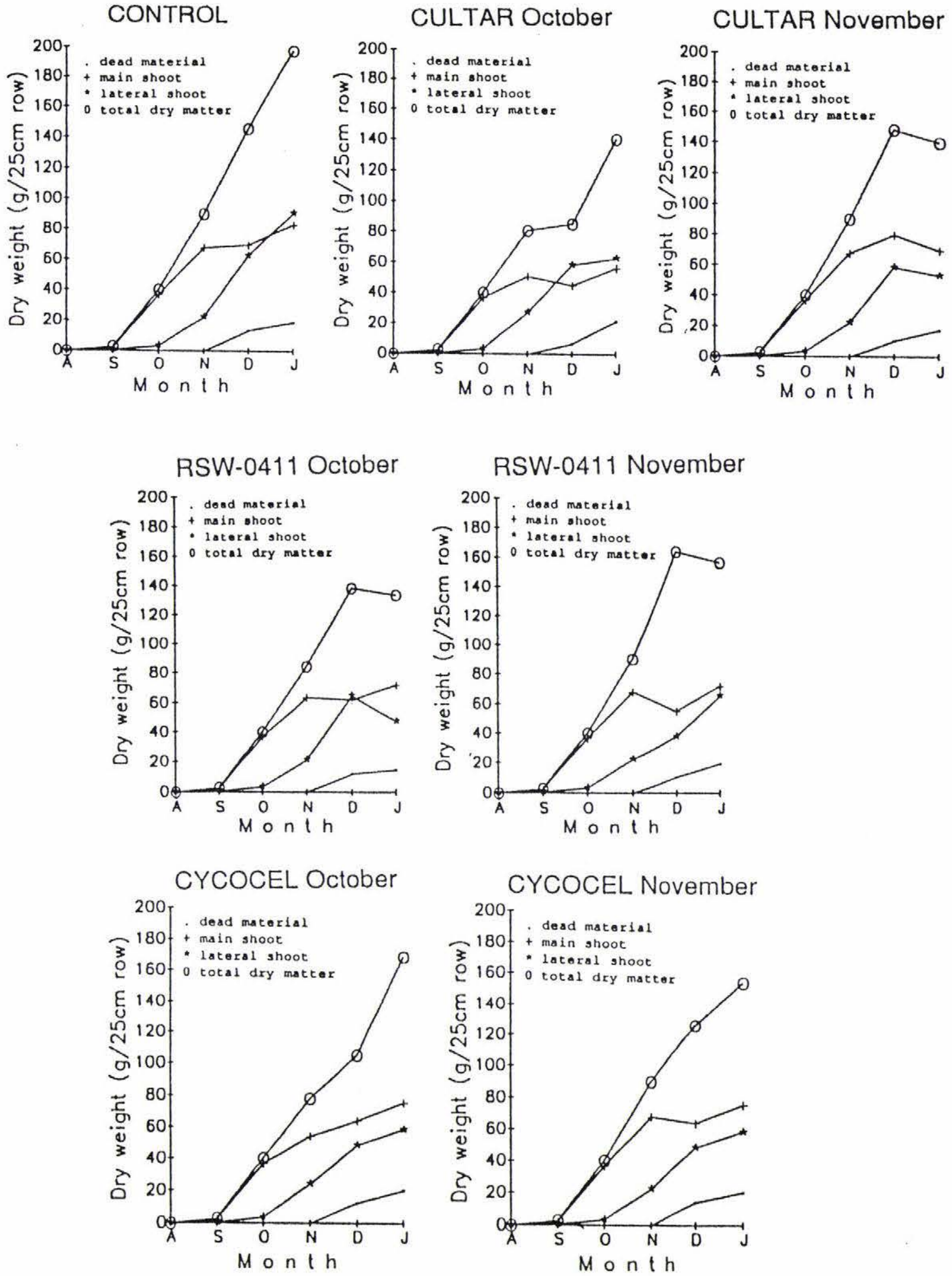

Figure 3.4. Effect of Cultar, RSW-0411 and Cycocel applied at higher rates on general plant growth of the second year seed crop (1989/1990).

significant differences in dry weight (Appendix 5.). Similarly, shoot proportions (including number of lateral shoots per main shoot, number of secondary lateral shoots per lateral shoot, lateral shoot weight per main shoot weight and secondary lateral shoot weight per lateral shoot weight) from application through to mid January (peak flowering) were not significantly affected by plant growth regulator application (Appendix 6.).

3.2. Flowering Pattern

Flower production in *Lotus corniculatus* extended over a period of three months, from the middle of November to late of February. Only one distinct peak was found during the flowering season in all treatments (Figures 3.5., 3.6. 3.7. and 3.8.). In nearly all treatments this peak was well defined and occurred on the same date (11 January 1990). However in areas sprayed with the high rate of RSW-0411, peak flowering was delayed 6 days. The application of Cultar at 1.0 kg a.i./ha in October (Figure 3.5) appeared to stimulate more early flowering with a resultant plateau effect. However, the date of peak flowering was not affected. An opposite effect occured when Cultar was applied at the low rate (0.5 kg a.i./ha). November applied Cultar at both 0.5 kg and 1.0 kg a.i./ha delayed flower production in the early season up to 10 days, but significantly increased flower production during the peak flowering period (5 and 11 January 1990). Plate 3.3. illustrates the differences in appearance of the control crop and the November applied Cultar treated crop at peak flowering. Interestingly, however, the differential effects of early and late application of Cultar were not carried over into the following year. The time and rate effects of residual Cultar (Figure 3.8) on the flowering duration and date of peak flowering were similar.

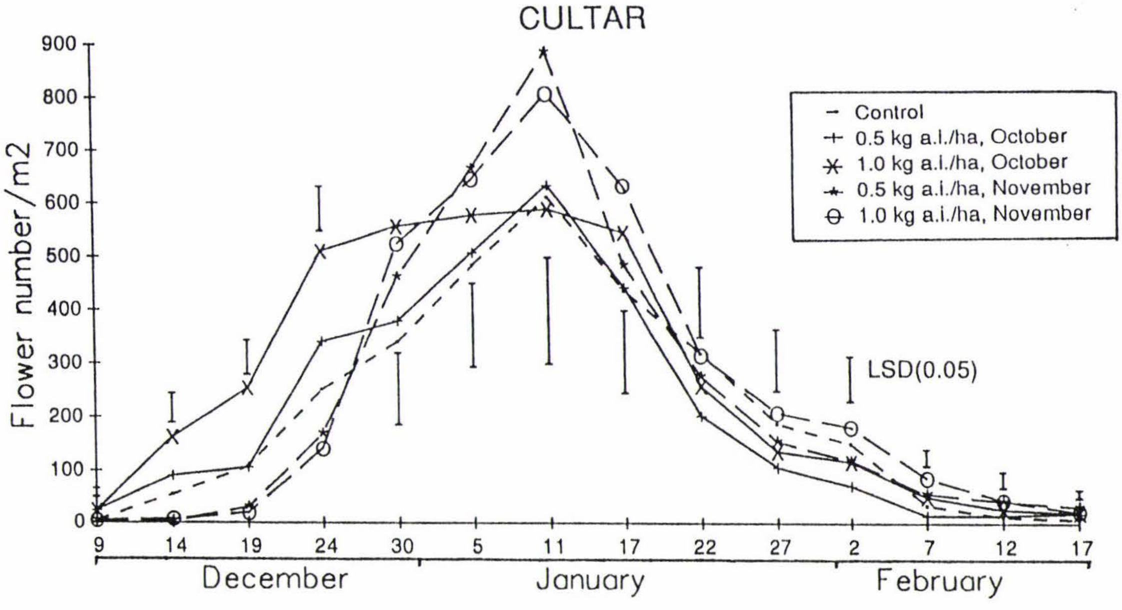

Figure 3.5. Flowering patterns of the second year *Lotus corniculatus* seed crop (1989/1990) as affected by Cultar applied at two different times and two different rates.

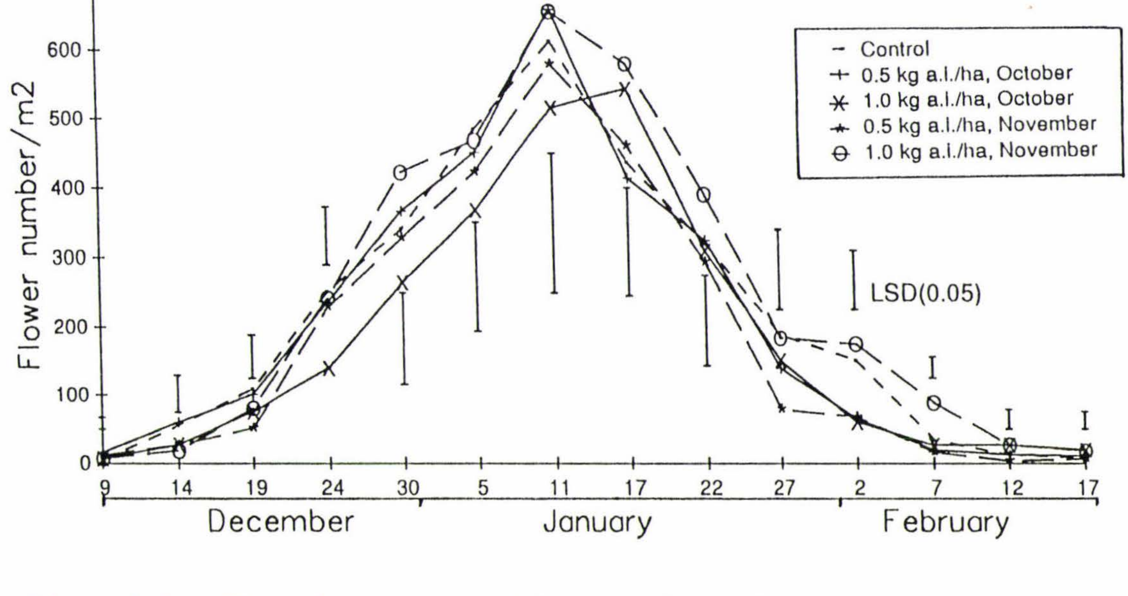

Figure 3.6. Flowering patterns of the second year *Lotus corniculatus* seed crop (1989/1990) as affected by RSW-0411 applied at two different times and two different rates.

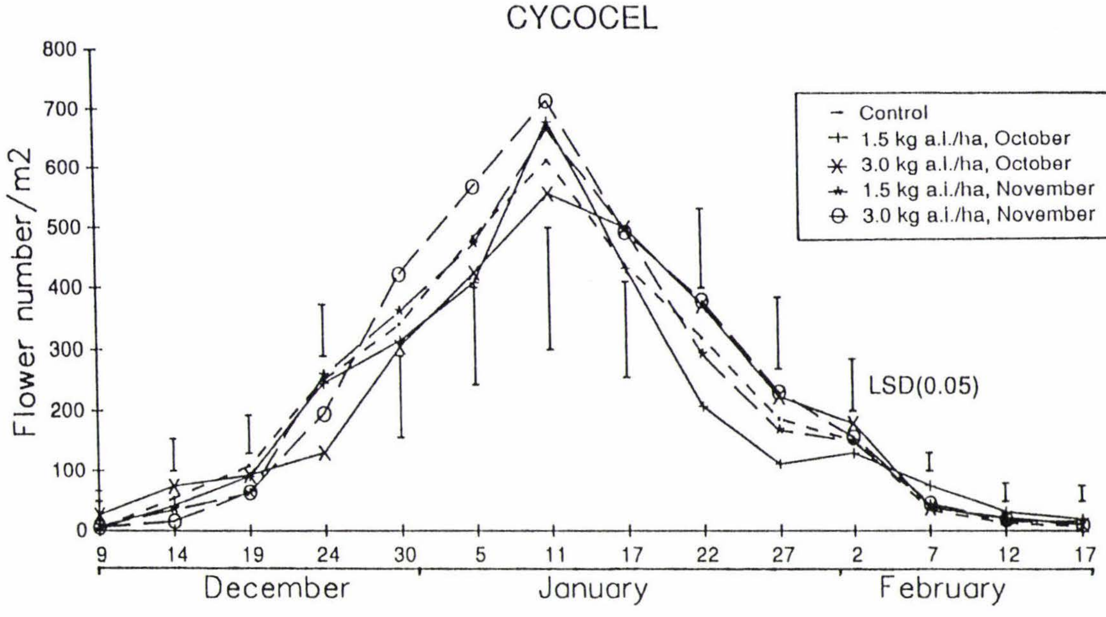

Figure 3.7. Flowering patterns of the second year *Lotus corniculatus* seed crop (1989/1990) as affected by Cycocel applied at two different times and two different rates.

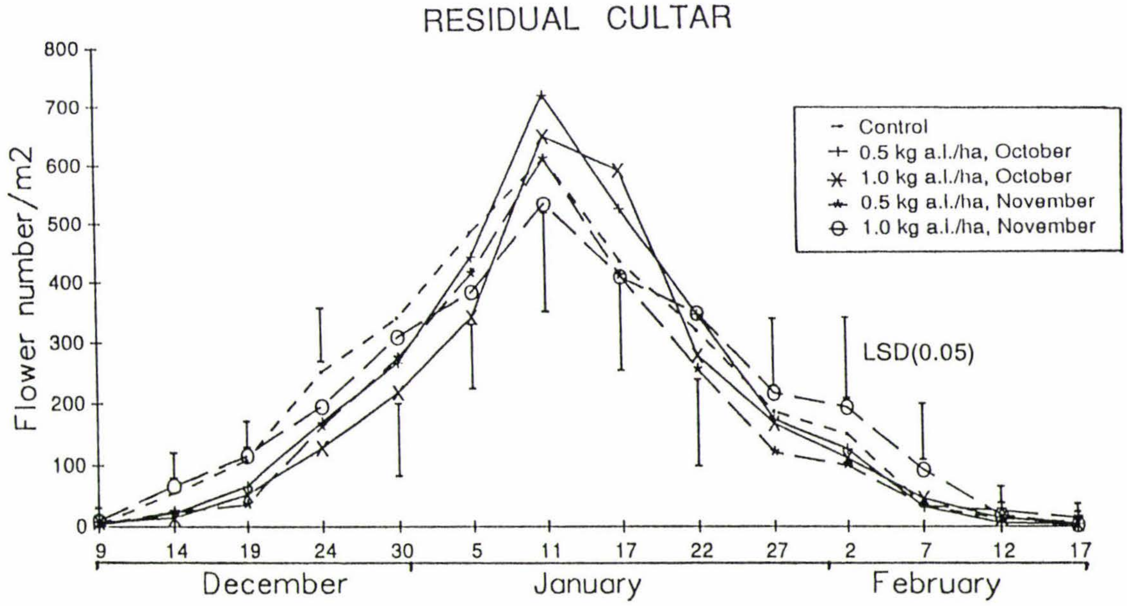

Figure 3.8. Flowering patterns of the second year *Lotus corniculatus* seed crop (1989/1990) as affected by residual Cultar applied at two different times and two different rates in the first year seed crop (1988/1989).

Plate 3.3. *Lotus corniculatus* crop at 12 January 1990 (one day after peak flowering) showing differences in flowering intensity of control plants (A) and plants treated with 1.0 kg a.i. Cultar/ha in November (B)

Table 3.1. shows the results of reproductive structure category and distribution in main shoot, lateral shoot and secondary lateral shoot at 13 January (two days after peak flowering). Total reproductive structures were not affected by plant growth retardant application, but there was a reduction ($P_{0.01}$) in the number of reproductive structures in secondary lateral shoots following October application of RSW-0411 (41%) and November application of Cycocel (49%). These reductions primarily resulted from lower numbers of secondary lateral shoots (Appendix 4.). The number of reproductive structures per secondary lateral shoots was not significantly different in either treatment (Table 3.1.). In contrast, Cultar applied in November increased ($P_{0.01}$) total open flower numbers at peak flowering by up to 67%, compared with the control. Other variables observed, *i.e.* number of reproductive structures on main shoots and on lateral shoots, total flower buds and total umbels, number of reproductive structures per main shoot, per lateral shoot and per secondary lateral shoot, were not significantly affected by application of of any of plant growth regulator treatments (Table 3.1.).

3.3. Reproductive Growth and Floral Abortion.

Floret bud abortion, flower abortion, floret or pod abortion, and ovule or seed abortion were investigated in open flowers formed at different periods during the season in 1990. Floret bud abortion was examined by taking a sample of 30 flower buds and a further sample of 30 open flowers 15 days later. Table 3.2. shows that the number of floret buds buds per flower bud was about six. Neither time of flower bud production nor application of plant growth regulators had a significant effect on this figure. By the time of open flower, about five florets were retained, about one floret bud having been aborted. It was observed that floret bud loss occurred in the early stages of flower bud development. No abortion was found when 50 tagged unopened

Table 3.1. Reproductive structure distribution on shoots and reproductive categories of *Lotus corniculatus* at 13 January 1990 (number per 25 cm row).

Treatment[0]		Mean Reproductive Structures[1]									Total[2]
		in Shoots			per Single Shoot			Categories			
Chemical	Time	MS[3]	LS	SLS	MS	LS	SLS	F.Buds	Flowers	Umbels	
Control		107.0	118.0	75.7ab	0.86	0.35	0.31	98.7	88.7*bc*	113.3	300.7
Cultar	October	142.7	142.3	54.0bc	1.03	0.56	0.29	120.3	88.0*bc*	130.7	339.0
	November	169.7	188.3	93.0a	1.11	0.45	0.34	147.3	147.7*a*	156.0	451.0
RSW-0411	October	127.0	127.7	44.3c	1.05	0.50	0.28	93.3	75.0*bc*	130.7	299.0
	November	103.0	122.7	58.7bc	0.91	0.49	0.33	79.3	71.7*bc*	133.3	284.3
Cycocel	October	100.0	119.0	59.0bc	0.89	0.41	0.30	99.7	65.3*bc*	113.0	278.0
	November	146.7	144.7	38.7c	0.93	0.45	0.25	109.3	102.7*c*	118.0	330.0
		NS	NS	**[4]	NS	NS	NS	NS	**	NS	NS
Mean		128.0	137.5	60.1	0.97	0.46	0.30	106.9	91.3	127.9	326
Cv		32	20	25	20	23	20	26	23	21	21

Note : [0] Application rate of Cultar and RSW-0411 were 1.0 kg a.i./ha, Cycocel was 3.0 kg a.i./ha. 1) Reproductive structures include flower buds, open flowers and umbels. [2] Total means total reproductive structures. [3] MS = main shoot, LS = lateral shoot, SLS = secondary lateral shoot, F.Bud = flower bud. [4] ** Values in the same column followed by the same letter are not significantly different at F-test level of 0.01, according to LSD(0.05). NS means no significant differences.

Table 3.2. Effect of plant growth regulators applied in October on number of floret buds per inflorescence (15 days before open flower), florets per blooming inflorescence and floret bud survival at different times of flower production. (1989-1990). (All Treatment X Time : NS)

Treatment1)	**Time of flower production**				**Treatment**
	16 Dec.	**31 Dec.**	**15 Jan.**	**30 Jan.**	**Means**
A. Floret buds per inflorescence					
Control	--	5.99	6.05	5.82	5.95NS
Cultar	--	5.83	6.40	6.04	6.09
RSW-0411	--	6.28	5.90	5.91	6.03
Cycocel	--	5.91	5.96	5.65	5.84
Time means(NS)	--	6.00	6.08	5.86	5.982)
B. Florets per inflorescence					
Control	4.62	4.21	5.02	4.72	4.64*bc***3)
Cultar	5.31	5.10	5.45	5.04	5.23*a*
RSW-0411	4.32	4.38	4.46	4.43	4.40*c*
Cycocel	4.88	4.76	4.69	4.49	4.70*b*
Time means(NS)	4.78	4.61	4.91	4.67	4.74
C. Floret bud survival (%)					
Control	--	70	83	81	78*ab* *
Cultar	--	88	85	84	86*a*
RSW-0411	--	70	75	75	74*b*
Cycocel	--	80	79	80	80*ab*
Time means(NS)	--	77	81	80	79

Note: 1) Cultar and RSW-0411 were applied at 1.0 kg a.i./ha, while Cycocel at 3.0 kg a.i./ha.
2) Overall mean. **3)*,**,***** Values in treatment means column or in time means line followed by the same letter are not significantly different at probability level 0.05, 0.01,and 0.00, respectively according to LSD(0.05). NS means no significant differences. These notes also apply to other tables in sub-section 3.3.

flowers , each with six floret buds, were observed again five days later (at least three florets open). Although the number of open florets per inflorescence was significantly increased by Cultar application in October, there was no significant effect of either time of flower production or plant growth regulator on floret bud survival at the time of flower opening.

Plate 3.4. illustrates the development of inflorescences from the open flower stage until 30 days after open flower (DAOF) showing some examples of the types of inflorescence abortion and floret or pod abortion which occurred. Table 3.3. further shows the development of florets per inflorescence or pods per umbel during the period from mid December to the end of January. When ten flowers with at least five florets were sampled, there was unexpected variation in the number of florets per flower at the time of flower opening and also as a result of plant growth regulator treatment. Five days later, however, this variation had largely disappeared. Floret or pod abortion occurred consistently throughout flower development during the flowering season. The time of flower production and plant growth regulator application had little effect on this abortion pattern. However, by 30 DAOF, only 3.4 pods per umbel remained compared with the initial 6.1 florets present per inflorescence at tagging (Table 3.3.).

The abortion of ovules or seeds during their development is clearly illustrated in plate 3.5. Ovules or seeds were considered to abort when they were considerably reduced in size or had shrivelled. Ovule or seed number during flower development was recorded and is presented in Table 3.4. Control plants produced about 50 ovules per ovary. October applications of RSW-0411 and Cycocel slightly improved ovule production (4-6% from control). However, season also modified ovule production, being slightly higher during early flowering (16 December), decreasing until peak flowering time (15 January), and increasing again in late flowers. These differences,

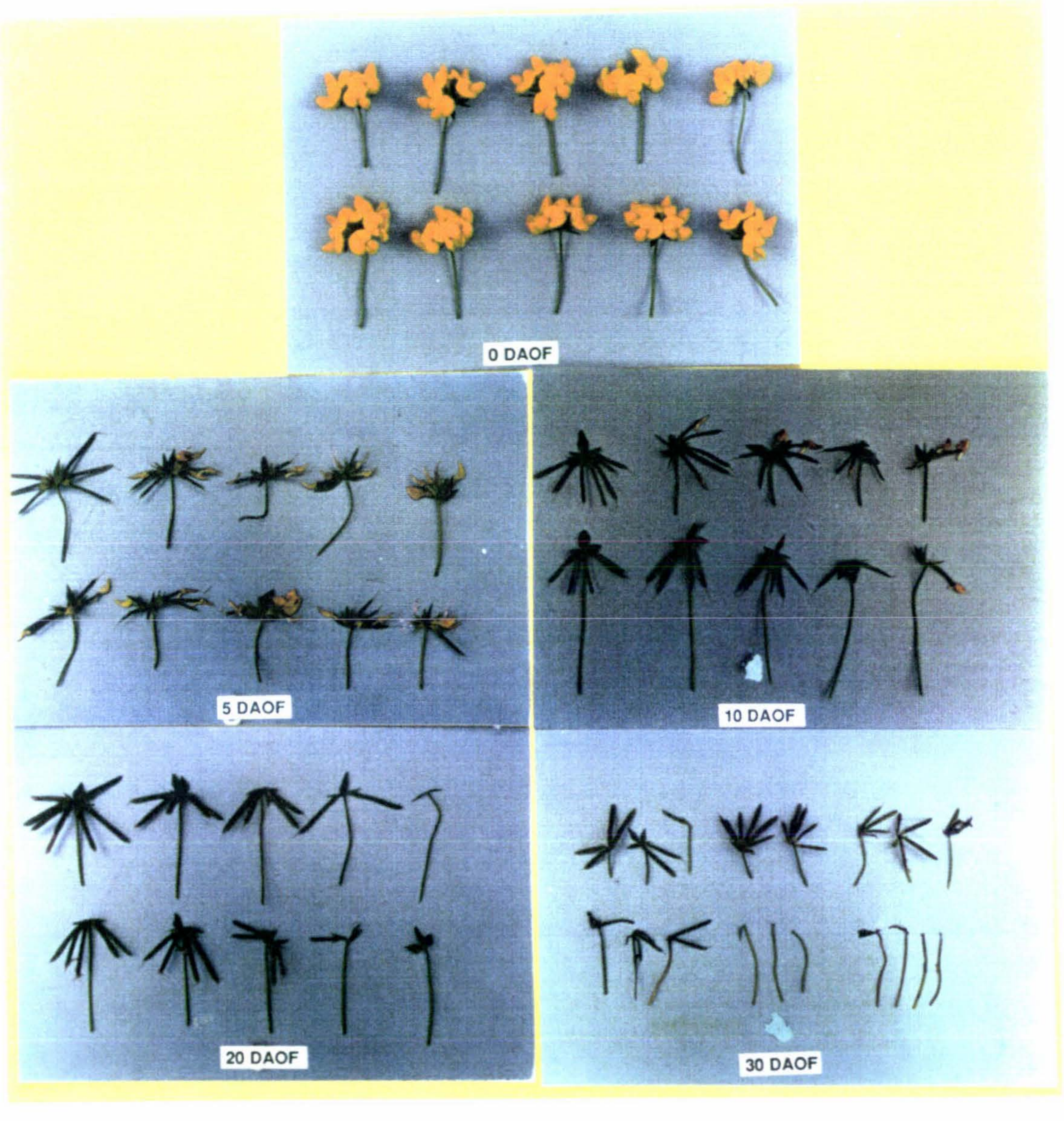

Plate 3.4. Flower development of *Lotus corniculatus*, showing floret or pod abortion, petioles without pods and decayed umbels.

Table 3.3. Effect of plant growth regulators applied in October on florets per inflorescence or pods per umbel measured at 0, 5, 10, 20, and 30 DAOF in inflorescences produced at four different times. (1989-1990). (All Treatment X Time : NS)

Treatment	Time of flower production 16 Dec.	31 Dec.	15 Jan.	30 Jan.	Treatment Means
A. 0 DAOF.					
Control	6.06	6.00	6.23	6.06	6.09*ab**
Cultar	6.33	6.10	6.43	6.13	6.25*a*
RSW-0411	5.90	6.26	6.23	5.70	6.03*b*
Cycocel	5.96	6.20	6.20	5.76	6.03*b*
Time means***	6.07*bc*	6.14*ab*	6.28*a*	5.92*c*	6.10
B. 5 DAOF.					
Control	5.66	5.53	6.06	5.76	5.76NS
Cultar	5.90	5.73	5.96	5.43	5.76
RSW-0411	5.40	5.70	5.90	5.80	5.70
Cycocel	5.40	5.93	5.76	5.63	5.68
Time means(NS)	5.59	5.74	5.93	5.66	5.73
C. 10 DAOF.					
Control	5.10	5.30	5.16	4.50	5.02NS
Cultar	5.40	4.96	5.13	4.60	5.03
RSW-0411	5.20	5.56	5.33	4.44	5.13
Cycocel	4.96	5.75	5.10	4.86	5.17
Time means*	5.17*a*	5.39*a*	5.18*a*	4.60*b*	5.09
D. 20 DAOF.					
Control	4.10	4.06	3.66	4.46	4.08NS
Cultar	3.23	3.33	4.20	4.10	3.72
RSW-0411	4.36	4.06	3.70	4.63	4.19
Cycocel	3.10	3.50	3.10	4.16	3.47
Time means*	3.70*b*	3.74*b*	3.67*b*	4.34*a*	3.86
E. 30 DAOF.					
Control	3.66	3.56	3.20	4.16	3.65NS
Cultar	2.66	3.36	3.43	3.00	3.12
RSW-0411	4.16	3.96	2.76	3.13	3.51
Cycocel	2.93	3.50	2.76	3.86	3.27
Time means(NS)	3.36	3.60	3.04	3.54	3.39

Note: See notes in Table 3.2.

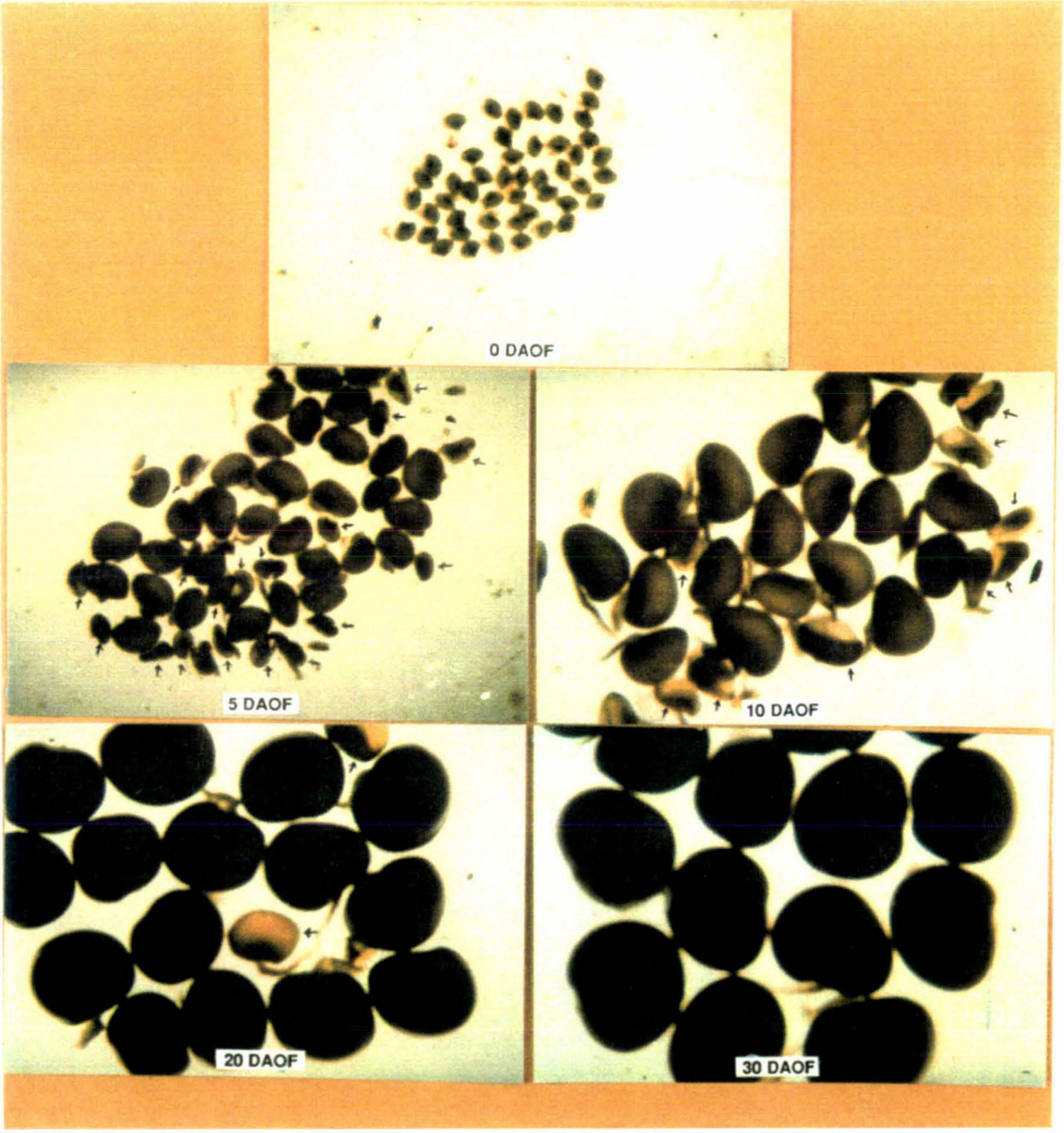

Plate 3.5. Seed development of *Lotus corniculatus*, showing some shrivelled seeds (arrows) at any developmental stage. The seeds were photographed with 20x magification from seed samples of early flowers (31 December 1989).

Table 3.4. Effect of plant growth regulators applied in October on number of ovules per ovary or seeds per pod measured at 0, 5, 10, 20, and 30 DAOF in inflorescences produced at four different times. (1989-1990). (All Treatment X Time : NS)

Treatment	Time of flower production				Treatment
	16 Dec.	31 Dec.	15 Jan.	30 Jan.	Means
A. 0 DAOF.					
Control	49.70	46.00	46.16	48.86	47.68*c* *
Cultar	50.13	48.76	47.46	48.20	48.64*bc*
RSW-0411	51.73	49.60	47.46	48.60	49.35*b*
Cycocel	53.43	52.00	46.90	50.46	50.70*a*
Time means* **	51.25*a*	49.09*b*	47.00*c*	49.03*b*	49.09
B. 5 DAOF.					
Control	34.33	33.76	26.96	34.73	32.45NS
Cultar	33.36	31.60	28.20	33.46	31.66
RSW-0411	40.13	29.70	30.00	31.50	32.83
Cycocel	41.20	35.60	28.83	36.70	35.58
Time means* **	37.26*a*	32.67*b*	28.50*c*	34.10	33.13
C. 10 DAOF.					
Control	29.16	27.80	18.86	20.73	24.14*a**
Cultar	28.56	24.46	17.76	18.76	22.39*ab*
RSW-0411	29.36	17.96	16.90	22.23	21.62*ab*
Cycocel	27.60	18.73	16.03	18.30	20.17*b*
Time means* **	28.68*a*	22.24*b*	17.39*c*	20.01*b*	22.08
D. 20 DAOF.					
Control	17.93	15.80	17.26	17.60	17.15NS
Cultar	17.73	16.86	12.53	15.60	15.68
RSW-0411	16.00	14.60	17.26	15.50	15.84
Cycocel	17.13	16.33	15.50	15.66	16.15
Time means(NS)	17.20	15.90	15.64	16.09	16.21
E. 30 DAOF.					
Control	16.06	13.66	15.53	14.76	15.01NS
Cultar	14.86	15.16	12.36	14.63	14.26
RSW-0411	15.90	13.00	14.53	15.20	14.66
Cycocel	16.46	16.36	14.40	14.63	15.47
Time means(NS)	15.85	14.55	14.21	14.81	14.85

Note: See note in Table 3.2.

however, were small. Seed abortion mainly occurred during the early stages of flower development. At 10 DAOF, an average of about 27 (55%) of ovules had aborted, while twenty days later (30 DAOF) only about 7 (15%) of additional ovules had aborted (Table 3.4). The pattern of seed abortion was not affected by plant growth regulator application, but the the time of flower production clearly modified the pattern. Ovule and seed abortion was greatest in flowers produced at peak flowering (15 January), approximately 63% having been aborted within 10 DAOF. The lowest abortion rate occurred in early flowers, only 44% being aborted in the same period of flower development, although the time means for seeds per pod 30 DAOF did not reflect this trend. On average, 14.9 seeds were set in a pod from 49 ovules per ovary by 30 DAOF. This represents a 30% survival. It is interesting that the rate of pod-length development (Appendix 7.) parallelled the seed abortion rate, pod development being slow in early formed umbels, and faster in late formed umbels.

Mean survival of flowers, florets per flower and ovules per ovary at 30 DAOF are shown in Table 3.5. Umbel survival increased significantly (P 0.001) in late (January) flowers. Early flowers produced in December only retained 39%-52% live umbels, while those produced in late January retained more than 87% live umbels. Since production time did not affect the survival of pods per umbel or seeds per pod, poor seeding potential in early flowers was mainly due to low umbel survival. Plant growth regulators reduced umbel survival (P 0.01) particularly in early flowers. However, no significant effect was found in the survival of total pods per umbel or seeds per pod following the application of plant growth regulators (Table 3.5). The impact of umbel survival according to time of umbel production was directly reflected in total seed survival. Again late flowers supported a greater percentage of seeds 30 DAOF but the reduction in potential yield was high (mean 84%)

Table 3.5. Effect of plant growth regulators applied in October on the survival of umbel, pods per umbel, seeds per pod and total seeds[1] (percent) measured at 0, 5, 10, 20, and 30 DAOF in inflorescences produced at four different times. (1989-1990). (All Treatment X Time : NS)

Treatment	Time of flower production 16 Dec.	31 Dec.	15 Jan.	30 Jan.	Treatment Means
A. Umbel survival.					
Control	60.0	71.6	93.3	95.0	80.0*a*
Cultar	26.6	43.3	75.0	81.6	56.7*b*
RSW-0411	40.0	46.6	78.3	83.3	62.1*b*
Cycocel	30.0	46.6	80.0	90.0	61.7*b*
Time means	39.2*c*	52.1*b*	81.7*a*	87.5*a*	65.1
B. Pods per umbel survival.					
Control	61	60	51	69	60NS
Cultar	42	55	53	49	50
RSW-0411	71	63	45	55	59
Cycocel	50	56	45	67	55
Time meansNS	56	59	49	60	56
C. Seeds per pod survival.					
Control	32	30	34	30	32NS
Cultar	30	31	26	30	29
RSW-0411	31	26	31	31	30
Cycocel	31	32	31	29	31
Time meansNS	31	30	30	30	30
D. Total seeds survival.					
Control	12	13	16	20	15
Cultar	4	7	10	13	9
RSW-0411	8	8	11	14	10
Cycocel	5	8	11	18	11
Time means***	7*c*	9*bc*	12*b*	16*a*	11

Note: [1] Survival was calculated based on the number of umbels, pods per umbel, seeds per pod and total seeds at 30 DAOF compared to their number at 0 DAOF. See also note in Table 3.2.

The effects of plant growth regulators applied in October on thousand seed weight at 30 DAOF by inflorescences formed at different times during December and January are shown in Table 3.6. Although treatments did not affect seed weight, the time of flower formation was important. The relationships previously described (Table 3.5) between time and umbel formation was also reflected in seed weight, early flowers (December) producing lighter seeds 30 DAOF than later formed flowers (January).

Table 3.6. Effect of plant growth growth regulators applied in October on thousand seed weight (gram, 0% SMC) measured at 0, 5, 10, 20, and 30 DAOF in inflorescences produced at four different times. (1989-1990). (Treatment X Time : NS)

Treatment	Time of flower production				Treatment
	16 Dec.	31 Dec.	15 Jan.	30 Jan.	Means
Control	0.3073	0.6706	0.8686	1.1346	0.7543NS
Cultar	0.3826	0.5226	1.0506	1.1286	0.7712
RSW-0411	0.4153	0.5240	0.9466	1.1300	0.7540
Cycocel	0.3300	0.6013	0.8093	1.1293	0.7175
Time means**	0.3588*d*	0.5797*c*	0.9188*b*	1.1307*a*	0.7492

Note: See note in Table 3.2.

3.4. Seed Yields and Seed Yield Components.

Crop conditions at harvest time was illustrated in Plate 3.6. Seed yield results presented in Table 3.7 (Appendices 12,13,14) show that both first and second year seed crops produced high seed yields. In 1989, there was no significant increase in seed yield following the application of plant growth regulators at two level rates and two phenological stages of plant growth, *i.e.* pre flowering bud (October) and the onset of flowering (November). The average yield from the 1989 harvest was 549 kg seed per hectare.

Table 3.7. Effect of plant growth regulators on seed yield[0], harvest recovery and harvest index of *Lotus corniculatus* seed crop grown in two consecutive years (1988-1990).

TREATMENT			1989 HARVEST		1990 HARVEST						
Chemical	Time	Rate	2 February (36DAPF) Seed Yield		21 February(41DAPF) Seed Yield (A)		Recovery[1]	Harvest Index[2]	28 February (47DAPF) Seed Yield		Increase from (A)
		kg a.i./ha	kg/ha	%Ctrl	kg/ha	%Ctrl	%	%	kg/ha	%Ctrl	%
Control			460	100	759*bc*	100	63	6.00*c*	982	100	31
Cultar	October	0.5	558	121	836*bc*	110	66	7.51*ab*	1086	111	30
		1.0	790	172	750*bc*	99	58	6.90*c*	953	97	27
	November	0.5	503	109	926*ab*	122	68	9.08*ab*	1168	119	26
		1.0	604	131	1030*a*	136	63	9.75*a*	1214	124	18
Cycocel	October	1.5	563	122	674*c*	89	73	5.72*c*	909	93	36
		3.0	605	132	696*c*	92	75	5.84*c*	975	99	40
	November	1.5	485	105	844*abc*	111	73	7.01*bc*	902	92	8
		3.0	394	86	724*c*	95	55	6.72*c*	1082	110	50
RSW-0411	October	0.5	--	--	772*bc*	110	75	6.59*bc*	876	89	13
		1.0	--	--	788*bc*	104	70	6.37*c*	870	89	9
	November	0.5	--	--	724*c*	95	67	5.66*c*	1028	105	42
		1.0	--	--	757*bc*	100	74	6.04*c*	925	94	24
Alar	October	2.0	629	136	--	--	--	--	--	--	--
		4.0	546	119	--	--	--	--	--	--	--
	November	2.0	526	114	--	--	--	--	--	--	--
		4.0	471	102	--	--	--	--	--	--	--
			NS[3]		*[3]		NS	**	NS		NS
Mean			549		796		68	6.86	998		27
Cv			28		14		19	18	17		61

Note: [0] Seed yields were expressed at 8% seed moisture content.
[1] Harvest recovery were calculated from seed yield potential based on the number of umbels at the first harvest (21 February 1990).
[2] Harvest index equals to seed yield at 0% moisture content divided by total dry matter harvested.
[3] *,**,*** Values in the same column followed by the same letter are not significantly different at probability level of 0.05, 0.01 and 0.00 respectively, based on LSD(0.05). NS means not significantly different. This note also applies to tables thereafter.

Plate 3.6 Crop condition at 15 February 1990 (six days before harvest) showing crop greenness and variation within a plot. At harvest the crop looks greener.

In 1990, however considerable differences in seed yield occurred between harvests on 22 February (41 DAPF) and on 27 February (47 DAPF). When the crop was harvested 41 DAPF, a beneficial effect of Cultar application at the onset of flowering (November, 1.0kg a.i./ha) was recorded, but other plant growth regulators and earlier application time of Cultar did not show promise. A significant increase of 36% in yield was obtained with 1.0 kg a.i.Cultar per hectare when applied in November. Cultar was also the only tested plant growth regulator which ameliorated harvest index. However, none of the tested plant growth regulators affected seed recovery percentage at harvest (hand harvesting). A mean of 68% of seeds were recovered at the first harvest time in 1990. In the second harvest (47 DAPF), no significant increase in yield was found in any treated plants, despite the fact that an overall average increase of 202 kg seed per ha, occurred following six days postponement of harvest, and resulted in a 27% increase in seed yield. Naturally, this was 'disappointing'!

Observations on seed yield components and seed yield potential per unit area are presented in Tables 3.8. and 3.9. Plant growth regulator effects on the number of pods per umbel were inconsistent (Table 3.8.). In the first year no effect on this parameter was found following the application of plant growth regulators. However, in the second year, all three chemicals reduced pod number per umbel by as much as 10% ($P_{0.05}$), in cases where the chemical was applied in October (Cycocel), in November at low rates (Cultar) or higher rates (RSW-0411). The mean number of pods per umbel was 4.6 in 1989 and 3.6 in 1990. Number of seeds per pod and seed weight were not affected by any treatment in either year. The average number of seeds per pod was 19.5 in 1989 and 15.6 in 1990. Thousand seed weights were 1.1049 g in 1989 and 1.222 g in 1990. However, considerable variation was observed among plots in the same treatment (Plate 3.7.). Total umbels per meter square was modified by plant growth regulators (Table 3.9.). Only Cultar applications increased total number of flowers produced during the flowering season or total umbels retained at 19 January (8 DAPF). At harvest, however, these initially beneficial effects were not sustained. The calculations of seed yield potential presented in Table 3.9. indicate that when seed yield potential was calculated based on total flowers produced during the flowering season, Cycocel applied at 1.5 l a.i./ha showed some yield suppression. However, none of the other treatments showed a potential yield enhancement compared to the untreated control. Seed yield potential means were 2139 kg, 1503 kg and 1199 kg/ha when calculated from the whole season flower production, and from total umbels at 8 DAPF (19 January) and at 41 DAPF (21 February), respectively.

Plate 3.7 An example of an X-ray photograph of harvested pods used to determine the number of seeds per pod, showing the variation which occurs between two replicates of pods from the same treatment (Cultar 1.0 kg a.i./ha plants treated in November). (A) A greater portion of seeds had matured, (B) a greater portion of seeds are shrivelled or are not yet mature.

Table 3.8. Effect of plant growth regulators on seed yield components at 2 February 1989 and 21 February 1990 harvests.

Treatment			Pods per umbel		Seeds per pod		1000 seed weight	
Chemical	Time	Rate	1989	1990	1989	1990	1989	1990
		kg a.i./ha					gram	
Control			4.73	3.81*abc*	17.9	16.5	1.056	1.202
Cultar	October	0.5	5.03	3.88*a*	20.7	14.7	1.113	1.288
		1.0	4.70	3.84*ab*	17.6	15.2	1.147	1.159
	November	0.5	4.77	3.43*e*	19.3	15.1	1.171	1.263
		1.0	4.83	3.76*abcd*	18.8	15.5	1.136	1.181
Cycocel	October	1.5	4.63	3.41*e*	19.4	14.9	1.123	1.181
		3.0	4.77	3.47*de*	20.5	15.5	1.105	1.233
	November	1.5	4.43	3.51*cde*	19.8	15.2	1.087	1.237
		3.0	4.53	3.66*abcde*	20.2	16.7	1.090	1.228
RSW-0411	October	0.5	--	3.41*e*	--	14.8	--	1.291
		1.0	--	3.81*abc*	--	15.8	--	1.217
	November	0.5	--	3.54*bcde*	--	16.1	--	1.213
		1.0	--	3.45*de*	--	16.1	--	1.191
Alar	October	2.0	4.40	--	20.1	--	1.092	--
		4.0	4.33	--	21.0	--	1.061	--
	November	2.0	4.50	--	18.7	--	1.108	--
		4.0	4.67	--	19.3	--	1.055	--
			NS	0.05	NS	NS	NS	NS
Mean			4.64	3.61	19.5	15.6	1.104	1.222
Cv			10	5	10	6	7	7

Note: See note 3) in table 3.7.

Calculated dry matter partitioning of the first harvest material is shown in Table 3.10. Total dry weight of plants in the untreated crop was 1177 g/m^2. Cultar application at the higher rate in October and at both 0.5 and 1.0 kg a.i./ha in November significantly reduced total dry weight at harvest (by 14% to 19%). A similar effect occurred with Cycocel application at 3.0 l a.i./ha in November. No significant effects of plant growth regulators were found on seed weight, reproductive weight, or on their contribution to total dry matter. On average, about 30% of total dry weight at harvest was allocated to the reproductive structures, with approximately one third of this amount contributed by the seeds. Seeds contributed an average of only 10% of total dry weight.

Table 3.9. Total reproductive structures, measured from the whole season (WS), at 19 January 1990 and at harvest (21 February 1990), and seed yield potential (1989-1990).

Treatment			**Reproductive Structures**			**Seed Yield Potential[1)]**		
Chemical	**Time**	**Rate**	**WS**	**19 Jan.**	**21 Feb.**	**WS**	**19 Jan.**	**21Jan.**
		kg a.i./ha	umbels/m^2			kg/ha		
Control			3013*cdefg*	2088*cd*	1650	2274*abc*	1661*abcde*	1267
Cultar	October	0.5	3101*cde*	2263*bcd*	1727	2283*abc*	1681*abcd*	1383
		1.0	3844*a*	2518*abc*	1918	2592*a*	1698*abc*	1291
	November	0.5	3396*abc*	2642*ab*	2151	2223*abc*	1729*ab*	1383
		1.0	3659*ab*	3027*a*	2417	2512*ab*	2096*a*	1672
Cycocel	October	1.5	2802*efg*	1968*d*	1576	1680*d*	1177*e*	931
		3.0	3026*cdef*	1840*d*	1534	2041*bcd*	1225*cde*	1039
	November	1.5	3009*cdefg*	1957*d*	1778	1964*cd*	1271*bcde*	1181
		3.0	3332*bcd*	2085*cd*	1751	2508*ab*	1565*bcde*	1310
RSW-0411	October	0.5	2857*defg*	1820*d*	1594	1866*cd*	1191*de*	989
		1.0	2537*f*	2093*cd*	1544	1846*cd*	1529*bcde*	1120
	November	0.5	2588*fg*	2028*cd*	1592	1799*cd*	1414*bcde*	982
		1.0	3364*abc*	2045*cd*	1577	2218*abc*	1359*bcde*	1041
			***	**	NS	*	*	NS
Mean			3118	2183	1755	2139	1503	1199
Cv			9	14	18	14	20	29

Note: 1) Seed yield potentials were calculated based on total reproductive structures on this table and the others seed yield components from the harvest in 1990. See also note 3) in table 3.7.

Table 3.10. Effect of plant growth regulators on dry matter distribution of *Lotus corniculatus* at harvest 21 February 1990

Treatment Chemical	Time	Rate	Seed (S)	Reproductive Structure(R)	Total Dry Matter(T)	S/R[1]	S/T	R/T
		kg a.i./ha		g/m^2				
Control			98.61	294.15	1177*ab*	33	8.5	25
Cultar	October	0.5	124.04	348.17	1035*bcde*	35	11.8	33
		1.0	115.18	342.85	1012*cde*	34	11.4	34
	November	0.5	131.36	378.39	953*e*	35	13.9	40
		1.0	153.06	446.68	984*de*	34	15.8	46
Cycocel	October	1.5	88.76	270.82	1090*abcde*	33	8.1	25
		3.0	101.94	287.34	1107*abcd*	35	9.2	26
	November	1.5	106.71	307.29	1110*abcd*	35	9.6	27
		3.0	103.33	307.35	1021*cde*	34	10.1	31
RSW-0411	October	0.5	89.13	276.21	1091*abcde*	32	8.3	26
		1.0	103.49	302.22	1143*abc*	34	9.1	26
	November	0.5	102.36	302.21	1192*a*	34	8.7	26
		1.0	96.83	290.40	1173*ab*	33	8.4	25
			NS	NS	*	NS	NS	NS
Mean			108.9	319.5	1083	34	10.2	30
Cv			28	24	8	6	30	27

Note: [1] S/R=seed weight per reproductive weight, S/T=seed weight per total dry matter weight, R/T=reproductive weight per total dry weight. * values followed by the same letter are not significant at P level of 0.05. See also note 3) in table 3.7.

3.5. Residual Effect of Cultar.

Measurements were made to determine whether Cultar application to a first year crop of *Lotus corniculatus* could be carried over as a residual effect to influence seeding in the following year. This was thought necessary to more critically examine the suggestion that Cultar in one year can influence seed yield in the following year. The results (Appendixes 8, 9 and 10) show that this is not the case. Cultar treatment in the first year had no significant effect on second year seed crops in terms of seed yield, yield components (umbel number, pods per umbel, seeds per pod or seed weight), total dry matter production or dry matter partitioning, despite the observation that the onset of early growth in the second year appeared to be delayed by 2-3 weeks in terms of visual total ground cover.

3.6. Seed Quality.

Seed quality from the first harvest of *Lotus corniculatus* seeds in 1990 is presented in Table 3.11. Application of plant growth regulators had no effect on the percentage of normal germination, abnormal seedlings, hardseededness, or dead seed. More than 90% of the seeds were viable, about 75% being capable of germination when seeds were tested. The level of hard seed was low (being approximately 20%). Presumably this reflects the scarifying effects of mechanical threshing of pods after harvest in reducing hard seed levels.

Table 3.11. Germination test of harvested seed *Lotus corniculatus* in 1990.

Treatment Chemical	Time	Normal Seedling	Abnormal Seedling	hard-seed	Dead Seed	Viable Seed
				percent		
Control		70.3	2.7	25.0	2.0	95.3
Cultar	October	65.7	4.0	27.0	3.3	92.7
	November	81.7	3.7	13.0	1.7	94.7
RSW-0411	October	69.0	3.0	26.7	1.3	95.0
	November	66.3	4.3	27.3	2.0	93.7
Cycocel	October	74.7	4.0	19.3	2.0	94.0
	November	68.0	3.3	27.0	1.7	95.0
CultarR	October	72.3	2.7	21.0	4.0	93.3
	November	74.0	5.0	17.3	3.7	91.3
		NS	NS	NS	NS	NS
Means		71.3	3.7	22.5	2.4	93.9
Cv		15	72	53	79	4

Note: [1)] Cultar, RSW-0411 and CultarR (Cultar Residue) were at application rate of 1.0 kg a.i./ha, while Cycocel at 3.0 kg a.i./ha.

CHAPTER FOUR
DISCUSSION

The application of plant growth regulators including Cultar, RSW-0411 and Cycocel reduced shoot height of birdsfoot trefoil without affecting node numbers, especially following application at the time when the plant was growing vigorously before flowering. Somewhat surprisingly, however, this effect was not carried over to peak flowering time in RSW-0411 or Cycocel-treated plants (Figure 3.1.). This reduction in plant height by the same plant growth regulators has also been reported by many workers, for example Hack and Lembrick (1985), Clifford and Hare (1987), and Ryback and Walczak (1988) in other species, as well as in *Lotus corniculatus* (White *et.al.*, 1987; Li and Hill, 1989b). However, Niemelainen (1987) found in red clover that Cultar, RSW-0411 and Cycocel applied at similar rates to those used in this experiment at the beginning of flowering had no clear effect on shoot height measured at peak flowering time.

Results in the present study show that application of Alar, RSW-0411 and Cycocel have no obvious benefit on birdsfoot trefoil seed production. Seed yield and seed yield components were not affected by Alar application. Although, Alar has been successfully used to increase seed yields in red clover seed crops (Jakesova and Svetlik, 1987, Christie and Choo, 1990), the present findings confirm the negative results in the same species *Lotus corniculatus* reported by White *et.al.* (1987). They found that Alar application under diverse combinations of conditions, including crop age, location, genotypes and cultivar, planting design, application schedule and treatment time, did not change seed yield. RSW-0411 did not improve branching, flower production or seed yield. Similar results have been found in another herbage legume, red clover (Niemelainen, 1987). In the case of Cycocel, the results were similar to RSW-0411 treatments, there being no beneficial effects on branching behaviour, flowering pattern or seed yield. Reports in other herbage legumes (white clover, by Marshall and Hides (1984); red clover by Niemelainen (1987) and Ryback and Walczak (1988)) also indicate that Cycocel application does not show promise for enhancing herbage legume seed production, even though it may benefit wheat and oat production (Kust, 1986). Since Cultar was the only chemical used in this study which showed potential value in birdsfoot trefoil seed production, further discussion will concentrate on the effects of this chemical.

The growth retarding effect of Cultar in this experiment was not associated with an improvement in lateral shoot development. This disagrees with the report by Li and Hill (1989b) who found that Cultar application in November increased the weight of lateral branches in proportion to the weight of main shoots. This conflicting result may be due to a combination of two factors, - the age of the plants and weather conditions during the experiments which were carried out in different years at an adjacent site. Energy reserves accumulated in the roots and the crown during the first year combined with more available sites on the crown for regrowth may have enabled second year plants used in this experiment to produce more main shoots and recover more quickly after grazing than the first year plants used in the experiment by Li and Hill (1989b). It is worth noting that in this experiment, main shoot production apparently occurred mainly during spring and and no new main shoots emerged after the first flowering time (cf. Figure 3.2.). This is a similar situation to that reported by Stephenson (1984), but is not entirely in agreement with results by Li and Hill (1988). In this latter case, although sward plants showing a similar effect to findings in the present study, new main shoot emergence did continue after first flowering in spaced plants. In addition, dry conditions in September followed by high rainfall in the following month (note that temperature was similar throughout the spring, Appendix 1.) might have promoted branching in the crowded main shoots already present rather than promoting the production of new main shoots. These factors, in combination, may have caused birdsfoot trefoil plants to become less responsive to plant growth regulator application.

Shoot development, in terms of shoot numbers monitored in this experiment shows the important role of branching on subsequent reproductive growth in birdsfoot trefoil (Table 3.1.). Lateral shoots together with secondary lateral shoots contributed about 60% of the total reproductive structures produced by the time of peak flowering although the number of reproductive structures on both shoot types was less than those on main shoots. This finding is similar to that reported by Hansen (1953) and Nelson and Smith (1968a,b), although these authors did not report the subsequent reproductive development of the plants. However, it is contradictory to results reported by MacDonald (1946), Li (1989) and Li and Hill (1988, 1989b) who emphasized the importance of shoots emerging from the crown (main shoots) whether the plants were grown in spaced or in row conditions. Since the plants used in this experiment and in Nelson and Smith's (1968a,b) experiments were similar in age and were grown under similar rainfall patterns, the two factors discussed in the above paragraph may presumably also be implicated as reasons for this difference. However, since in the present experiment variation was high, there is still a need for further experiments with more replication and larger sample size to clarify if these factors are truly important.

Results in this study show that none of the plant growth regulator treatments affected flowering period of birdsfoot trefoil, which extended over three months. In the case of Cultar, the results disagree with those found in recent trials by Hampton *et.al.* (1989) who suggested that Cultar reduced the flowering duration of *L. corniculatus* and *L. uliginosus* crops. This variation is possibly due to differences in the last grazing time (August in this study, but September in Hampton's study) which might have been reflected differences in plant growth conditions after the chemical was applied. Observation in a birdsfoot trefoil crop in plots adjacent to this experiment cut in early October suggested that the start of flowering was delayed by about 2-3 weeks.

Protracted flowering duration, however, is considered to be less important for seed production in this crop compared to increased flowering intensity. In this study, only Cultar had the potential to increase flowering pattern. Cultar application during the vegetative stage promoted early flowering, while application at the start of flowering resulted in a sudden increase in inflorescence numbers at peak flowering. This result conforms well with those reported by Li (1989).

Surprisingly, seed yields (second harvestin 1990) were not improved despite the increased flowering pattern. This is contrary to findings in Li's experiment (1989), where seed yields were increased by Cultar application. There are three possible explanations which may have been important in this lack of improvement in seed yields in the present study. Firstly, although high Cultar application in October increased early flowering, inflorescence abortion in early flowers was almost double that in untreated control plants (Table 3.5). Secondly, although peak flowering was increased in November Cultar treated plants, attack by caterpillars which may have affected flowers at that time was observed more severely in plants in this treatment than in other plants. Insect control with Maverick on January 3 was apparently ineffective due to subsequent high rainfall, and the next spray not applied until

January 16 when flowering of Cultar treated plants was similar to that of the untreated control. Fungal attack of pods by *Botrytis cinerea* was visually similar between plants October treated with plant growth regulators and the untreated control (Appendix 11.), but this effect may have been greater in November Cultar treated plants due to insect infestation. In addition, most umbels in November Cultar treated plants were on the top of the canopy which might have received more direct sunlight and shattered more readily. Finally, seed yields in the second year were high, ranging from 870 to 1214 kg/ha, (cf. only 457-637 kg/ha in Li's (1989) experiments). That no significant improvement in seed yields was achieved suggests that lack of assimilate supply due to reduced vegetative growth without enhancement of photosynthesis activity might have limited reproductive growth which lead to high flower or pod abortion.

The effects of plant growth regulators on seed yield in the second year in this study are conflicting for harvests taken at 41 days after peak flowering (DAPF) and 47 DAPF. Significant improvements were achieved with Cultar application in November at the first harvest, but this advantage failed to reach significant levels at the second harvest. These conflicting results may be explainable by the following reasons. Firstly, variation in the stage of seed development within or between plots was high as illustrated in Plate 3.7. Moreover, at the second harvest, considerably smaller sample areas were available for harvesting due to previous vegetative harvests in plots used for monthly growth analysis and first harvest seed samplings. Also, some physical destruction to subsequent harvest areas of the plots which occurred during the first harvest operation, may have reduced 'available' yield of seed at the second harvest. Finally, there might also have been variation in *Botrytis* infestation, pod shattering and moisture stress between plots. All these factors confounded differences in seed yield between harvests. Despite this difficulties, it is interesting to note that seed yield in the second harvest is about 27% higher (P 0.01) than in the first harvest. In fact, the first harvest timing (41 DAPF) had been delayed six days from the 35 DAPF suggested by Li (1989) due to cool wet weather and the fact that negligible pod shattering was observed. Increased seed yield at the second harvest (47 DAPF) was possibly because even a 6-day delay advantaged seed development and maturity in the late flowers, which exhibited low inflorescence abortion. Moreover, weather conditions of high relative humidity (minimum RH was 65% only on one day) and cool temperatures between the two harvest times (Appendix 2) were not conducive to pod shattering of mature pods. Studying pod shattering in a bulk sample of mature pods (light brown in color) exposed to RH 49% or above at 32°C for 6 days, Metcalfe *et.al.* (1957) found that pod shattering was low (4.7% of dehisced pods), but almost 90 % of the pods had shattered under RH 40%. This emphasizes the apparent advantage of delaying harvest of *Lotus corniculatus* seed crops, providing weather conditions are not favourable for pod shattering. Another interesting aspect of this study was the greater seed yield obtained in the second year than in the first year. This result is supported by other reports (McGraw and Beuselink, 1983; White *et.al.*, 1987; Gataric and Alibegovic-grbic, 1989), suggesting that more precise controlled management of second year crops may be more important than first year seed crop management.

A major problem in seed production of birdsfoot trefoil is the difficulty in recovering a large portion of seed from a massive bulk of vegetative material at harvest. The use of desiccants to alleviate this problem has been suggested by Wiggans *et.al.* (1956). Li (1989) has discussed the advantage of using the plant growth regulator Cultar to reduce excessive vegetative growth and thereby improve harvest index. Results in this trial agree with Li's results in that reduction of vegetative growth and increases in harvest index occurred as a result of Cultar application, despite the fact that no improvement in seed yield recovery was obtained. This was because the crop was harvested by hand. Investigations on the use of machine harvester efficiency appear necessary to examine whether Cultar can ameliorate seed yield recovery. Application of other plant growth regulators, including RSW-0411 and Cycocel used in this study, and Alar and mepiquat chloride used in work by White *et.al.* (1987), have shown no obvious advantage in increasing either harvest index or seed yield.

Reports on the effects of plant growth regulators on seed yield components in herbage plants are conflicting. Hampton and Hebblethwaite (1985a, b) found that Cultar increased the number of seeds per spikelet in perennial ryegrass. Such results, however, are contrary to findings with birdsfoot trefoil reported by Li and Hill (1989b) who found that Cultar treatment reduced the number of pods per umbel from an equivalent number of florets per inflorescence. Li and Hill considered that Cultar apparently retards plant growth without improving the plant's photosynthetic efficiency or modifiying assimilate partitioning in favour of reproductive growth, and suggested that the failure of more florets to develop into mature pods was as a result of lack of assimilate supply to reproductive growth. This is likely to be important since considerably more inflorescences were produced in treated plants than in the untreated control. Results in the present study, however, show inconsistent effects of Cultar. There were no effects of Cultar in the first year trial. Despite the fact that there was a reduction in the number of pods per umbel in the low rate-October treatment with Cultar in the second year, plant growth was not reduced in this treatment. Reductions in the number of pods per umbel were also found in some Cycocel and RSW-0411 treatments. These occurred without visual retardation of plant growth. This phenomenon suggests that the absence of growth simulation of competitor vegetative organs seems likely to be important for the success of floret development. However, if Li and Hill's (1989a) suggestion is correct the apparent low pods per umbel in the second year crop (3.6) compared with 4.6 in the first year should, as a result of higher number of inflorescences produced in the second year, have been reflected in higher seed yield.

Seed yield component values in *Lotus corniculatus* in the present study were similar to those in other reports (MacDonald, 1946; Seaney and Henson, 1970; Li, 1989), with the exception of flower numbers in the second year which were apparently high. With this exception, seed yield potential at harvest (Table 3.9) based on the values obtained in this study (1200 kg/ha) is high, but is comparable with Seaney and Henson's report (1970), especially when plants are grown under environmental conditions favourable for seed production. Seed yield potential at harvest time is obviously a more realistic method of assessing actual seed yield (R^2 = 0.74) compared with calculations based on total numbers of reproductive structures one week after peak flowering (1500kg/ha, R^2 = 0.54) or on total inflorescence numbers produced during the whole season (2140 kg/ha, R^2 = 0.24). Poor correlation coefficients in these last two calculations compared with actual seed yields strongly implicate the variable levels of reproductive abortion found in this study. However, actual seed yield is still 20-30% less than seed yield calculated at harvest time, only about 800-1000 kg seed/ha being recovered during hand harvesting. This yield is still considerably better than commercial machine harvested seed yields in New Zealand (200-350 kg/ha) as reported by Li (1989).

Reproductive abortion is apparently a common feature in birdsfoot trefoil which occurs throughout reproductive development, but particularly during the very early stages of inflorescence development before flower blooming. This has also been reported by other workers (Giles, 1949; Bubar, 1958; Joffe, 1958; Seaney and Henson, 1970). Flower bud abortion was not monitored in the present study although it was observed to occur. However, results throughout the flowering season suggest that flower buds consistently abort 'one' floret bud before the flower opens, a situation which was apparently unaffected by plant growth regulators. Soon after flower opening, pollination failure may also become a major cause of potential seed loss in this species since it is an incompletely self-incompatible and self-sterile plant (Silow, 1931; Seaney, 1964; Dobrowski and Grant, 1980a, b). However, even when each flower is outcrossed, a proportion of florets still fail to develop pods (Stephenson, 1984). At other stages of floret development, the present study reveals that throughout the flowering season only about 30% of the 29-72 ovules in an ovary develop into seeds, and only about half of the florets in an open flower develop into live pods at 30 DAOF. In this regard there were no apparent effects from plant growth regulator application. Similar results have been reported by Hansen (1953) and Seaney and Henson (1970) in the case of seed abortion, and Stephenson and co-workers (1986, 1988) in the case of floret abortion.

Although flower abortion is originally explainable by Darwin's theory of evolution (Stephenson and Winsor, 1985), the precise mechanisms of flower bud abortion is still unclear. Several factors have been considered to be responsible for reproductive abortion in a wide range of plant species. These include photosynthetic activity (Schou *et.al.* 1978; Greene, 1989), assimilate competition between vegetative and reproductive growth and among reproductive structures (Kollman *et.al.* 1974; Chanprasert *et.al.*, 1988; Ho, 1988), and nutrient deficiency, particularly boron (Dugger, 1983). However, Joffe (1958) and Russelle and McGraw (1988) have suggested that nutrient deficiency is apparently not the cause of abortion in *Lotus corniculatus*. In a study on the effects of plant growth regulators on seed production in birdsfoot trefoil, Li and Hill (1989b) suggested that lack of assimilate supply may reduce the number of pods per umbel from equal numbers of florets per inflorescence. In the present study, although the results suggest that lack of assimilate supply is the apparent cause of reproductive abortion, the mechanism involved might be considered to be different. It is possible that the apparent lack of assimilate supply to the flowers as a result of vegetative growth competition has a major effect on abortion levels in early flowers.

Th present study show that plants tend to abort developing flowers or umbels rather than to reduce the proportion of florets per inflorescence which develop into mature pods in live umbels or to reduce the proportion of ovules in an ovary which develop into seeds in live pods. Flower abortion occurred as early as 10 DAOF and continued until 30 DAOF, although the greatest abortion occurred between 10 to 20 DAOF. That the highest rate of seed abortion occurred in the early period of ovule development suggests that lack of pollination, fertilization failure or intense competition among the fertilized ovules may occur. However, pod abortion occurred consistently throughout flower development, and approximately 2-3 pods were aborted from about 6 florets during the 30-day period from open flower. Considering that no live pods were found without any seed inside, this suggests that seed abortion may be the main cause of pod abortion, and apparently pods with fewer seeds tend to abort. This result is supported by Stephenson and co-workers (1986,1988) who found that while natural patterns of pod abortion occur, plants still produce mature pods that contain significantly more seeds compared to the random pattern of pod abortion (conducted by hand thinning florets on some inflorescences prior to any abortion). They further suggest that birdsfoot trefoil selectively aborts those pods with fewest seeds and consequently increases the average quality of its offspring.

Results in this study show that seed weight in early flowers at 30 days after open flower (DAOF) was only about one third of those in late flowers. This variation may be because seeds from early flowers have not completed their development, while seeds from late flowers had apparently reached maturity by that time (as shown by their pod color and X-ray photographs of the pods). In other words, more time may have been needed for seeds from early flowers to attain maturity than seeds from late flowers. This suggestion agrees well with results reported by Anderson (1955), Wiggans *et.al.* (1956), Winch (1958) and Beuselink and McGraw (1988). However, results by Li (1989) and Li and Hill (1989a) have suggested that seeds form flowers originating early in the season develop faster than seeds from flowers originating late in the season. This situation may have been caused by differences in temperature during seed development, as suggested by Long *et.al.* (1989) who found that in warmer temperatures (30/22°C day/night) seed development was faster than under cooler temperature conditions (27/13°C), but maximum seed weight was lower. Since flowers studied in this and other experiments were formed before maximum average temperatures were reached (Appendix 2), early flower seed development may have been slower than seed development in late flowers. The converse situation occured in the study reported by Li (1989)

The results from the present experiments and other studies suggest that the application of Cultar at 1 kg a.i./ha is considered to be an 'acceptable' rate. However simple recommendations of the correct application time are still difficult to make since age of crop and weather conditions during plant growth may modify the potentially beneficial effects of Cultar. In the first year seed crop, Li (1989) found that applications at the vegetative stage up to beginning of flowering all similarly improved seed yields. Care should be taken not to apply Cultar too early in the vegetative stage since it may severely inhibit plant growth. Similarly, application at or after pre peak flowering may be non beneficial or deleterious. Late Cultar applications, even though they may promote small lateral branch formation do not apparently improve seed yield since daylength and temperature in the late season may well be no longer suitable to induce these small branches to become fertile. In the second year seed crop, Cultar application at the beginning of flowering appears to be the best recommendation. Application at the vegetative stage, even though it may increase early flowering, is apparently not beneficial since a large portion of the flowers are likely to abort by harvest time. Again, late application is presumed to have no beneficial effect in improving seed yield, since, as with the first year crop, weather conditions in the late season are a likely constraint. This situation still requires investigation. Moreover, since Cultar application in the previous year is not carried over to second year seed production, chemical reapplication in the second year appears important and warrants further investigation.

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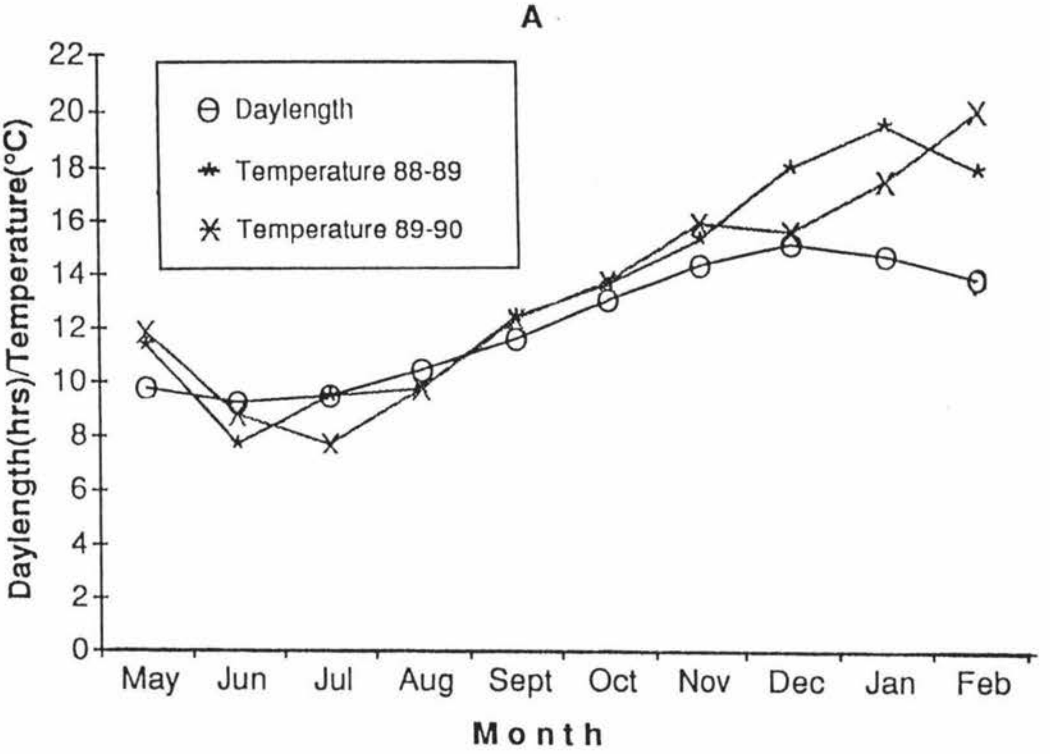
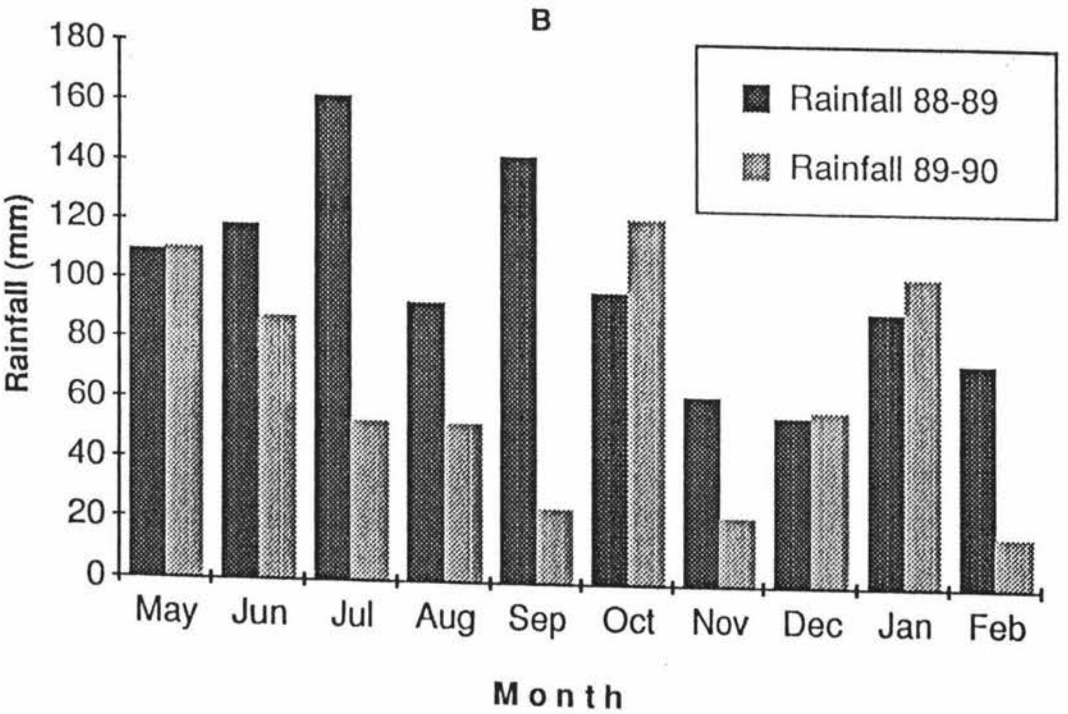
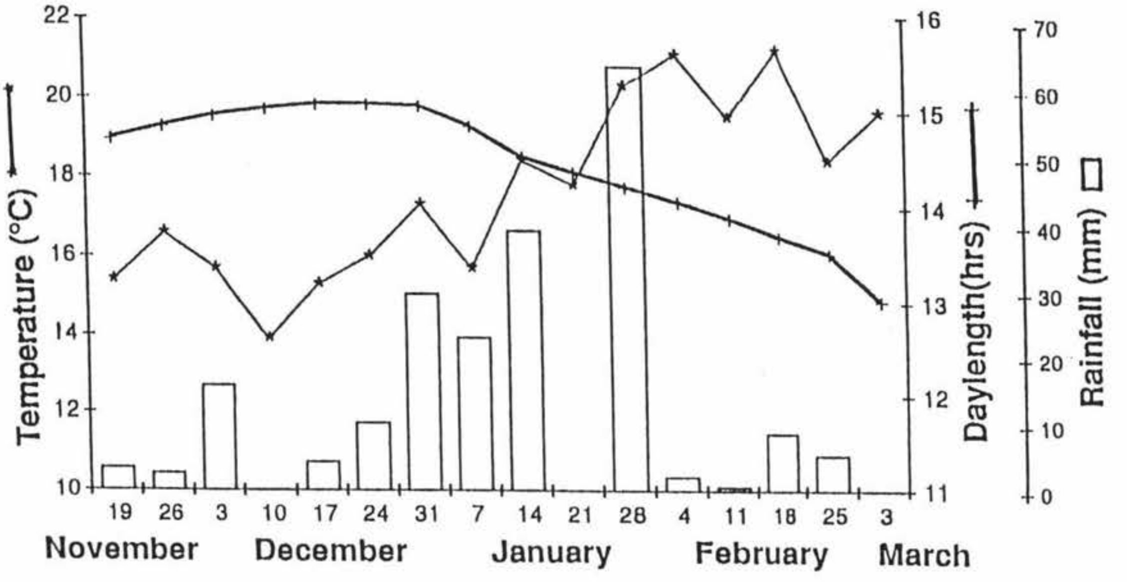




Appendix 1. Monthly daylength and temperature changes (A) and monthly rainfall (B) at Palmerston North, New Zealand, during 1988-1990.



Appendix 2. Detailed weather data at Palmerston North, New Zealand during the flowering period in 1989/199.

Appendix 3. Main shoot length and the number of nodes in the longest main shoot of the second year *Lotus corniculatus* seed crop (1989/1990).

Treatment		Shoot length (cm)					Node number				
Chemical(1)	Time	Sep	Oct	Nov	Dec	Jan	Sep	Oct	Nov	Dec	Jan
Control		5.6	39.2	81.2a	102.0a	111.6a	5.1	9.7	16.3	20.9	21.7
Cultar	October	--2)	--	52.7c	69.0c	80.3c	--	--	14.1	19.4	20.2
	November	--	--	--	82.8bc	89.5bc	--	--	--	19.8	21.7
RSW-0411	October	--	--	63.6bc	86.0b	102.1ab	--	--	15.6	19.7	22.1
	November	--	--	--	91.7ab	100.7ab	--	--	--	22.0	21.8
Cycocel	October	--	--	70.3ab	93.8ab	98.9ab	--	--	15.6	21.3	22.7
	November	--	--	--	94.0ab	104.2ab	--	--	--	20.3	21.0
				**3)	**	*			NS	NS	NS
Mean				63.2	84.4	98.2			15.4	19.5	21.6
Cv				9	11	9			10	11	11

Note: 1) Application rate of Cultar and RSW-0411 were 1.0 kg a.i./ha, Cycocel was 3.0 kg a.i./ha. 2) --, Data had not been measured yet, since the treatment had not been applied. 3) *,** Values in the same column followed by the same letter are not significantly different at F test level of 0.05 and 0.01, respectively, according to LSD(0.05). These notes also apply to appendixes 4, 5 and 6.

Appendix 4. Effect of plant growth regulators applied at higher rates on the monthly development of numbers of plants, main shoots, lateral shoots and secondary lateral shoots.

Treatment		Plants					The number of Main Shoots					Lateral Shoot					SLS(1)
Chemical	Time	Sep	Oct	Nov	Dec	Jan	Sep	Oct	Nov	Dec	Jan	Sep	Oct	Nov	Dec	Jan	Jan
Control		16.3	16.0	16.0	13.7	15.0	197	254	280	173	129	30	153	432	474	335	242*ab*
Cultar	October	--	--	16.0	14.7	11.0	--	--	297	192	136	--	--	452	47	255	189*bc*
	November	--	--	--	14.0	15.0	--	--	--	218	150	--	--	--	586	414	272*a*
RSW-0411	October	--	--	16.8	12.7	11.0	--	--	301	182	121	--	--	426	495	268	164*c*
	November	--	--	--	12.7	13.7	--	--	--	189	113	--	--	--	505	271	178*bc*
Cycocel	October	--	--	13.1	13.0	13.3	--	--	223	183	111	--	--	483	389	290	203*bc*
	November	--	--	--	14.0	12.7	--	--	--	162	162	--	--	--	432	329	157*c*
				NS	NS	NS			NS	NS	NS			NS	NS	NS	*
Mean				15.5	13.6	13.1			275	185	132			448	479	309	201
Cv				31	33	30			28	25	27			17	16	21	19

Note: 1)SLS: the number of secondary lateral shoot. See also notes in Appendix 3.

Appendix 5. Effect of plant growth regulators applied at higher rates on the monthly development of shoots, dead material and total dry weight of *Lotus corniculatus* in 1989/1990.

Treatment		Main Shoots					Total Lateral Shoots					LSW[1]	SLSW
Chemical	Time	Sep	Oct	Nov	Dec	Jan	Sep	Oct	Nov	Dec	Jan	Jan	Jan
Control		2.791	36.79	67.87	69.75	83.15	0.075	3.468	22.37	62.47	90.86	67.12	23.746
Cultar	October	--	--	57.26	44.70	56.25	--	--	27.27	34.27	62.90	45.62	17.283
	November	--	--	--	79.66	69.44	--	--	--	58.51	53.00	45.15	7.850
RSW-0411	October	--	--	63.27	61.94	71.83	--	--	21.28	64.67	47.57	38.65	8.913
	November	--	--	--	76.69	71.92	--	--	--	76.52	65.39	49.63	15.767
Cycocel	October	--	--	54.05	55.07	67.88	--	--	23.97	37.94	77.24	58.17	19.071
	November	--	--	--	63.92	75.37	--	--	--	48.51	58.57	47.04	11.533
				NS	NS	NS			NS	NS	NS	NS	NS
Mean				60.61	64.53	70.83			23.73	54.70	65.08	50.20	14.88
Cv				24	22	18			22	30	28	26	45

Note: 1) LSW=lateral shoot weight, SLSW=secondary lateral shoot weight. See also notes in Appendix 3.

Appendix 5. continued.

Treatment		Dead Material		Total Dry weight				
Chemical	**Time**	**Dec**	**Jan**	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**
Control		13.35	23.72	2.866	40.26	90.24	145.6	197.7
Cultar	October	6.77	21.59	--	--	81.10	85.8	140.7
	November	10.13	17.25	--	--	--	148.3	139.7
RSW-0411	October	12.02	14.64	--	--	84.54	138.6	134.0
	November	10.57	19.36	--	--	--	163.8	156.7
Cycocel	October	12.31	24.07	--	--	78.02	105.3	169.2
	November	13.90	20.09	--	--	--	126.3	154.0
		NS	NS			NS	NS	NS
Mean		11.29	20.10			83.50	130.5	156.0
Cv		31	21			17	22	16

Appendix 6. Effect of plant growth regulators applied at higher rates on the monthly development of the proportion of lateral shoot to main shoots and secondary lateral shoots to lateral shoots of *Lotus corniculatus* in 1989/1990. (in percentage).

Treatment		Lateral Shoot per Main Shoot										SLS per LS1)	
Chemical	Time	Number					Weight					Number	Weight
		Sep	Oct	Nov	Dec	Jan	Sep	Oct	Nov	Dec	Jan	Jan	Jan
Control		15	60	160	277	279	2.7	9.4	33	91	102	73	38
Cultar	October	--	--	153	258	193	--	--	49	76	111	76	50
	November	--	--	--	281	279	--	--	--	74	82	66	30
RSW-0411	October	--	--	145	269	224	--	--	35	101	68	62	50
	November	--	--	--	289	246	--	--	--	101	85	67	53
Cycocel	October	--	--	221	215	266	--	--	46	73	109	72	44
	November	--	--	--	267	209	--	--	--	79	77	48	40
				NS	NS	NS			NS	NS	NS	NS	NS
Mean				170	265	224			41	85	90	66	44
Covariance				19	21	31			35	23	33	19	30

Note : 1) SLS per LS means secondary lateral shoot per lateral shoot. See also notes in Appendix 3.

Appendix 7. Effect of plant growth regulators applied on October in pod length development measured at 0, 5, 10, 20 and 30 DAOF in inflorescences produced at four different times. (1989/1990).(All Treatment X Time : NS)

Treatment 1)	Time of flower production 16 Dec.	31 Dec.	15 Jan.	30 Jan.	Treatment Means
A. 0 DAOF.					
Control	6.60	6.56	6.50	6.40	6.52NS
Cultar	6.73	6.63	6.43	6.13	6.48
RSW-0411	6.66	6.73	6.50	6.16	6.52
Cycocel	6.80	6.73	6.56	6.00	6.53
Time means*3)**	6.70*a*	6.67*ab*	6.50*b*	6.18*c*	<u>6.51</u> 2)
B. 5 DAOF.					
Control	10.20	13.03	11.50	13.73	12.12NS
Cultar	9.13	11.23	10.26	12.20	10.71
RSW-0411	10.50	11.76	10.66	13.30	11.56
Cycocel	9.26	11.96	10.80	12.90	11.23
Time means***	9.78*b*	12.00*a*	10.81*b*	13.03*a*	<u>11.40</u>
C. 10 DAOF.					
Control	16.30	18.13	20.36	21.10	18.98NS
Cultar	16.03	17.26	18.43	20.60	18.08
RSW-0411	17.20	16.83	19.90	22.26	19.05
Cycocel	16.66	19.83	19.03	21.76	19.33
Time means***	16.55*c*	18.02*bc*	19.43*b*	21.43*a*	<u>18.86</u>
D. 20 DAOF.					
Control	23.76	24.56	26.06	24.66	24.77NS
Cultar	20.86	23.36	22.90	22.56	22.43
RSW-0411	25.20	25.13	25.20	22.26	24.45
Cycocel	25.43	26.33	24.76	21.66	24.55
Time means*	23.82*ab*	24.85*a*	24.73*a*	22.79*b*	<u>24.05</u>
E. 30 DAOF.					
Control	22.83	24.66	25.96	25.03	24.63NS
Cultar	23.26	25.83	24.73	22.83	24.17
RSW-0411	23.63	23.40	24.50	22.20	23.43
Cycocel	22.80	25.06	22.96	23.46	23.58
Time means*	23.13*b*	24.74*a*	24.54*a*	23.38*b*	<u>23.95</u>

Appendix 8. Residual effect of Cultar application in the 1988/1989 crop on seed yield and harvest index of the subsequent year *Lotus corniculatus* seed crop (1989/1990)

Application		First Harvest (A)[1]			Second Harvest (B)	
Time	Rate	Seed Yield	Recovery	Harvest Index	Seed Yield	Increase from (A)
	kg a.i./ha	kg/ha	%	%	kg/ha	%
Control		758.9	62.7	6.0	981.9	30.6
October	0.5	716.6	71.6	6.0	969.5	42.1
	1.0	751.2	77.3	5.1	1066.3	43.5
November	0.5	796.4	65.8	6.9	1035.4	30.3
	1.0	721.7	76.2	5.2	975.0	35.1
		NS[2)]	NS	NS	NS	NS
Means		748.9	70.7	6.0	1005.0	36.3
Cv		19	18	21	18	22

Note: [1)] A: First harvest, conducted at 21 February 1990 (41 DAPF)· B: Second harvest, conducted at 27 February 1990 (47 DAPF). [2)] Values in the same column are not significantly different at probability level of 95%.

Appendix 9. Residual effect of Cultar application in the 1988/1989 crop on seed yield components and seed yield potential of the subsequent year *Lotus corniculatus* seed crop (1989/1990).

Treatment		Reprod. Structures per m^2 (1)		Pods/	Seeds/	1000 Seed	Seed Yield Potentials	
Time	Rate	(A)(2)	(B)	Umbel	Pod	Weight	(A)	(B)
	kg a.i./ha					gram	kg/ha	
Control		3013	1650	3.81	16.50	1.2027	2275	1267
October	0.5	2915	1441	3.82	14.70	1.2517	2079	1034
	1.0	2632	1601	3.57	15.43	1.1183	1612	1003
November	0.5	2500	1527	3.76	16.27	1.3157	2005	1220
	1.0	2911	1450	3.53	15.77	1.2037	2017	993
		NS	NS	NS	NS	NS	NS	NS
Means		2794	1534	3.70	15.75	1.2184	1998	1175
Cv		14	23	7	13	7	27	34

Note: (1) Reproductive structures per m^2 include flower buds, open flowers and umbels. (2) (A) Total flower production during the whole season, (B) total reproductive structures at tha first harvest 21 February 1990. NS means values in the same column are not significantly different at probability level of 95%.

Appendix 10. Residual effect of Cultar application in the 1988/1989 crop on dry matter distribution of the subsequent year *Lotus corniculatus* seed crop (1989/1990)

Application		Seed (S)	Reproductive Structure (R)	Total Dry Matter (T)	S/R	S/T	R/T
		kg/ha			percent		
Control		986	2942	1177	33.1	8.53	25.4
October	0.5	1017	2949	1105	34.0	9.10	26.7
	1.0	1053	2944	1184	35.1	8.69	24.4
November	0.5	1129	3173	1100	35.8	10.61	29.9
	1.0	1002	2786	1119	35.7	8.90	24.8
		NS	NS	NS	NS	NS	NS
Means		1037	2959	1137	34.7	9.17	26.23
Covariance		35	30	9	7	33	29

Note: 1) (A) First harvest, conducted at 21 February 1990 (41 DAPF), (B) Second harvest, conducted at 27 February 1990 (47 DAPF). 2) Values in the same column are not significantly different at probability level of 95%.

Appendix 11. Raw data of *Botrytis cinerea* investation (percent) on 30 DAOF pods from flowers produced during peak flowering time 1989/1990

Treatment 1)	Block I	II	III	Means
Control	28	25	24	26
Cultar	22	23	39	28
RSW-0411	29	52	25	36
Cycocel	55	43	19	39

Note: 1) Cultar and RSW-0411 were applied at 1.0 kg a.i./ha, while Cycocel at 3.0 kg a.i./ha.

Appendix 12. Raw data of harvested seed yield in 1989

TREATMENT			BLOCK			
Chemical	Time	Rate	I	II	III	AVERAGE
		kg a.i./ha		kg/ha		
Control			430.9	273.8	676.1	460.1
Cultar	October	0.5	556.3	468.8	648.4	557.8
		1.0	694.9	703.6	972.9	790.4
	November	0.5	572.2	517.8	419.7	503.2
		1.0	693.4	489.1	629.8	604.1
Alar	October	2.0	552.3	637.0	697.3	628.8
		4.0	579.4	439.6	620.7	546.5
	November	2.0	205.6	746.8	624.5	525.6
		4.0	339.1	635.4	437.6	470.7
Cycocel	October	1.5	509.2	406.6	773.1	562.9
		3.0	837.9	465.7	512.5	605.4
	November	1.5	466.0	315.8	673.8	485.2
		3.0	436.6	432.9	312.5	394.0

Probability F-test = 0.31; Cv = 28%; LSD(0.05) = 256.3 kg/ha

Appendix 13. Raw data of seed yield of the first harvest in 1990

TREATMENT			BLOCK			
Chemical	Time	Rate	I	II	III	AVERAGE
		kg a.i./ha		kg/ha		
Control			817.2	675.5	784.0	758.9
Cultar	October	0.5	928.6	749.2	831.6	836.5
		1.0	747.2	693.0	810.7	750.3
	November	0.5	1073.0	824.8	879.2	925.7
		1.0	1226.0	988.7	875.8	1030.2
RSW-0411	October	0.5	678.5	777.4	858.8	772.2
		1.0	827.8	684.1	851.4	787.8
	November	0.5	639.3	741.5	790.1	723.6
		1.0	834.8	719.9	717.5	757.4
Cycocel	October	1.5	564.6	716.9	739.4	673.6
		3.0	635.9	677.7	775.6	696.4
	November	1.5	842.3	981.0	709.5	844.3
		3.0	599.2	871.5	701.4	724.0

Probability F-test = 0.03; Cv = 14%; LSD(0.05) = 187.7 kg/ha

Appendix 14. Raw data of seed yield of the second harvest in 1990

TREATMENT			BLOCK			
Chemical	Time	Rate	I	II	III	AVERAGE
		kg a.i./ha		kg/ha		
Control			945.2	1020.0	980.4	981.9
Cultar	October	0.5	1087.6	890.7	1280.1	1086.1
		1.0	1005.4	774.1	1079.5	953.0
	November	0.5	1303.8	987.7	1212.9	1168.1
		1.0	1407.5	1170.4	1063.2	1213.7
RSW-0411	October	0.5	763.1	722.3	1142.5	876.0
		1.0	896.0	655.0	1058.8	869.9
	November	0.5	887.1	1014.6	1181.0	1027.6
		1.0	800.8	1178.3	796.2	925.1
Cycocel	October	1.5	880.0	877.8	968.5	908.8
		3.0	965.0	784.8	1174.2	974.5
	November	1.5	936.7	977.7	791.3	901.9
		3.0	863.0	1264.9	1121.4	1082.1

Probability F-test = 0.26; Cv = 17%; LSD(0.05) = 279.7 kg/ha