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A STUDY OF VEGETATIVE AND REPRODUCTIVE DEVELOPMENT IN CAUCASIAN CLOVER (*Trifolium ambiguum, M.Bieb.*) CV. MONARO

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1993

A STUDY OF VEGETATIVE AND REPRODUCTIVE DEVELOPMENT IN CAUCASIAN CLOVER

(*Trifolium ambiguum, M.Bieb.*) CV. MONARO

A thesis presented in partial fulfilment
of the requirements for the

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FAHAM EFENDI

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ABSTRACT

A STUDY OF VEGETATIVE AND REPRODUCTIVE DEVELOPMENT IN CAUCASIAN CLOVER (*Trifolium ambiguum, M.Bieb.*) CV. MONARO

(FAHAM EFENDI, 1993)

SUPERVISORS : 1. Dr. Peter Coolbear
2. Prof. Murray J. Hill

A four year established stand of Caucasian clover (*Trifolium ambiguum,M.Bieb*) cv. Monaro grown on Fine Sandy Loam with moderate fertility was studied to assess the vegetative and reproductive development and the effect of defoliation on seed yield and yield components from September 1991 to June 1992.

As in many other traditional herbage legumes, an indeterminate growth habit and a protracted flowering period can be a factor limiting seed production in this clover. From the examination of the vegetative growth and flowering behaviour, it was found that the protracted flowering pattern in this clover is mainly caused by continuous production of reproductive shoots from the crowns rather than continuing inflorescence emergence in one stem as results indicated that inflorescences produced were mainly associated with the number of reproductive shoots available at the time of inflorescence emergence.

Reproductive growth commenced at the beginning of October when most visible inflorescence buds were formed and subsequent flowering occurred about 8 weeks later. This reproductive growth was found to be the most concentrated on reproductive shoots/stems formed in November to December 1991. These reproductive shoots contributed the majority (70%) of proportion to total inflorescences.

Inter-row cultivation decreased the number of reproductive shoots produced as well as decreasing the number of inflorescences at harvest. Inflorescences originating from main crowns produced a higher number of floret buds and seeds per inflorescence than those originating from secondary crowns as the main crowns have their own strong taproot system and are more mature. Throughout the flowering season, *T. ambiguum* consistently abort about 10 % flower buds before the flowers open. On average of 60% of the open florets developed into live pods at maturity, but only one from two ovules in an ovary usually developed into seeds. On average of about 72% of pods had one seed, 11% of pods with two seeds and 16% were without seed.

Both in early and middle flowers, seed development studies revealed that maximum yield of high quality seed was obtained only when seeds attained their maximum dry weight at 30 days after pollination, at the time when seed moisture content had fallen to 30 to 40%. At this time the pods were yellowish brown in colour. To ensure the production of good quality seed with high yield in *T. ambiguum* it is necessary, therefore to wait crop until at least 34 days after pollination for harvesting the seeds. Seeds harvested early at day 14 to 22 did not retain their viability suggesting that these seeds was still immature and could not withstand desiccation. It was found that pod shattering begun at about 8 days after maximum dry weight (mass maturity) was reached. Heavy rainfall and strong dry wind was found to enhance the rate of shattering. Hardseededness was observed firstly in freshly harvested seeds when seed moisture contents were close to 20%. The levels of hardseededness was higher after drying. Inflorescences set later in the season produced more hard seeds due to higher temperatures and increased humidity during maturation stage.

The results of this experiment have confirmed the high seeding potential of *T. ambiguum* (cv. Monaro) and this should facilitate the production of adequate seed supplies of this cultivars. The average seed yield potential, potential harvestable seed yield and actual seed yield was 893 kg/ha, 707 kg/ha and 427 kg/ha respectively. This might be attributed to the facts that the crop examined in this study was a four year established sward which was mature enough to produced a high yield.

Another study was done involving late defoliation treatments designed to investigate their possible effects on plant growth and seed production. Cutting the plant to the ground level showed an obvious harmful effects on seed yield in *T. ambiguum*. The results show that October and November cutting resulted a 67 and 72% decrease in actual seed yield compared to uncut control. This results support Steiner's view (**in press**) that the plant morphology of *T. ambiguum* limits its ability to recover from defoliation.

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Bismillahirrahmaanirrahiim
In the nama of Alloh the most gracious and merciful

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New Zealand, May 1993

Faham Efendi

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CHAPTER 1

GENERAL INTRODUCTION

1.1. General description

Caucasian clover (*Trifolium ambiguum, M.Bieb.*) which is also well known as Kura, Honey or Pellet clover in the USA is a rhizomatous perennial legume with a potentially wide range of adaptation throughout high mountain environments in the middle latitudes including cold, temperate and intermontane areas, as well as continental rangeland and steppes (Bryant, 1974). It is believed that the plant is indigenous to the Caucasian region of the USSR, Eastern Turkey and Northern Iran (Zohary, 1970) where it is utilised for hay and as a pasture legume (Hely, 1957; Komarov, 1945). Literature on this clover is limited and has been concisely reviewed by Bryant (1974).

Caucasian clover has received considerable attention for agricultural purposes in USSR, Czechoslovakia, Canada, USA and in Australia. Agronomic evaluation in New Zealand has shown that *Trifolium ambiguum* cv. Summit (Paljor, 1973), cv. Treeline (Stewart, 1979) and cv. Monaro (Gurung, 1991) exhibit several characteristics which identify it as promising plant for eroded slopes as well as valuable pasture clover for high country environments.

1.2. Agronomic potential and agricultural value

The potential of this clover as an important alternative pasture plant is clear because of its early seasonal production, persistence under heavy seasonal grazing, its vigorous rhizome development, and its adaptability to grow and persist on low pH (<5) soil where other clovers (*e.g. Trifolium repens*) fail (Agabayan, 1960; Bryant, 1974).

Following its introduction in the USA in 1911, Pellet (1946) reported the following advantages of this clover: firstly, once established, it provides highly productive and long-lived permanent pasture for hay, forage and silage; secondly, it is profuse flowering with nectar readily available; thirdly, its habit of spreading from underground rhizomes ensures an increase in the stand; and lastly, its extensive root system which makes it a good proposition for oversowing in highly eroded sites for soil conservation. In addition, it is a long-lived perennial plant which is deep rooting, drought resistant and winter hardy (Komarov, 1945).

In New Zealand, clovers play an essential role as a forage pasture in most farming systems. However, under the harsh conditions of New Zealand hill and high country, there are only a few pasture species which are able to persist and make a significant contribution to pastures (Scott *et al.,* 1983). Under low fertility, low pH, low winter temperature and seasonal moisture deficit, the growth of traditional legumes such as *Trifolium subterraneum, Trifolium repens* and Lotus corniculatus are restricted (Chapman and Macfarlane, 1985; Scott *et al.,* 1983). With the demand to develop alternative productive pastures under marginally productive areas, therefore, the potential uses of caucasian clover as a forage legume have become recognized.

Caucasian clover is also resistant to many of the viruses common to other temperate clovers (Burnett and Gibson, 1975; Jones *et al.,* 1981). Stewart (1979) observed that *Trifolium ambiguum* persisted in an area which was heavily infested with grassgrub (*Costelytra zealandica*) where both *Trifolium repens* and *Trifolium hybridum* were killed. *Trifolium ambiguum* was reported to be a host for the clover cyst nematode (*Heterodera trifolii*), but it was demonstrated to be much more resistant to it than *Trifolium repens* (Norton and Iselly, 1967).

This clover compares favourably in feeding value with other traditional legumes (Speer and Allinson, 1985). Its acceptability to all form of livestock (Pellet, 1945; Larin 1956), its non-oestrogenic nature (Anonymous, 1977), its high concentration of nitrogen and minerals, and its digestibility (FitzGerald, 1980; Davis, 1981; Allinson *et al.*, 1983) are some valuable characters which make this clover superior to other traditional legumes in many circumstances.

1.3. General agronomic and management problems

Despite its promising characteristics, however, this clover has not been developed commercially and has found only limited used outside its indigenous habitat (Townsend, 1970; Hely, 1971). This clover is difficult to establish because of slow seedling growth and no work appears to have been specifically directed toward determining the growth pattern of this clover.

As in other newly introduced legumes, the difficulty in achieving a substantial amount of high seed yield is frequently reported as a major factor limiting its more widespread use in pasture systems. Because of slow growth, or perhaps other reasons, seed yields the first year after planting are as low as 70 kg per hectare. Seed yields in later years are reported to range from 100 - 600 kg per hectare (Bryant, 1974; Voloshenko *et al.*, 1979; Stewart and Daly, 1980; Steiner, **in press**). It has been suggested that characteristics contributing to its low and unreliable seed yield include poor pollination, erratic and protracted flowering, and poor seed set (Hampton *et al.,* 1990). Above all, there is little information available on suitable agronomic management practices for both herbage and seed production.

1.4. Objectives of the study

This study was conducted as a result of the demand for a better understanding of this clover and the need for the development of appropriate management strategies to improve seed production. The objectives of this study are :

1. To define the morphological characteristics of *Trifolium ambiguum, M.Bieb.* during regrowth periods
2. To determine potential seed yield, and to examine possible causes of low seed yield in field conditions.
3. To describe in detail the sequence of seed development which can be used to determine optimum harvest time.
4. To determine the response of aerial and underground plant components to different times of defoliation and to identify some of the effects of defoliation on reproductive growth and seed yield.

This study was carried out in one growing season on a four year old established stand of *Trifolium ambiguum* from September 1991 to July 1992 on the AgResearch Grasslands Research Unit, Aorangi, Palmerston North, New Zealand.

CHAPTER 2

LITERATURE REVIEW

2.1. Taxonomy

Taxonomically, *Trifolium ambiguum, M.Bieb.* is closely related to *Trifolium hybridum, Trifolium repens* and *Trifolium montanum* (Bryant, 1974) (see **Appendix 2.2**). With both *T. hybridum* (Kiem, 1953; Evans, 1962) and *T. repens* (William and White, 1976) interspecific hybrids have been developed, but so far they generally infertile. The classification as stated by Komarov (1945), Towsend (1970) and Zohary (1970) is as follows :

Family	***Leguminosae***
Sub family	***Papilionatae***
Section	***Eumoria***
Genus	***Trifolium***
Sub genus	***Trifoliastrum***
Species	***ambiguum***

2.2. Genetical characteristics

T. ambiguum is a plant of wide morphological variations (Bryant, 1974). This clover is one of a few species in this genus exhibiting natural polyploidy (Kannenberg and Elliott, 1962). Their cytological examination revealed diploid, tetraploid and hexaploid races with a basic somatic number of 2N = 16. They suggested that although most morphological, floral and agronomic characters generally changed with ploidy level, the only accurate means of distinguishing among ploidy levels were cytological examinations. The morphological features related to ploidy levels will be discussed in the later sections of this chapter (see **Section 2.6**).

In terms of desirable agronomic performance, Kannenberg and Elliott (1962) suggested that among different ploidy levels, the hexaploid collections were superior. Interploidal fertility in *T. ambiguum* has been observed by these workers and they suggested that, in general, fertility between ploidy levels increased directly with ploidy; the lowest average seed production was in 2N X 4N crosses. Optimum ploidy level may be higher than 6N, but Bryant (1974) considered that 8N strains so far have produced little of agronomic significance, besides such forms have not been found in the nature.

2.3. Origin and distribution

As described briefly in **section 1.1**, *Trifolium ambiguum* is a perennial legume which is believed initially to have distributed throughout the Crimea, Caucasian Russia (Azerbaydzan, Armenia, Georgia), Eastern Turkey and Northern Iran. The species is adapted to mountain slopes, valleys and screes, and to drier grassy steppes (Bobrov, 1950; Hossain, 1961; Zohary, 1970, Bryant, 1974). Within its native habitat, this clover has not been domesticated and found only limited use (Towsend, 1970; Hely, 1971). The characteristics of its habitat suggest that this clover can tolerate acid soils, low levels of nutrients, random summer frost and summer droughts better than available domesticated legumes (Bobrov, 1950; Zohary, 1970; Baysal, 1974; Spencer, *et al.,* 1975).

Spasmodic testing and evaluation have been done in Russia (Bobrov, 1950), Czechoslovakia (Vachek and Ded, 1956), USA (Keim, 1954; Towsend, 1970), and Australia (Hely, 1957; Costin and Winbush, 1963). In Australia, this clover was first introduced in 1931 from Georgia, USSR, Turkey, Iran, USA and England by CSIRO, however, evaluation did not proceed beyond the nursery stage until the mid's 1950s when naturally occurring ploidy groups were recognized (Hely, 1957). In the period 1962 - 1968, various forms representative of all three ploidy groups were tested against *T. repens, T. fragiferum, T. hybridum* in the Snowy Mountains of New South Wales at 1580 m above sea level (Costin and Winbush, 1963; Hely 1963, 1972; Bryant, 1971, 1974). The best performing diploid and tetraploid survivors from these field test were then selected and polycrossed by Hely (1972) seed supplies developed to form the basis of the registered cultivars, Summit (diploid) and Treeline (tetraploid) (Barnard, 1972).

In New Zealand, *T. ambiguum* was initially introduced by Mr. A.H. Nordmeyer and tested at the Forest and Range Experiment station, Rangiora (Paljor, 1973). From this evaluation, it was shown that *T. ambiguum* (cv. Summit) exhibited several characteristics which identified it as promising plant for reclothing eroded slopes (Paljor, 1973). However, agronomic studies indicated that this clover may also have potential as a pasture legume, especially where environmental extremes such as drought, cold temperature, nutrient deficiencies reduce the productivity of traditional herbage legumes (Paljor, 1973; Gurung, 1991).

2.4. Habitat and edaphic factors

Caucasian clover originates from both moist and dry sites, even dry slopes of volcanic origin throughout Turkey and Northern Iran as high as 3170 m, where 4 -

5 months of snow cover is succeeded by late summer to autumn drought (Bryant, 1974). Bryant (1974) also indicated variations in frost tolerance between lines and between chromosome races, the hexaploid lines being most affected by frost during growing season. Field observations in Australia indicated that this clover prefers to grow on non calcareous soils of the steppes and on the meadow soils of the mid latitude mountains where white clover failed completely (Barnard, 1972; Bryant, 1974). Agabayan (1960) has reported that *T. ambiguum* is able to grow at very low pH levels (*e.g.* 4.9) where other introduced *Trifolium* species have failed.

Barnard (1972) reported that *T. ambiguum* (cv. Summit) grew well at low pH (5.1) and restricted phosphate levels where it was found to grow and persist better than *T. repens*. Its ability to grow under low phosphate conditions is probably largely due to it massive root and rhizome system enabling a greater volume of soil to be explored (Stewart, 1979). The ability to grow at low phosphate levels does not mean that this clover can't respond to high levels of phosphate. Under field conditions, it has been found that this clover was responsive to phosphate applications and to compete better with grasses (Agabayan, 1966; Barnard, 1972; Paljor, 1973; Meares, 1975;). Hexaploid Caucasian clover (cv. Monaro) has been ranked highly with regard to efficiency in phosphate utilization (as dry matter produced per unit P added) and is reported to have marginally lower internal and external phosphate requirement than other ploidy groups (Spencer *et al.,* 1980).

Some variations appear to occur between lines in their ability to withstand waterlogging and free surface water. Field survival in excess of 80% has been observed in three lines (2N (CPI 2264), 4N (CPI 6884), and 6N (CPI 20871)) after inundation for 40 days in early spring by water up to 15 cm deep (Bryant, 1974).

2.5. Agricultural value and potential uses

2.5.1. Potential herbage production

There are few pasture legume species which are able to persist and give significant contribution to pastures in medium to high altitudes (800- 1500m), low winter temperature areas, and in areas which have long periodic summer droughts. Under such conditions, the growth of traditional herbage legumes (*e.g. T. subterraneum, T. repens, T. hybridum and T. montaneum)* are limited (Dear and Zorin, 1985).Though *T. ambiguum* has been proved to be promising to be grown under these conditions, little is known about its potential herbage/dry matter production as few assessments have been carried out.

Working in the Victorian montane environment, Spencer *et al.* (1975) found that *T. ambiguum* establishment is extremely slow, especially under competitive conditions, and that herbage production is very poor in comparison with that of *T. repens*. The same results were also reported by William and Caradus (1978) with the same species and White and Meijer (1978) in comparison with that of lucerne.

Under infertile high country environments, Stewart and Daly (1980) reported that the performance of *T. ambiguum* compared favourably with that of *T. repens,* while *Lotus pedanculatus* was better. Little difference between *T. ambiguum* and *T. repens* was noted during establishment without maintenance superphosphate in a Haldon soil at Hunua, North Canterbury. However, over the next 3 years *T. ambiguum* (cv. Prairie) out yielded *T. repens* (cv. Huia) by an average of 2.6 ton dry matter/ha/year (Daly and Mason, 1987). They suggested that even though some lines seem to be superior than others, in general, *T. ambiguum* was very productive in the very fertile lowland soil, moderately productive at the dry hill sites and less so in the infertile high country environment (Daly and Mason, 1987).

During research in a high altitude region (1150 m) of South-Eastern Australia, Dear and Zorin (1985) found that *T. ambiguum* was well adapted to the cold winter and dry summers. All the lines tested (2N, 4N and 6N) persisted throughout the experimental period, whereas *T. repens, T. pratense* and *T. montanum*, had disappeared by the fourth year even though these cultivars out yielded in the first year, (Dear and Zorin, 1985). Their measurement of herbage production indicated that the most promising ecotypes tested for high altitude regions were cv. 'Monaro', 'Kirovakan ' (both hexaploid), 'Alpine' and Forest (diploids), producing the equivalent of 5550 kg/ha, 3770 kg.ha, 2700 kg/ha and 2940 kg/ha respectively in the 4 months (Dear and Zorin, 1985). Among those lines cv 'Monaro' and 'Alpine' exhibited superior vigour and warrant further evaluation as promising pasture legumes.

2.5.2. Likely value for erosion control in New Zealand

Exposed subsoil due to heavy rainfall are common features of mountain slopes throughout region of New Zealand. The instability of plant communities and soils is complicated by the fact that most mountain ranges in New Zealand are geologically immature leading to high erosion potentials irrespective of vegetation cover. Indications from experiments in the USA and Australia are that *T. ambiguum* could be a suitable plant for erosion control as this species are best adapted to colonization and persistence on such sites (Costin and Winbush, 1963; Stewart, 1979) due to its deep rooted, spreading or rhizomatous habit. In addition to this, by introducing such legumes, the soils can build up nitrogen status (Paljor, 1973). Once legume growth has been established, other nutrient limitations may be corrected (Spencer *et al.,* 1980).

2.6. Description of the plant

2.6.1. Below ground parts

The general morphological characteristics of *T. ambiguum* have fully described in the botanical review by Komarov (1945) (see **Appendix 2.1**), Herman (1954), Hossain (1961), Zohary (1970), and Towsend (1974). However, the description of the root and rhizome system as well as variations within species were poorly explained in these reviews.

The root system of *T. ambiguum* has been described by Bryant (1974) as having a strong deep semi woody often branching taproot from which many branched rhizomes grow. These rhizome eventually give rise to daughter plants both terminally and from nodes. Depending on the growth stage, the dimensions of the root system vary, but a fully developed root system could well exceed 1 metre in depth and 0.75 metre in width. Dear and Zorin (1985) noticed that all main crowns of *T. ambiguum* produced a strong taproot system comprising 4 to 6 thick roots, to 30 - 40 cm deep. Compared with white clover, red clover and zigzag clover, roots of *T. ambiguum* are extended to a greater depth and are more fibrous and stouter (Spencer *et al.,* 1975). These features in the root system of *T. ambiguum* may explain why this clover is more resistant than traditional herbage legumes.

Stewart (1979) found that the number of daughter plants was highly correlated with the number of rhizomes produced. He reported that the number of rhizomes, the number of nodes, internode length and the length of rhizomes also exhibited a ploidy level effect. The number and the length of rhizomes tend to increase with increasing ploidy levels (Kannenberg and Elliot, 1962; Baysal, 1974). Stewart (1979) reported that diploids have the highest number of nodes and tetraploid has the least and hexaploid in between.

2.6.2. Above ground parts

There is considerable variation in the above ground morphology of *T. ambiguum*, in terms of size, shape and growth habit, all of which are influenced by genotypic/ploidy levels and environment. The type of stand formation as the results of vegetative spread is quite variable. In some cases, the mother plant develops to an appreciable size via the formation of a crown composed of many leaf petioles (Speer and Allinson, 1985). In these cases, few daughter plants develop. Some *T. ambiguum* plants initiate vigorous rhizomes and daughter plant development, ultimately, resulting in a compact stand. The stands can be 1 - 1.5 m in radius with the mother plant at the centre of the crown.

The ploidy levels of *T. ambiguum* were also found to affect leaflet shape and leaflet area. The mean leaf area and leaflet length change with ploidy level (Kannenberg and Elliot, 1962; Stewart, 1979). Leaflet shape, given by the ratio of leaf length to width, indicates that diploid have round leaflets, hexaploid have large elongated leaflets and tetraploid the most elongated narrow leaflets (Dear and Zorin, 185). Generally, plant height and size increases with higher ploidy levels (Meares, 1975).

2.6.3. The root-shoot ratio

The proportion of the plant components below the ground to the above the ground component has been described by Paljor (1973), Meares (1975), Spencer *et al.* (1975) and Stewart (1979) to be higher than in *T. repens.* Although their reports showed differences which may attributable to diverse experimental conditions, it is possible to draw some general conclusion from their work.

Generally, the proportion of *T. ambiguum* below the ground increases as the plant matures and establishes rhizome systems, while in *T. repens* (cv. Huia) the proportion below the ground decreases as the plants produce stolons. At three weeks of age 30 - 35% of the dry matter of seedlings of *T. ambiguum* was below the ground, while in *T. repens* had slightly less at 25 - 30 % (Paljor, 1973). After 3 months at about the time *T. ambiguum* initiated rhizome production, the proportion of the plant below the ground had increased to 50 - 60% in *T. ambiguum* and decreased to 20 - 25% in *T. repens* (Meares, 1975). In 17 months old mature plants, by which time *T. ambiguum* had produced a mass of rhizomes, the below ground proportion had increased to 70 - 80%, while in *T. repens* it had further decreased to 10 - 15% (Spencer, 1975). Meares (1975) found that harsh environmental conditions increase the below the ground proportions by 5 - 10%.

2.7. Reproductive structure

2.7.1. Flowers

The inflorescence of *T. ambiguum* is a typical umbel consisting of varying numbers (usually 60 - 170) of florets attached by a short pedicle to a long peduncle. Pedicle is short (up to 1/3 length of calyx tube). Each floret consist of a 10 nerved calyx.

The corolla may vary from white tinged pink and changes to rose pink after anthesis. Flowers are sweet smelling with good nectar content which is attractive to honey bees and other kind of insects (Barnard, 1972) (**Appendix 2.2**).

2.7.2. Pods

Several pods are born in the tip area of the peduncle. Pods are relatively short compared to *T. repens,* and are cylindrical, yellowish brown to dark brown at maturity. Usually the *T. ambiguum* flower has 2 ovules per ovary but only about 60% of these ovules develop into mature seed (Kannenberg and Elliot, 1962; Barnard, 1972).

2.7.3. Seeds

The seed size of *T. ambiguum* is bigger than other clovers, especially in hexaploid cultivars. Measurements by Dear and Zorin (1985) showed that thousand seed weight of *T. ambiguum* vary considerably in different strains and cultivars ranging from 1.13-1.39 g (diploid), 1.62-2.25 g (tetraploid), and 2.11-2.63 g (hexaploid).

The rate of hardseededness seems to be influenced by both genetic differences and environmental conditions during seed development. For example, the percentage of hard seed at ripening of white clover, alsike clover and red clover is very high (more than 70%), while that of crimson clover is very low (<40%). In *T. ambiguum* up to 40-60% hard seed have been reported (Bryant, 1974) and the percentage of hard seed in seed lots of the same clover can be as high as 75% (Redkina, 1978). The cultivars developed in Australia (*e.g. cv. Summit* and *Treeline*) are reported to have only 15-60% hard seed (Bryant, 1974).

Hardseededness in *T. ambiguum* has been reported to be easily broken by scarifying seed with abrasive materials (*e.g.* sand paper). Australian workers have successfully increased germination percentages from 15% to 90% by mechanical scarification (Bryant, 1974). In New Zealand, Gurung (1991) found that mechanical scarification increased germination of *T. ambiguum* (cv. Monaro and Alpine) from 8% to 70%. Sulphuric acid was used as a scarification agent by Redkina (1978), who found that such treatment could increase germination in *T. ambiguum* up to 100%. Speer and Allinson (1985) suggested an optimum scarification treatment of concentrated sulphuric acid for 24 minutes. Scarification treatment may cause all viable hard-seeds to germinate; however, the percentage of germination also depends on seed quality and age of seeds.

2.8. Growth and development of the plant

2.8.1. Germination and seedling establishment

The germination of *T. ambiguum* is controlled by environmental conditions, especially temperature and moisture stress. Working with cv. Summit, Bryant (1974) suggested that the optimum temperature for germination was 15°C with initial germination occurring at 4°C after 10 days while at 15°C 90% of seeds have germinated after 7 days. He also noticed that pre-chilling treatment at -5°C did not alter the final germination percentage, but delayed germination by several days. He suggested that this might be a protective mechanism to protect seeds from false break.

A considerable variation in seedling vigour partially due to seed size has been reported (Bryant, 1974; Kannenberg and Elliot, 1962; Paljor, 1973). Kannenberg and Elliot (1962) obtained one thousand seed weights of 0.1510, 0.1716 and 0.2986 g for diploid, tetraploid and hexaploid respectively. These workers found a high correlation between these weights and radicle length, dry weight and water content with C.P.I. 10803 (hexaploid) having a significantly higher seedling dry weight than other selections and *T. repens.* However, after this initial boost due to high seed weight, all *T. ambiguum* selections tend to be very slow to establish (Spencer *et al.,* 1975; Meares, 1975). Further detailed assessment of *T. ambgiuum* are needed to understand the reason for this slow establishment. The selection C.P.I. 10803 (hexaploid) appears to be superior in this respect than other collections and *T. repens* (Costin and Windbush, 1963; Meares,1975). However, observation in Canterbury, New Zealand, in an oversowing trial at Mesopotamia showed that establishment of *T. ambiguum* (cv. C.P.I. 10803/hexaploid) has been much poorer than that of *T. repens* (cv. Huia) and *Lotus pedunculatus* (cv. Maku/tetraploid) (White and Daly, 1975; Meares, 1975). This suggest that seedling vigour also varies considerably with chromosome race, where it may, at least partially, be related to seed size as well as environmental conditions during establishment.

Meares (1975) suggested that rapid root penetration is an important factor in seedling establishment in dry areas and cold location subject to frost and lift. A small application of nitrogen has been shown to aid enhanced establishment by increasing growth and effective nodulation (Hely, 1963; Evan, 1966).

2.8.2. Reproductive growth

The lack of a reliable seed source has been put forward to be the main reason why scientific and commercial interest in *T. ambiguum* has been limited. Reports suggested that *T. ambiguum* is a shy seed yielder and little is known about the management of their seed production (Bryant, 1974; Hampton *et al.*, 1990). Most of the information available on clover seed production is concerned with traditional herbage legume such as *T. repens, T. pratense,* and *Lotus species.* Limited studies have been done to investigate *T. ambiguum* especially in seed production. So far in New Zealand, *T. ambiguum* is being grown only for research purposes and there have been few published results. Therefore, in this discussion, relevant information on seed production in most traditional clover species will be integrated.

2.8.2.1. Flowering

Having an indigenous habitat in high elevation areas and regions with cold winters, it seems that *T. ambiguum* appears to have strong photoperiod and vernalization requirement for commencing reproductive growth (Meares, 1975). However, observations in the USA (Iowa, latitude 42° North), various treatments with photoperiod, temperature, water, nitrogen and age could not induce out of season flowering (Kiem, 1953). This suggest that the level of maturity and/or genetic difference may be responsible to its response. Townsend (1970) found a high correlation (r = 0.85) for date of flowering of individual plants in two subsequent years indicating that flowering is regulated by seasonal factors. In Australia (Canberra, latitude 34° South), Hely (1957) observed that flowering behaviour was erratic suggesting that some plants require long days to initiate flowering. There have been no problem in flower inducement in New Zealand (Canterbury, latitude 43° South) (Nordmeyer, 1975 *in* Meares, 1975). A photoperiodic requirement for cv. Prairie was born out when it was observed that a few hours of artificial lighting each evening caused more regular flowering behaviour (Nordmeyer *in* Stewart, 1979).

Stewart (1979) found that flower initiation in *T. ambiguum* was much earlier at a low elevation than medium and high elevation which could be related to temperature differences. This suggest that higher temperatures could have a strong effect in accelerating floral initiation, in addition to the photoperiodic effect.

In all ploidy types of *T. ambiguum* peak flowering usually occurs in late spring and early summer (Kannenberg and Elliot, 1962; Towsend, 1970; Meares, 1975). In Australian conditions, usually the first flower buds can be seen in mid-October with peak flowering in November (Dear and Zorin, 1985). Towsend (1970) reported a highly variability in flowering dates within ploidy levels with diploid earlier than tetraploid which were also earlier than hexaploid. The same results have also been found by Dear and Zorin (1985). The earlier flowering of the high altitude diploid forms may be a response to the short growing season, just as ecotypes of *T. subterraneum* evolved in dry areas flower early (Smetham, 1973 *in* Gurung, 1991) to escape the summer drought. However, in its native habitat vegetative reproduction through rhizome production is its main form of perennation (Bryant, 1974). In the domestication of C.P.I. 2284 to form the cv. Summit, Hely (1972) selected for free flowering and high seed yields and Bryant (1974) reported the existence of dense even flowering stands in Australia (latitude 42° South) and he suggested that with cv. Summit, two seed harvest in one season may be possible.

2.8.2.2. Pollination and fertilisation

Kannenberg and Elliot (1962) and Kiem (1953) reported absolutely no self compatibility within any of the ploidy levels, however Towsend (1970) found that some self compatibility did exist. 56% of a diverse set of plants (in term of genetic make up and origin) set no seed at all. Most of the rest set only one or two seed per head and only 2% of the population had a self seed set of more than 15 seed per head (Townsend, 1970). In cross pollinated inflorecences Kannenberg and Elliot (1962) reported that the number of seeds per head can be 35-145 and 43-133 for diploid and hexaploid respectively. The exact mechanism of self-incompatibility in *T. ambiguum* is still unclear at the present time, although many workers have suggested in some clover species that both morphological and physiological barriers may be involved (Kannenberg and Elliot, 1962; Daly and Mason, 1985). According to Atwood (1943) there are two"interference zones" active in preventing the growth of the incompatible pollen, one on the stigma and the other about three quarter of this distance down the style. Pollen on the stigma does not germinate until the cuticulae covering the stigma papillae is ruptured and releases the mucilaginous secretion beneath. Presumably, pollen germination in "rubbed" flower heads results from the combined release of mucilaginous matter by mechanical damage and the transfer of pollen grains from dehisced anthers to stigma surfaces. The degree of self-incompatibility is determined primarily by genetic make up and may be modified by environmental conditions (Thomas, 1981; Pasumarty 1990).

Because of a high degree of self-incompatibility within all ploidy levels, cross pollination is essential to obtain high seed yields of *T. ambiguum* (Kannenberg and Elliot, 1962; Hely, 1963; Towsend, 1970). Nevertheless, this clover is freely crossed pollinated by different moth, butterflies and honey bees. These pollinators are strongly attracted to the flowers and their rich nectar, moreover, unlike Zig-zag clover, Caucasian clover has a short corolla tube, therefore, cross pollination is not a problem if pollinators are present. Bryant (1974) has demonstrated that 4-5 beehives per hectare was optimum for cv. Summit during the dense flowering period.

Kannenberg and Elliot (1962) have shown that seed set of crosses between ploidy levels of *T. ambiguum* was very low. In another species of *Trifolium,* Hagberg (1957) found that a contamination as little as 4% diploid *T. pratense* reduced the seed yield of tetraploid *T. pratense* by 50%. Therefore, as *T. ambiguum* is probably similar, it would be essential to avoid contamination of ploidy levels to maximize seed yield (Stewart, 1985). Seed set is low within wild ecotypes (Hely, 1971), however because flowering and seed production are responsive to selection, the attaintment of high seed yield through breeding programmes is not likely to be a problem.

2.8.2.3. Seed development

Studies on seed development in several traditional herbage legumes have carried out by many workers (Hyde, 1950; Hyde *et al.,* 1959; Win Pe, 1978; Kowithayakorn and Hill, 1982; Hare and Lucas, 1984**b**), however, a detailed account of seed development in *T. ambiguum* has never been reported. This discussion, therefore, aims to review information on *T. ambiguum* seed development but this will be supplemented by documented results on seed development in more common domesticated herbage legumes (*e.g.* white clover, red clover, maku lotus and lucerne). During the course of seed development, seed quality is concerned with physical, physiological and biochemical changes in the seed. In most studies, attention has been paid to the two main aspects of seed development *viz.* the relationship of moisture content to dry weight and the development of viable seeds from the fertilization to maturity. Hyde (1950) and Hyde *et al.* (1956) have described the development of the clover seed (white clover and red clover) and proposed three developmental stages :

a. The growth stage (Stage I):

During a period of 10 days after pollination, there is a rapid increase in seed weight. The growth rate is logarithmic due to cell division in the embryo, endosperm and testa. The moisture content of seed is high and approximately constant (75 - 80%) and generally no viable seed is detected during this stage.

b. Food reserve accumulation stage (Stage II):

This stage takes up 10 - 14 days following the growth stage. It is characterized by a constant rate of growth which is presumably determined by the food transfer rate from the parent plant into the seed. The dry weight of the seed increases about threefold and at the end of the stage reaches its maximum dry weight. The actual amount of water in the seed decreases slightly and fresh weight falls to about 63%. In the end of this stage, the seed is structurally complete and attains maximum viability and vigour.

The period at which maximum grain development is first achieved (Aldrich, 1943) or at which maximum dry weight is first reached (Hyde, 1950; Austin, 1972) has been referred to as "Physiological maturity" (Harrington, 1972) or "Morphological maturity" (Anderson, 1955). Studies of several herbage legumes have shown that maximum viability was reached 4-7 days before seeds reached maturity (Hyde *et al.,* 1959; Win Pe, 1978; Kowithayakorn and Hill, 1982). However, recent work by Ellis and Filho (1991) with spring and winter cultivars of barley and wheat has demonstrated what seed physiologist have always known : that physiological development is not complete at maximum dry weight achievement. These workers have shown that maximum vigour and potential seed longevity is achieved several days after physiological maturity in all cultivars tested. They proposed that the term "mass maturity" be adopted instead of physiological maturity.

c. The ripening stages (Stage III):

This stage last 3 to 7 days from the completion of the growth stage. During this period the seed dries out rapidly and shrink in size. The seed dry remains relatively constant, but the moisture content falls to approximately half (from about 63% to 10%) as the seed reaches equilibrium with the relative humidity of the surrounding environment until the seed becomes suitable for harvest. The time required for this stage is very dependent on weather conditions (Delouche, 1980).

The germinability continues to decline as more hard seed are formed. This phenomenon is typical of many legumes. As the seed develops, their testa become impermeable to water and gasses. The seeds are unable to germinate and termed as "hard" (Hyde *et al.,* 1959). A high number of hard seeds occur when the seed is ripe due to loss of moisture. In white clover, the permeability of seed decreases when the moisture content is approximately 25% and the seed coat becomes impermeable at 14% moisture content (Hyde, 1959) and generally total germination percentage begins to decline. Win Pe (1978) suggested that the onset of hardseededness seems to be depend upon climatic conditions due the period of seed development and seed ripening. Hardseededness is positively related to the temperature during seed formation and seed moisture content is the main factor influencing hardseededness after seed maturation (Argel and Humpreys, 1983**a** and **b**). Working with lucerne (Kowithayakorn and Hill, 1982) reported that only a low percentage of hard seed appears during the ripening stage, but a higher percentage is found in seed after a period of storage.

2.8.2.4. Seed yield and yield components

Clover species are well known for their fluctuations in seed yield. Generally, the seed production capacity of clover species represents the cumulative expression of four principal components : number of flower heads per unit area, number of florets per head, number of seeds per floret, and seed weight (Thomas, 1981; Maldonado, 1985; Pasumarty, 1990). These components all differ in their relative contribution to total seed yield and change with genetic variability within species as well as with the environmental conditions (Zaleski, 1961; Thomas, 1981).

Huxley *et al.* (1979) reported that the major components of yield in white clover (*T. repens*) were the number of inflorescences per unit area and seed weight which accounted for 40% and 59% of the diversity in seed yield respectively. These workers suggested that variation in the number of seeds per floret (seed set) made a comparatively negligible contribution to variation in seed yield and was independent of inflorescence number. However, Gaspar *et al.* (1981) and Haggar *et al.* (1963) determined correlation coefficients between seed yield and associated characters and found that seed yield was positively correlated with flower heads per branch, seed set and seed weight. Binek (1983) also found that removal of up to 48 % of the inflorescences from white clover (cv. Podkowa) did not reduce seed yield. Compensatory increases of 58-78% occurred in both number and weight of seed per head, although seed weight altered little (less than 8%). This evidence strongly suggests that seed set is also a major component of seed yield in white clover and in other clover species.

Dessureaux (1951) analyzed the seed setting ability of several clones of Ladino clover and found that clones differed significantly in the number of ovules per floret, seed set per floret and percentage of florets bearing seed. Thomas (1981) reported that the mean number of ovules per floret in white clover (cv. Grassland Huia) was 5.5, while Clifford (1979**a**) found the average number of seeds per floret to be only 2.2. A population of Ladino clover, in which the ovule number per ovary averaged 4.6, yielded an average of 2.4 seeds per floret (Dessureaux, 1951). Working with white clover (cv. Grassland Huia), Maldonado (1985) reported that actual seed set was only 30-40% potential seed set (2.0 - 2.3 out of a total of 6-7 ovules per ovary). Many clover species potentially have for more than 50% seed set (Thomas, 1981). This suggests that by increasing seed set per ovary, there is the potential for doubling seed yield under conditions of optimal head density. Nevertheless, the reasons for poor seed set have not been fully understood.

Although the detailed accounts of seed yield components are not available and yields are still not consistently and reliably obtained, the seed production problem in *T. ambiguum* has recently received some attention from several workers. Bryant (1974) reported that in spite of 50% losses due to heavy rain, yields of *T. ambiguum* (cv. Summit) of up to 95 kg per hectare were obtained, while cv. Prairie produced a seed yield in excess of 200 kg per hectare which was considered to have been grown in a marginal environment for seed production. This indicates that potentially, the actual seed yield is likely to be greater than 200 kg per hectare. Several reports (refer to **Section 1.3**) suggest that with more appropriate management techniques and more understanding of the nature of the plant the seed yield of *T. ambiguum* has the potential to be greatly increased.

2.9. Management for seed production

Each component of seed yield is controlled by genetic and/or environmental factors which can be improved by manipulating plant genetic and/or agronomic management (Thomas, 1981). Therefore, the main objectives of good management for seed production after successful establishment, irrigation, fertilization weed and pest control is to promote the maximum numbers of growing points and subsequent flower head development over the shortest period of time to ensure high seed yields and a minimum seed loss at harvest.

2.9.1. Plant establishment and density

An optimum plant density should be established to achieve maximum seed yields. In white clover, maximum seed yield can be obtained at a low plant density at about 10 plants per metre square after the development of large vigorous plant stand in the second season (White, 1990). In red clover, similar results was achieved at population density of 17 plants per metre square at 60 cm row spacing (Clifford, 1974). Optimum plant density is obtained when there is enough spaces for stolons/stems to develop flowering sites and spacious enough for light penetration to the reproductive point to promote flower head development (Clifford, 1980; Thomas, 1980; Pasumarty, 1990). The same principles may applied to *T. ambiguum*, however, this clover is a rhizomatous plant which tends to spread by producing underground rhizomes. It is, therefore, very difficult to establish optimum populations at normal sowing rate like in other non-rhizomatous clovers. An alternative technique to provide space for the development of rhizomatous clovers is to sow in wide rows (Clifford, 1980, 1985; Whelan and White, 1985).

The optimum seeding rate for seed production has not been assessed in any detail as this clover is still grown on an experimental basis in New Zealand (Hare *pers. comn.*). Preliminary studies at the Lincoln University Research Farm, for seed production from rhizomatous clovers (*e.g.* zigzag clover, red clover and caucasian clover) have been established at 0.75 m row spacing. In Australia, excellent establishment of cvs. Summit and Treeline were obtained from a seeding rate of 1.9 kg per hectare; in a row of 45-47 cm wide, while to control erosion, a broadcasting technique is used by sowing at the rate of 4.5 kg per hectare. This rate has resulted in seedling densities as high as 15 plants per square metre (Bryant, 1974).

2.9.1.1. Inter-row cultivation

Because inflorescence production is an intrinsic function of growth (Thomas, 1961), total floral expression is likely to be inhibited by canopy development (Clifford, 1980). Thus the seed production management of a particular cultivar must take into account of the natural branching habit (Hoglund and William, 1984) and allow sufficient space for reproductive shoot development. This requirement is more adequately controlled through adjustment to row spacing than seeding of broadcast crops (Clifford, 1985).

T. ambiguum is a rhizomatous legume which can produce a very dense rhizomes below ground and a vegetative stand above ground over four years. It is believed that the reproductive stems/shoots should be encouraged to grow as it is these stems which become the main seed bearing stems, however, if too many shoots are produced (over dense stand), many of these stems either remain vegetative or fail to branch and then produce few umbels per unit area (Hare, 1983, 1984). Inter-row cultivation technique has never been studied in *T. ambiguum*, but has been reported to improve seed yield of Kent wild white clover by 23% (Lewis *et al.*, 1984). Working with *L. pedunculatus*, Hare (1983) found that environmental conditions and the timing of applying inter-row cultivation is of importance and this technique reduced rhizome stem competition and increased seed yields from crown stems. Further he suggested that stems developing close to the plant crown appear to have a higher proportion of reproductive stems than stems growing further away from the crown. Therefore, if rhizome growth is curtailed by cultivation during the spring to early summer, more nutrients will be available to develop reproductive stems from plant crowns. It is crown stems and not rhizome stems which appear to be of importance for reproductive development. As *T. ambiguum* has a similarity with maku lotus in term of growth habit, research on the effects of inter-row cultivation on seed yield is considered to be advantageous.

2.9.2. Defoliation and seed production

As in many traditional herbage legumes, the management system that has been commonly used in New Zealand consist of the control of growth by defoliation. Defoliation is considered to be a disturbance to normal plant development and is defined as removal of any part of the plant, including the removal of growing point (Harris, 1978). This can promote the vegetative expansion of axillary buds, thus, increasing branching and bud density as well as the number of possible sites for floral development (Zaleski, 1961).

In general, the severity of defoliation and therefore the response of the plant may be considered in terms of *frequency* (the time interval between successive defoliation), *intensity* (the proportion removed at particular defoliation) and *timing* (the number of defoliation carried out in relation to the developmental stages of plant and the season of the year (Harris, 1978; Sheath, 1978; Maldonado, 1985).

Early defoliation (prior to flowering), generally increases seed yields which is reported to be mainly due to the production of a higher number of inflorescences over uncut plants. Thomas (1961) suggested that defoliation during the vegetative phase of photoperiodically responsive plants allows increased light reception by the stolon to initiate floral development. This was achieved in white clover (cv. Huia) by cutting in mid-November (Clifford, 1980; Clifford *et al.*, 1985). This time of defoliation allows 30 days in which the plant growth is maximized after cutting (Brougham, 1962), and both increasing temperatures and day length occur to maximize growth and thereby floral expression. Defoliation at the vegetative stage in zigzag clover is reported to cause flowering emergence much earlier than late defoliation (after flowering) (Towsend *et al.*, 1968) and resulted in increased seed production. A significant increase of 30% over uncut was reported by Robertson and Amstrong (1964) in earlier cut plants in the same clover. Similar results were reported in subterranean clover (30% increase) (Collins, 1978) and in *T. ambiguum* (cv. Monaro), where seed production increased by about 40% (gurung, 1991). However, in red clover late defoliation increased seed yields compared to early defoliation (Clifford, 1979**b**) due to later development of flowering stems. This indicates that the response of plant to defoliation varies between species and even between cultivars within species.

In many circumstances, however, defoliation after the reproductive stage reduces seed yield. Several workers (*Lotus corniculatus,* Anderson and Metcalfe, 1957; Bader and Anderson, 1962; Sheath, 1978; *T. repens,* Maldonado, 1985) have established that defoliation which removes shoot apices will delay heading considerably because basal axillary buds on the shoot then require further time to develop into inflorescences. Moreover, those legumes are a long day plant and late defoliation during reproductive phase will push the flowering period into times of decreasing day length. This shift of flowering will result in reduced floral initiation and reduction in most yield components (Thomas, 1961; Clifford, 1979**a**). No flowering occurred in zigzag clover after late cutting (after flowering), while in white clover (cv.Huia), about 50% reduction in seed yield is also reported (Clifford, 1979**a**).

In order to achieve a more uniform and contracted flowering pattern, several defoliation treatments have been investigated in *Lotus corniculatus* (Anderson and Metcalfe, 1957; Bader and Anderson, 1962). However, none of these have been successful in achieving higher seed yield compared to uncut controls in all circumstances.

2.9.3. Harvesting

To achieve high seed yield, correct harvesting timing is essential in any seed production management system. Because of the extended flowering period from October to January (Dear and Zorin, 1985; Gurung, 1991) and the high possibility of pod shattering (Khoroshailov and Federenko, 1954), it is difficult to define the proper time for seed harvest since varying proportions of buds, flowers and immature seeds, ripening and shattered pods are present. This problem is typical in indeterminate clovers. Thompson (1979) suggested practical parameters commonly used to identify the best time of harvesting. These area first, when the number of seed heads are greater than at any time during the growing season, and second, the colour and consistency of the seed.

Optimal times for the harvest of herbage legumes seeds can be determined by monitoring the changes which occur during seed development (*e.g.* seed moisture content, seed weight and pod colour). Studies on several herbage legumes have shown that maximum viability was reached 4-7 days before seeds reached physiological maturity (Wiggans *et al,* 1956; Hyde *et al.,* 1959; Win Pe, 1978; Kowithayakorn and Hill, 1982; Hare and Lucas, 1984**a**). This suggests that in order to obtain high seed yield it is not necessary to wait for harvest until all pods fully dry and dark brown. Mature seeds may have been developed in dark green pods and light brown seeds and these seed pods do not easily shatter under a dry environment. Optimising harvest time is, therefore based on the compromise between the presence of immature pods containing seeds which are still non-viable, of low weight and quality, and losses of seed numbers due to shattering.

Hare and Lucas (1984**a**) suggested that three basic pieces of information should be available in estimating optimum harvest time, such as the time required to achieve maximum dry weight, changes in seed moisture content in relation with external flower appearances and the onset of seed shattering. In *Lotus corniculatus,* after several years of experience, Lanchashire *et al.* (1980) indicated that the seeds can be harvested when 70 to 80% of the pods have turned brown. Hare and Lucas (1984**a**) pointed out that the optimum time to commence harvesting in maku lotus was 2 to 4 days after seed physiological maturity (maximum dry weight) when the seeds had 35% moisture content, the pods were light brown, and 3 to 4% pod shattered. This occurred about 30 to 40 days after pollination. Lanchasire *et al.* (1980) suggested that to reduce seed loss due to shattering, it is advisable to cut the crop in the evening, night or early morning while the dew is present. As environmental conditions vary with time and sites, the problem is to identify developmental stages reliably.

2.9.4. Other cultural practices

To clarify matters already discussed in the previous sections, several practices can be integrated to obtain high seed yield in combination to those mentioned before. Though *T. ambiguum* grows well under low soil fertility, application of nutrients and fertilizer can improve seed yield. Introduction of insect pollinators into seed field is the most effective means of increasing seed set and seed yield (Bryant, 1974). In warm season a considerable improvement to seed set could be achieved by maintaining about 10 to 12 beehives per hectare during the dense spring flowering period. Also a higher seed yields are obtained from plant growing under irrigation especially during flowering (Clifford, 1986), especially after defoliation to enhance its recovery. Moisture stress has been reported to affect seed set and pod-filling in *Lotus corniculatus* (Lancashire *et al.,* 1980), so irrigation at this critical stage may be necessary.

2.10. Concluding remarks

Trifolium ambiguum is an example of a plant resource whose potential benefit for pasture establishment has only begun to be assessed. It is a genetically and morphologically heterogenous species and is adapted to a wide range of environmental conditions as well as tolerant of many climatic and edaphic extremes where domesticated or traditional legumes species lack persistence. Its value in erosion control and revegetation has already been demonstrated and two cultivars have been developed specifically for this purpose *e.g.* cv. Summit (diploid) and Treeline (tetraploid) (Bryant, 1974).

Several lines and cultivars has displayed definite potential. Among these lines, cv. Monaro and Kirovakan (both hexaploid) and cv. Alpine and Forest (diploid) are potentially promising (Stewart, 1979; Dear and Zorin, 1985; Gurung, 1991). Although early establishment is low and early herbage production is poor, the long term productivity of *Trifolium ambiguum* has been demonstrated to be satisfactory, but further investigations still need to be done in an extended field trials.

The important thing should be noted is that despite its demonstrated agriculture value, *Trifolium ambiguum* has been reported to be a poor seed yielder. As a result, its further acceptance and its wider use both by growers or even researchers is currently restricted by the lack of reliable seed supplies. Unfortunately, the likely causes of such problem have not been identified in any detail. Seed production is the final stage in a sequence of plant processes which are controlled by genetics, environmental factors and often by the growers. Humpreys (1978) mentioned that the potential seed yield of forage legume crops is often limited by the characteristics of the plants themselves such as long sequence of inflorescence production, uneven seed ripening, and dropping or shattering of pods/seeds. Such characteristics can lead to seed harvesting inefficiency, since only small proportion of the total potential seed yield is available for recovery at one harvesting. However, with increased knowledge and understanding of those morphology and physiological processes which control seed yield and all their attributes, its suitable management could be developed in the near future.

CHAPTER 3

PRELIMINARY STUDY OF VEGETATIVE AND REPRODUCTIVE MORPHOLOGY OF CAUCASIAN CLOVER

3.1. INTRODUCTION

As vegetative growth is a prerequisite for subsequent reproductive growth, the relationships between these two characteristics have an important role in determining the final seed yield of a crop. In most clover species which have an indeterminate growth habit, conditions favourable for vegetative growth are often not the same as those which favour reproductive growth (Lorenzetti, 1981). This growth habit results in plant flowering over an extended period. Consequently, during flowering, young flower buds, fully open flowers, young pods and ripe pods ready to dehisce can be present simultaneously on an individual plant. This causes poor recovery of seed yield and low quality. Moreover, the dehiscent nature of the pods means that while immature pods are still developing, mature pods may already suffer heavy losses of seed from shattering before harvesting time. Therefore it is practically impossible to recover all the potential harvestable seed yield because a certain amount of seed is unavoidably lost either due to many immature pods being harvested or due to seeds being lost through pod dehiscence (Seaney and Henson, 1970; McGraw and Beuselink, 1983; Mc Graw *et al.,* 1986).

Another physiological reason suggested as a general limitation to obtaining high seed yield in most clover species is that assimilate partitioning rates to reproductive growth are often very low. The partitioning of assimilate among aerial plant components has been studied in several legumes *e.g.* in *Lotus corniculatus* (Beuselink, 1983; McGraw *et al.,* 1986), alfalfa, red clover and ladino clover (Smith, 1962; 1964; Greub and Wedin, 1971). In *L. corniculatus* McGraw *et al.*

(1986) found that even when plants were grown in a good environment for seed production, only about 12% of available assimilate was translocated to the seeds. This low partitioning to seed is caused by low partitioning to reproductive growth and small seed size in relation to other organs (McGraw and Beuselink, 1983). This suggests that competition between reproductive and vegetative components as well as between reproductive parts can seriously affect final seed yield.

Abortion has also been commonly reported to occur in legumes at all stages of reproductive development. A review in *L. corniculatus* by Seaney and Henson (1970) mentioned that only about 40% of the 20 - 70 ovules in an ovary develop into mature seeds. In addition, Stephenson (1984) found in the same species that only one out of three flowers produced a mature fruit and that three of every five which initiated fruits aborted. He suggested that a lack of assimilate and strong competition between developing inflorescences rather than pollination failure was responsible for the low reproductive output in this species. Atwood (1943) suggested that the abortion of some developing seeds might also arise as a result of competition for nutrients within the inflorescence. Whether such problems occur in *T. ambiguum* is still unknown, but obviously, this does not favour high seed production (Li, 1989; McGraw and Beuselink, 1983).

Thomas (1987) suggested that in an indeterminate plant, a long flowering period may be caused by any one of a number of factors including : (1), the sequential development of different shoot orders (*i.e.* main shoots *vs.* lateral shoots/branches), (2), the delayed development of flowers on a long single stem/stolon in which there are several vegetative nodes between successive flowers (*e.g.* white clover), and (3), the sequential development of shoots originating at different times which may also be responsible for creating a long flowering period. In this situation, early shoots are usually the first to flower because of earliness of formation and often larger size (*e.g.* in maku lotus: Li, 1989), while flowering in late-formed shoots is delayed resulting in a long flowering duration. Whether such problems occur in Caucasian clover is not yet known, there having been no detailed study on vegetative and reproductive growth patterns related to seed yield in this species. To improve the harvestable seed yield in this clover, therefore, understanding crop growth is necessary before recommending the most suitable management practices which encourage both vegetative and reproductive growth to allow the attainment of maximum seed yield. The present study was carried out to obtain information on vegetative and reproductive growth morphology of Caucasian clover (cv. Monaro) and their likely contribution to final seed yield.

3.2. MATERIALS AND METHODS

3.2.1. Experiment site and area

This experiment was carried out in one growing season on a four year established stand of Caucasian clover (cv. Monaro) from September 1991 to March 1992 on the AgResearch Grassland Unit, Aorangi, Palmerston North, New Zealand. The soil type is Fine Sandy Loam with moderate fertility. Details of prevailing temperature, day length/sunshine hours and wind speed were obtained from climatic data ex AgResearch Grassland Division, Aorangi, Palmerston North which were recorded at the trial area (**Appendix 3.2**).

Since some plots in the field were seriously infested by weeds (*e.g.* white clover, red clover, a range of grasses and broadleaf weeds), on 12 November 1991 plots were sprayed with the herbicide "Fusilage" (chemical group : carboxylic acid derivative) at the rate of 2.4 litres/ha in 240 litres of water per hectare. The purpose of this application was to control the growth of annual and perennial grasses. On 2 November 1991, herbicide "Asulox" (chemical group: carbamate) at the rate of 1.5 litres/ha in 150 litres of water per hectare was also sprayed to control red and white clover growth. Spot spraying was carried out to avoid harmful effects to caucasian clover. Hand weeding was also carried out anytime when necessary during field observations. To avoid a high variation in results, samples were not taken from areas which were badly infested by weeds (see **section 3.2.2.3**). No irrigation, fertilizer and pollinator introduction was applied during this study.

3.2.2. Field observations and measurements

3.2.2.1. Aerial components

At the beginning of the experiment (15 October 1991), the sward was inter row cultivated with a 60 cm row spacing (**Plate 3.1**). In perennial legumes there is a close relationship between plant density and subsequent seed yields. As has been discussed in **section 9.2.1**, *T. ambiguum* is a rhizomatous legume which have produced very dense rhizomes over four years. The idea behind this practice is that the reproductive stems/shoots should be encouraged to grow as it is these stems which become the main seed bearing stems. However, if too many shoots are produced, many of these stems either remain vegetative or fail to branch and then produce few umbels per unit area due to competition for space, light and nutrient (Hare, 1983). In Maku lotus, Hare (1983) found that the main crowns appear to produce a higher proportion of reproductive stems than from rhizomes further away from the crown and inter-row cultivation will encourage the main crown to develop more reproductive stems. Inter-row cultivation techniques have never been studied in *T. ambiguum*, but have been reported to improve seed

Plate 3.1. Inter-row cultivation on 15 October 1991 with 60 cm gap between rows

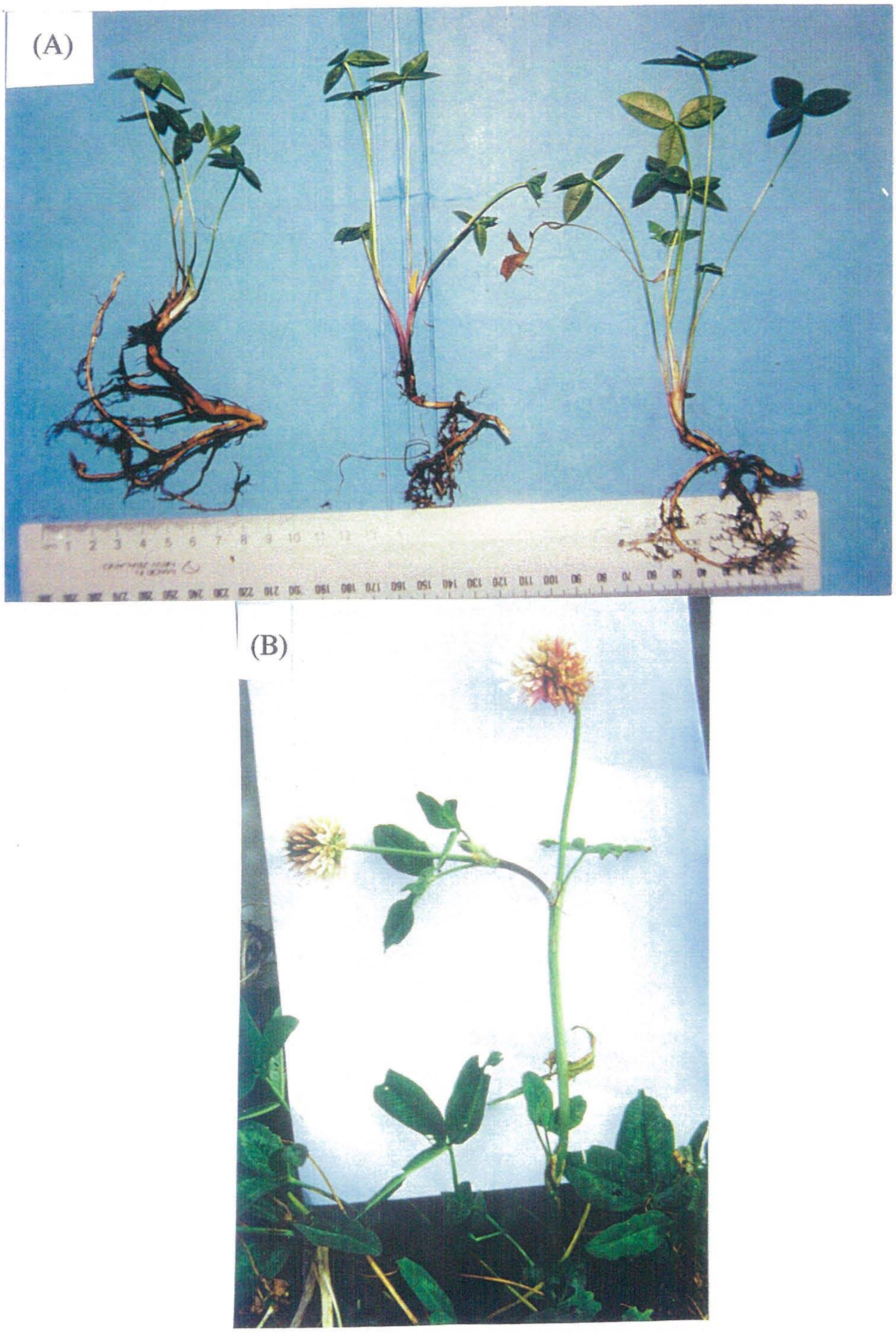


Plate 3.2. The morphology of vegetative shoots (A) and reproductive shoot bearing inflorescences (B)

(A)

(B)

Plate 3.3. **The morphology of main crown with woody taproot and rhizomes (A) and (B) different sizes of secondary crowns.**

yield second year of Kent wild white clover by 23% (Lewis *et al.*, 1984) and has been adopted as part of management practices to improve seed yield of white clover in New Zealand (Clifford, 1977; 1985). As *T. ambiguum* has similarity in terms of growth habit with Maku lotus, it is expected that inter-row cultivation will give a higher seed yield by producing more reproductive shoots and inflorescences from main crowns per unit area.

The area was divided into subplots for destructive and non-destructive sampling (**Appendix 3.1**). The growth cycle covered the period September 1991 to March 1992. On each plot a grid pattern of 0.5 X 0.5 m was randomly allocated in the rows (Total 5 grids) and sampled every two weeks to provide material for destructive aerial component analysis. Each sample was cut to ground level and then each component was separated into different categories of vegetative and reproductive parts such as the number of vegetative and reproductive shoots, the number of inflorescence buds, open flowers and mature flowers, the number of leaves and leaf area index and the dry matter of each aerial component.

There were several types of shoots which can be separated on size, morphology and position on the plant such as :

(1), *Crown shoots,* a shoot arising from anywhere on the stem tissue of the crown.

(2). *Rhizome shoots/daughter shoots,* a shoot developing from a rhizome axillary or terminal apex position at or below the soil surface associated with nodal rooting with its apex emerging above the soil surface.

(3). *Reproductive shoots*, a shoot arising from the side of a crown. This shoot/stolon can be easily distinguished from other types of shoots from its length and the development of nodes and internodes as well as its visible inflorescence buds in the early reproductive stage.

The division of plant parts used during dissection in this study are represented diagrammatically in **Figure 3.1** and visually in **Plates 3.2.** and **3.3.**

The Leaf area index (LAI; m^2 leaf/m^2 ground area) was measured by counting the total number of expanded green trifoliate leaves of the cut sample and then a sub-sample of 40 trifoliate leaves were taken and leaflets individually plucked from the petiole and the leaf area then determined on a "Hayashi" photocell planimeter. From these measurements, the total green leaf area of the cut sample and hence leaf area index were measured. Dry matter determinations for aerial components were carried out by drying the material in an air drying oven at 80^0C for 24 hours.

3.2.2.2. Underground components

On the grid position where aerial component samples were taken (**section 3.2.2.1**), a sod sample of 25 X 50 X 20 cm was dug after each cut to provide material for underground component analysis (total 5 sods/sampling date). Each sod was individually soaked in the water for at least 30 minutes, washed free of soil and then disentangled. Within each sod sample, the underground components consisted of primary crowns and taproots, secondary crowns and taproots, rhizomes and fibrous roots. *Primary crown* is the dominant crown with strong and woody taproot system in a multi crown plant or a composite organ consisting of an aggregate of stem bases (crown) and an associated primary taproot, while a *secondary crown* is

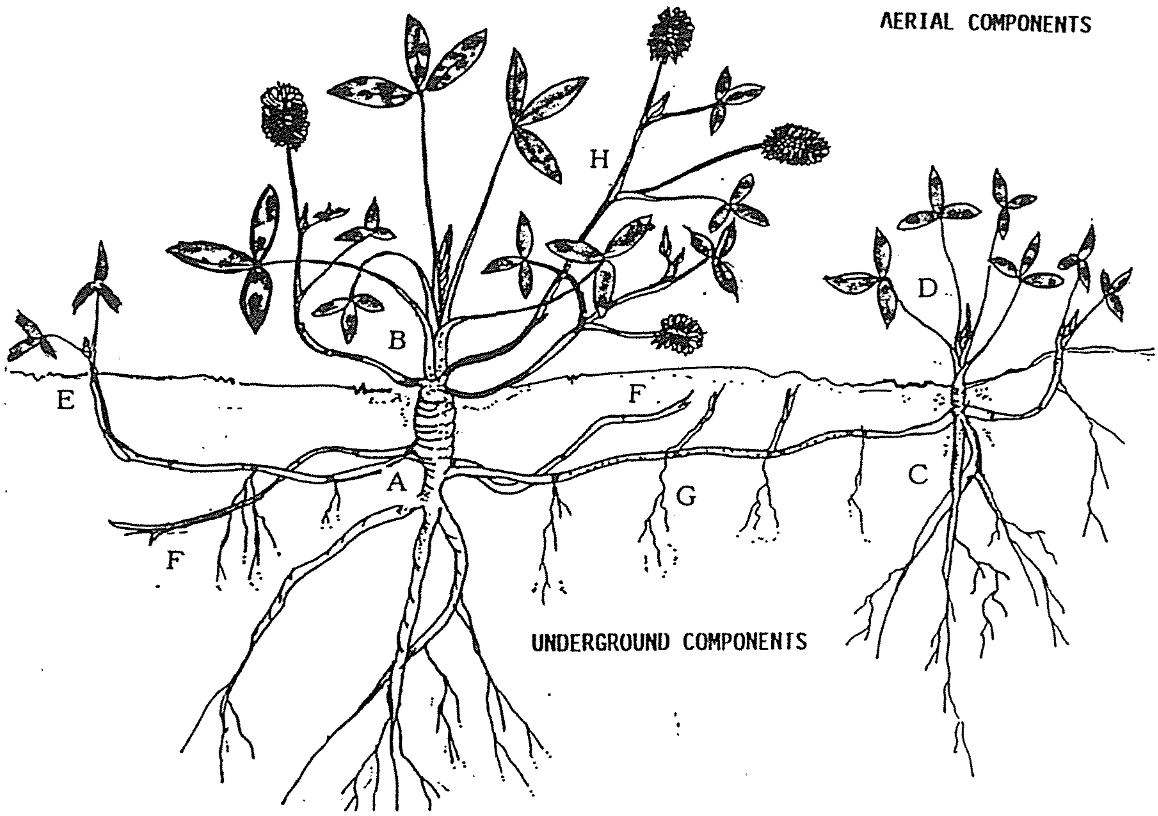


Figure 3.1. The diagrammatic representation of the morphology of Caucasian clover (*Trifolium ambiguum, M.Bieb*)

The division of plant parts :

A. Primary crown and taproot
B. Primary crown shoot
C. Secondary crown and taproot
D. Secondary crown shoot
E. Rhizome shoot
F. Rhizome shoot initial
G. Rhizome and fibrous root
H. Reproductive shoot and inflorescence

a small crown developed from a swollen node within the rhizomes system at which a woody root system develops. Drying all underground component for dry weight determination was also conducted in an air oven at 80^0C for 24 hours.

To minimize possible sampling effects on remaining plant growth, sod samples were taken alternately (detailed strategy see **Appendix 3.1.**). There are 22 quadrants one of which was randomly taken every two weeks. To minimize sampling variation, only quadrants which had no serious weed infestation were taken for sampling (about 11 quadrat samples were taken). Another quadrat was used for seed yield analysis at harvest (see **section 3.2.2.3**). Unless specifically stated, plant component data were considered on a per 1m row basis.

3.2.2.3. Reproductive growth determinations

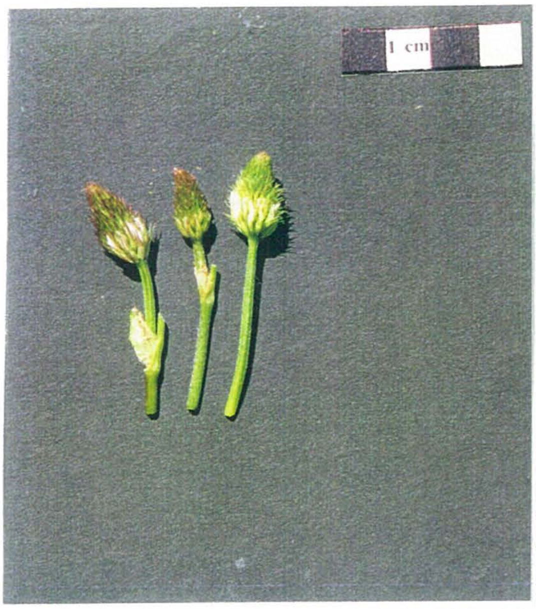
(1) Flowering pattern

The start of flowering, flowering duration, peak flowering and completion of flowering were observed on 5 permanent quadrants of 2 X 1.5 m rows by counting the number of newly opened inflorescences every 5 days. Generally, it took 5 days for flowers to fully open. These inflorescences were mainly white when fully open but later changed to predominantly pink within 5 days. This was used as a check to prevent double counting. Another check was done by tagging 5 to 10 inflorescences which had been counted and had their florets open and the development stage of these flowers checked when the next counting was undertaken, so that flowers at a similar stage could be ignored in new counts.

(2) Study of reproductive abortion

The number of visible floret buds and the number of florets per inflorescence in specifically allocated areas of the nondestructive sampling areas of 5 subplots (see **Appendix 3.1**) were recorded to assess the importance of reproductive abortion at different floret or pod development stages. On 1 November, 20 November, 10 December 1991 and on 1 January 1992, 15 visible buds/subplot emerged at different times and different positions (*i.e.* primary crown *vs.* secondary crown) (total 15 X 5 X 4 X 2 = 600 inflorescences) were randomly identified in the allocated plots by tagging their peduncles with different coloured plastic wires. The morphology of main crowns and secondary crowns is presented in **Plate 3.3**. This overall tagged population of approximately 600 inflorescences was used to determine the time required for the development of inflorescences emerging from different times and positions, from bud stage to open flower and maturity and to examine the changing pattern of each seed yield component.

The number of floret buds per inflorescence were counted from 3 inflorescences/subplot (15 buds) taken on each tagging date (total 3 X 5 X 4 X 2 = 120 buds), while the number of florets per inflorescence was measured from 15 open inflorescences obtained when more than 90% of tagged inflorescences were blooming (about 10 to 17 days from bud stage) (total 15 X 4 X 2 = 120 inflorescences). After all tagged inflorescence became dark brown (about 30 to 40 days after blooming stage), 15 inflorescences per treatment were taken for determination of pods per inflorescence (total 3 X 5 X 4 X 2 = 120 ripe inflorescences).



(A)

(B)

(C)

Plate 3.4. **General appearance of inflorescences in the bud stage (A), the opening stage (B) and the ripening stage (C)**

All inflorescences in each treatment were pooled and the number of seeds per pod was counted from 4 X 100 pods randomly taken from these inflorescences . It was found that some pods had no seed, one or a maximum two seeds. The percentage of pods falling in each of these three categories was calculated in each treatment. Seed weight was recorded on a-1000 seed weight basis adjusted to a seed moisture content of 10%. This was carried out by measuring 4 replicates of 100 seeds per treatment.

(3) Seed yield and yield components at harvest

Measurement of seed yield components, potential harvestable seed yield and actual seed yield was carried out from inflorescences obtained at harvest in the remaining destructive sampling quadrants which were not seriously infested by weeds (see **section 3.2.2.2** and **Appendix 3.1**). A visual assessment was made to determine harvest timing based on the percentage of mature inflorescences in the plot. Mature inflorescence were identified by the brown colour of the pedicles/seed heads (see **Plate 3.5**) and harvest date was decided when more than 90% of the inflorescences were mature (approximately 34 days after peak flowering). In each subplot, a quadrat of $0.25m^2$ (total 5 quadrants) was taken for determination of seed yield components and seed yield per 1m row. On each replicate, the number of inflorescences were separated into unripe inflorescences including buds and open inflorescences (pink/white or light brown) and mature inflorescences (dark brown). These total inflorescences were used to calculate potential seed yield (PSY), and only ripe inflorescences were used to obtain potential harvestable seed yield

(PHSY). After harvesting, samples from each replicate were kept in paper bags and dried at room temperature for 7 weeks. Ten ripe inflorescences were randomly taken from each replicate (total 5 X 10 inflorescences) to calculate the number of pods/seeds per inflorescence. The number of seeds per pod was counted from 100 pods per replicate (total 5 X 100 pods) dissected from these inflorescences.

Seed yield per unit area was calculated from the seed yield components recorded at harvest according to the following formula : Seed yield = P X E X N X S

Where:

P = the number of inflorescences/unit area

E = the number of florets/inflorescence

N = the number of seeds/floret

S = individual seed weight

The remaining harvested inflorescences per replicate were then hand threshed and total actual seed yield per 1m row was measured. Sample of 10 X 100 seeds per replicate were counted and weighed. Thousand seed weight and seed yield were expressed at an adjusted moisture content of 10%.

3.2.3. Statistical analysis

Data for most characters were analyzed by calculating standard errors of means ($\pm$SE) and t-test. The SAS computer program (1991) was also employed in seed abortion studies to calculate Least Significant Differences ($LSD_{0.05}$) where analysis of variance (ANOVA) indicated significant differences at $P < 0.05$ level. Graphs were prepared using The Quattro-pro program (1991).

3.3. RESULTS

3.3.1. Meteorological conditions

In the 1991/1992 growing season, temperatures were warmer than the 60 year average during the early part of the season (August- September), but temperatures were cooler than average for the rest of the season (October - January), with the exception of January (**Appendix 3.2**). Similarly, the number of sunshine hours was lower than average throughout the season, except in October. Apart from November, rainfall throughout the trial period was generally lower than average (**Appendix 3.2**).

3.3.2. Shoot system, morphology and plant growth

Caucasian clover has a vegetative shoot comprising several petioles arising from the crowns and nodes immediately above the crown or from the rhizome nodes. All vegetative shoots behave similarly in terms of size and growth, so they are termed as vegetative shoots or daughter shoots in this study (**Plate 3.2A**). Each shoot usually bears no nodes or branches and the height of vegetative shoot is determined by the length of its petioles. The general appearance of a vegetative shoot is much shorter than the reproductive shoot/stem. The performance of vegetative and reproductive shoots is illustrated in **Plates 3.2 A** and **B**. In Caucasian clover, there are no lateral structures which could substantially influence flowering pattern and the length of the flowering pattern is determined almost entirely by the numbers of reproductive shoots produced at certain times (**Figures 3.5 and 3.6**).

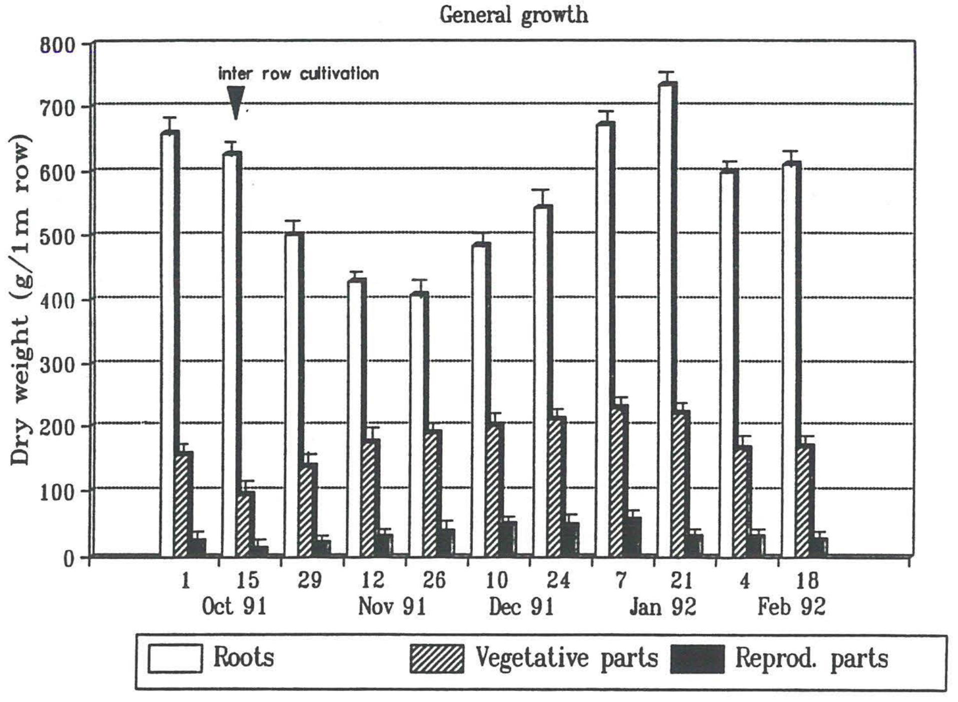


Figure 3.2. Dry matter accumulation in vegetative and reproductive components of Caucasian clover (cv. Monaro) during 1991-1992. Data presented are means of five replications. Vertical bars represent SE's calculated for individual means

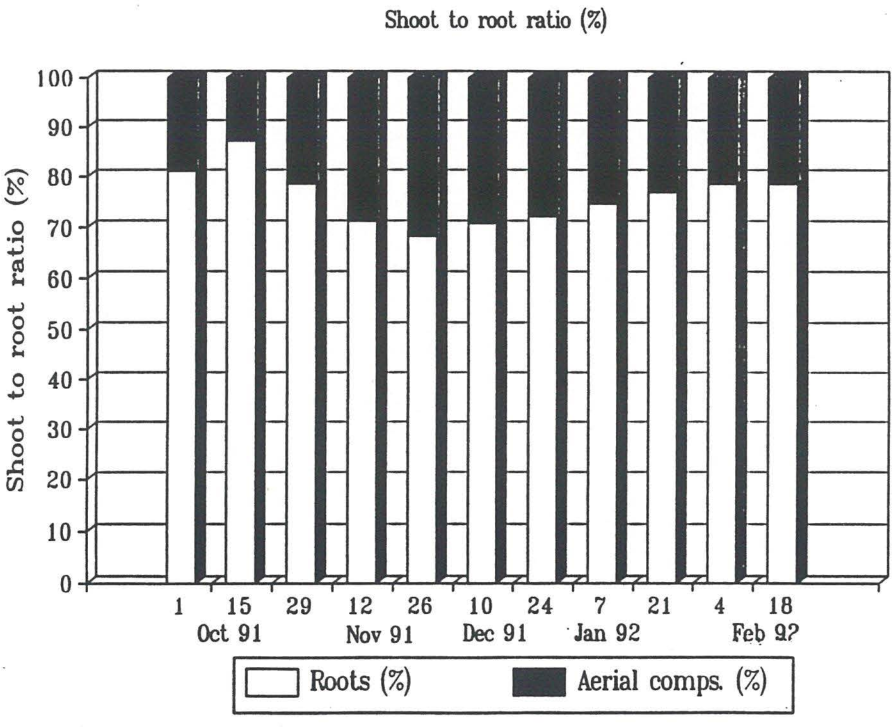


Figure 3.3. Shoot to root ratio of Caucasian clover (cv. Monaro) on each sample date during 1991-1992. Data presented are means of five replications

At the beginning of this experiment, substantial growth occurred and a small number of inflorescences were produced before inter-row cultivation was done on 15 October 1991 (**Figure 3.2; Plate 3.1**). A breakdown of growth of the aerial components shows that vegetative shoots are the main contributors to the increase of dry matter. Dry matter of vegetative parts between the first sampling date following cultivation on 15 October to 26 November 1991 increased rapidly at a growth rate of 15.9 g/1m row/week (**Figure 3.2**). However, in the same duration, a reduction in dry matter of underground components was observed following inter-row cultivation. However, from 26 November 1991 to 21 January 1992, a linear growth rate of 40.8 g/1m row/week was achieved in underground components. Root dry matter was comparatively constant at the beginning and the end of the experiment.

Reproductive components (including inflorescence buds, open flowers, green pods and mature pods) represent less than 7% of the total dry matter of aerial and underground parts, and less than 20% (*e.g.* 10 November) of total aerial components throughout the season (**Figure 3.2**). This Figure also shows that the maximum dry weight of reproductive parts was achieved about 12 weeks following inter-row cultivation (10 December) with total weight of 54.2 g/1m row. It was observed that flowering did not terminate the vegetative development of plant shoots. When reproductive shoots bore inflorescences new vegetative shoots continued to emerge throughout the reproductive season. Such phenomena defines Caucasian clover as a plant with an indeterminate growth habit.

The proportion of plant components above ground to below ground throughout the season is presented in **Figure 3.3**. Inter-row cultivation reduced shoot-root ratio from 1:4.2 down to 1:6.7 (**Figure 3.3**). The proportion of below the ground components decreased as the plant produced stolon and shoots. At 6 weeks after

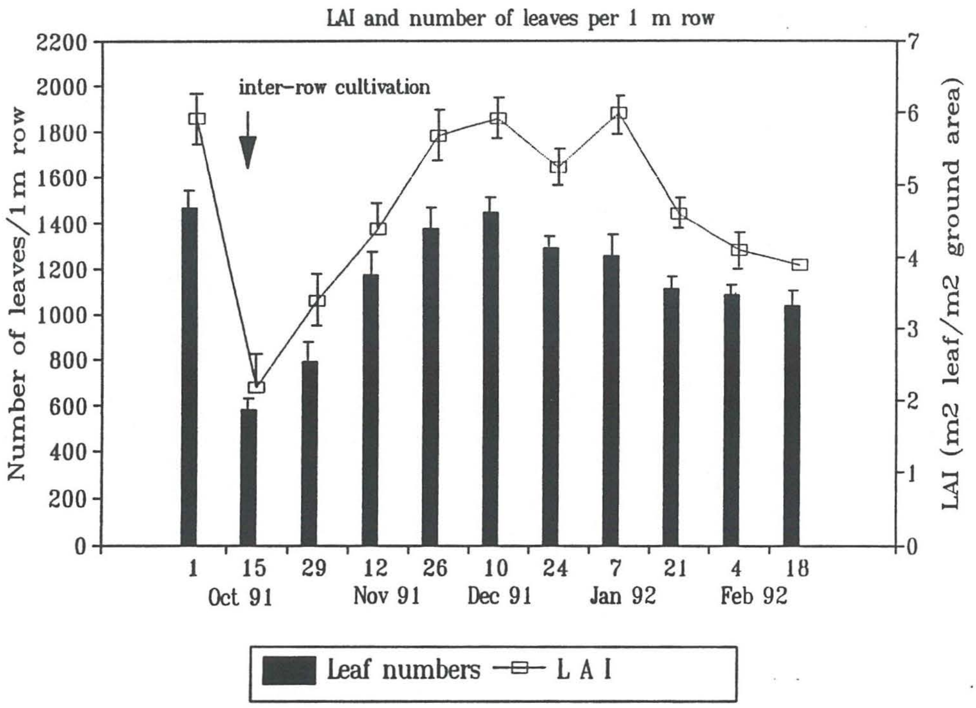


Figure 3.4. Leaf area index (LAI) and the number of leaves of Caucasian clover (cv. Monaro) on each sample date. Data presented are means of five replications. Vertical bars represent SE's calculated for individual means

cultivation the highest shoot-root ratio was achieved at 1:2.1 (**Figure 3.3**). Towards the end of the season, the proportion declined to about 1:3.5 (**Figure 3.3**).

The means of the two-weekly leaf area indices (LAI) data and the number of expanded green trifoliate leaves are presented in **Figure 3.4**. Before inter-row cultivation, a closed canopy had been achieved and cultivation reduced the LAI from 5.9 to 2.2 (**Figure 3.4**). The maximum LAI was restored after about 6 weeks of regrowth following cultivation from mid October to the end of November. This increase was associated with an increase in the number of leaves at the rate of 132.3 leaves/1m row/week (**Figure 3.4**). This figure also shows that LAI tends to decline from early January towards the end of the 1991/1992 season.

3.3.3. Reproductive shoots and flowering pattern

The production of reproductive shoots at each sampling date is shown in **Figure 3.5**, while the proportion of reproductive shoots originating from main and secondary crowns at peak flowering is presented in **Table 3.1.** It is clearly shown that inter-row cultivation reduced the number of reproductive shoots from about 88 to approximately 32 shoots/1m row and that these numbers recovered rapidly within 8 weeks following cultivation (the end of December). From early January to mid February, the number of reproductive shoots began to decline as these shoots possibly attained maximum inflorescence production and wilted (**Figure 3.5**). **Table 3.1.** shows that 64% of reproductive shoots emerged from main crowns with an average of 7.4 reproductive shoots/crown. Secondary crowns contributed around 36% of total reproductive shoots with only 1.7 reproductive shoots/crown.

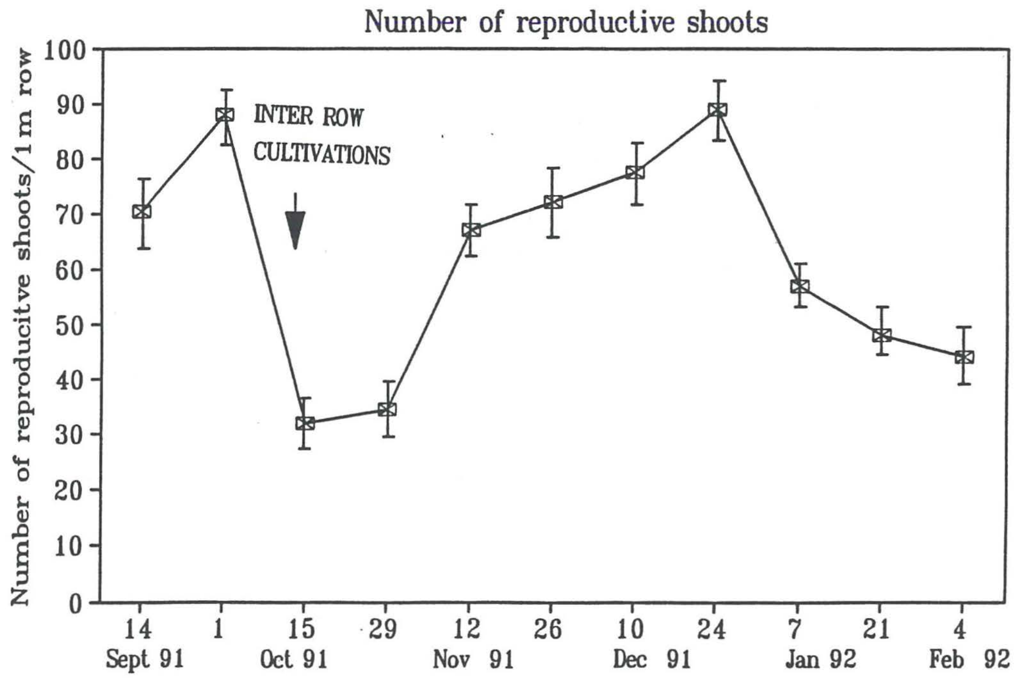


Figure 3.5. Number of reproductive shoots of Caucasian clover (cv. Monaro) on each sample date during 1991-1992. Data presented are means of five replications. Vertical bars represent SE's calculated for individual means

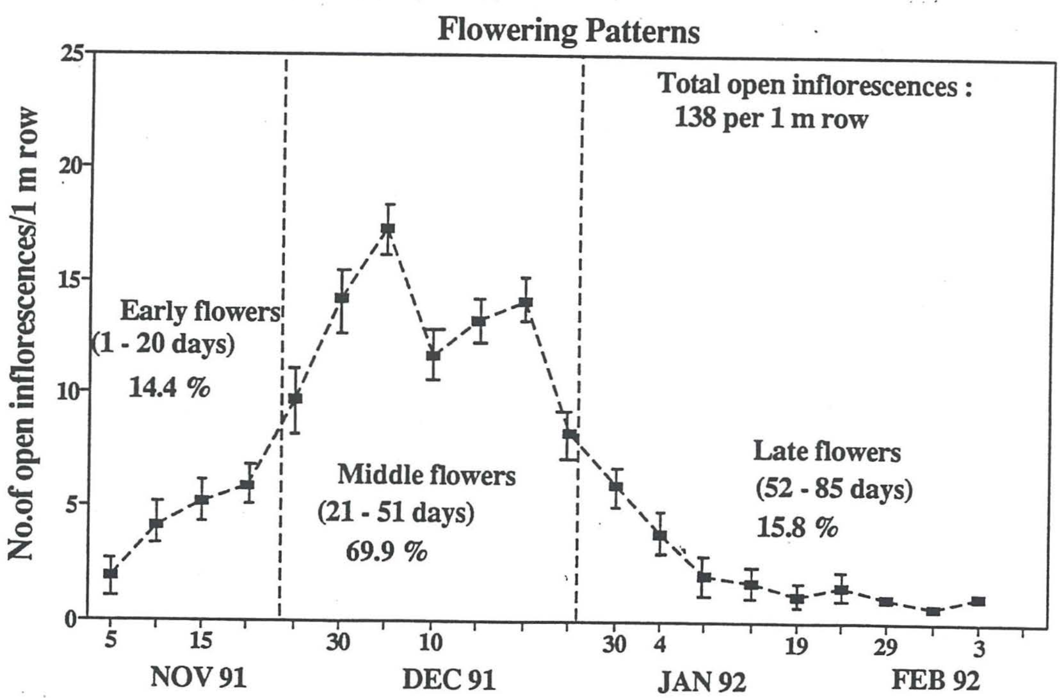


Figure 3.6. Flowering pattern of Caucasian clover (cv. Monaro) during 1991-1992. Data presented are newly opened flowers and means of five re[illegible]ations. Vertical bars represent SE's calculated for individual means [illegible] when larger than the symbols used

Although first inflorescence appearance was detected in the field in early September, the first appearance of open flower on permanent quadrat started on 5 November 1991. **Figure 3.6.** shows that Caucasian clover has two peak flowering periods on 5 December and 20 December and completed flowering in early February 1992. Although flowering occurred over a period of approximately 12 weeks, over 69.9% of total flowering occurred in a period of 4 weeks. The high rate of appearance of newly open inflorescences during peak flowering can be associated with the production of reproductive shoots (**Figures 3.5 and 3.6**). It appears that the first appearance of peak flowering was caused by the massive increase in reproductive shoots from 29 October to 12 November. Since no significant increase in reproductive shoot numbers was observed from 12 November to 10 December, it is possible that the second peak of flowering may have been caused by the simultaneous flush of open inflorescences from the later formed nodes from the same reproductive shoots. Early flowers and late flowers comprised 14.4 and 15.8% of the total flowers respectively.

Table 3.1. The number of reproductive shoots originating from main and secondary crowns at peak flowering (1m row)

Positions	No. crowns (1 m row)	No. repro ductive shoots (1m row)	No. Repro ductive shoots per crown	Proportions to total (%)
Main crown	7.6 (± 0.75)	56.6 (± 5.46)	7.4 (± 0.44)	64 (± 6.2)
Secondary crown	18.6 (±2.13)	31.4 (± 3.31)	1.7 (± 0.11)	36 (± 8.4)

Note : Data presented are means of five replicates. In brackets are standard error of individual mean (± SE).

3.3.4. Study of floral abortion

The number of floret buds per inflorescence produced from main crowns was significantly higher than from inflorescences produced from the secondary crowns (**Table 3.2A** and **Plate 3.5**). The overall mean number of floret buds per inflorescence was 110. Time of inflorescence production also had a significant effect on this figure. Inflorescences emerged on 1 November 1991 bore the highest number of floret buds per inflorescence (127/inflorescence) followed by inflorescences tagged on 20 November with 114 floret buds per inflorescences. Inflorescences tagged on 10 December and 1 January averaged around 100 florets.

By the time of inflorescence opening (about 10 to 17 days from the floret buds stage), about 90% of floret buds were retained. Inflorescences emerged on 1 November still retained the highest floret number with 115 florets per inflorescence (**Table 3.2 B**). There were no significant differences in floret bud survival of either times or positions of inflorescence production on floret primordia survival at the time of inflorescence
opening (**Table 3.2 C**).

Table 3.2. Floret buds per inflorescence, florets per inflorescence, floret bud survival, pods per inflorescence, floret survival and seeds per inflorescence of Caucasian clover (cv. Monaro) at different times and position of inflorescence production

A. Floret buds per inflorescence :

Positions	Time of inflorescence emergence				Position means
	1 Nov	20 Nov	10 Dec	1 Jan	
Main crown	139.6	129.4	112.6	97.8	119.9**a**
Secondary crown	114.4	98.6	87.9	98.6	99.9**b**
Time means	127.0**a**	113.7**b**	100.3**c**	98.2**c**	109.9

Notes : Data presented are means of 15 inflorescences; LSD (P=0.05) : Position means = 9.24; Time means =10.66; Times X Positions = NS

B. Open florets per inflorescence :

Main crown	123.8	118.6	105.8	86.5	108.7**a**
Secondary crown	106.8	78.8	84.4	86.6	89.2**b**
Time means	115.3**a**	98.7**b**	95.1**b**	86.6**b**	99.0

Notes : Data presented are means of 15 inflorescences; LSD (P=0.05) : Position means = 14.62; Time means = 12.84; Times X Positions = NS

C. Floret bud survival to open floret stage (%) :

Main crown	88.7	91.7	94.0	88.5	90.7^{NS}
Secondary crown	93.4	79.9	96.0	87.8	89.3
Time means^{NS}	91.1	85.8	95.0	87.0	90.0

D. Mature pods per inflorescence:

Positions	Time of inflorescence emergence				Position means
	1 Nov	20 Nov	10 Dec	1 Jan	
Main crown	62.8	82.6	92.6	52.8	72.7**a**
Secondary crown	57.6	55.6	69.2	65.8	62.1**b**
Time means	60.2**b**	69.1**ab**	80.9**a**	59.3**b**	67.4

Notes : Data presented are means of 15 inflorescences; LSD (P=0.05) : Position means = 6.72; Time means = 11.94; Times X Positions = NS

E. Open floret survival to mature pod stage (%) :

Main crown	50.7	69.6	87.5	61.0	67.2[NS]
Secondary crown	53.9	70.6	82.0	76.0	70.6
Time means	52.3c	70.1**b**	84.8**a**	68.5**b**	68.9

Notes : Data presented are means of 15 inflorescences; LSD (P=0.05) : Position means = NS; Time means = 8.9; Positions X Times = NS

F. Seeds per inflorescence :

Main crown	66.9	96.8	108.4	63.5	83.9**a**
Secondary crown	63.4	63.7	74.2	72.6	68.5**b**
Time means	65.2**b**	80.3**ab**	91.3 **a**	68.0 **b**	76.2

Notes : Data presented are means of 15 inflorescences; LSD (P=0.05) : position means = 13.92; time means = 11.82; Times X Positions = NS.

Notes (for A, B, C, D, E and F) :

1. Values in time mean rows or position mean column followed by the same letters are not significantly different according to LSD test (P=0.05).
3. NS means no significant differences.

The percentage of open floret survival to the mature pod stage was counted based on the number of pods per inflorescence at the maturity stage compared to the number of florets at the opening stage and the results are presented in **Tables 3.2 D and E**. It is clear that inflorescences produced on shoots originating from the main crown bore significantly more pods than secondary crown inflorescences. **Tables 3.2 E** and **F** further show that the percentage of pod set (floret survival), pod numbers as well as seed numbers of middle inflorescence produced near peak flowering (10 December) was the highest compared to other times of inflorescence production with 84.4% of florets developing pods. Inflorescences produced in January had the lowest percentage of floret survival and pod numbers with 68% and 59.2 pods per inflorescence respectively. There were no significant differences in floret survival between inflorescences produced from main crowns and secondary crowns.

Percentage of empty pods, one seeded pods, two seeded pods, seeds per inflorescence, thousand seed weight and seeds per pod were counted and presented in **Tables 3.3 A, B, C, D,** and **E**. Apart from the effect on seed per inflorescence, the position of inflorescence production had no effect on all the parameters measured. It is also shown that both early flowers (1 November) and late flowers (1 January) had a significantly higher percentage of empty pods than middle flowers (20 November and 10 December). The overall means of percentage of empty pods was 16.4%. Similar trends was also found in the effects of time of inflorescence production on percentage of pods bearing two seeds (11.4%). Both early and late flower groups bore significantly higher percentages of two seeded pods (**Table 3.3 B**).

Times of inflorescence production had significant effects on the number of seeds per inflorescence (**Table 3.3 B**). The total number of seeds per inflorescence was significantly higher in main crowns than those produced in secondary crowns. As inflorescences produced on 10 December had the lowest percentage of empty pod, these inflorescences also had the highest number of seeds with 91.3 seeds per inflorescence (**Table 3.3 B**). Neither times or positions of flower production had any effect on thousand seed weight, seeds per pod or percentage of pods bearing one seed (**Tables 3.3 C and D**).

Table 3.3. Percentage of empty pods, two seeded pods, one seeded pod, and thousand seed weight at harvest ripening at different times and position of inflorescence emergence in 1991-1992

A. Percentage of empty pods (%) :

Positions	Time of inflorescence emergence				Position means
	1 Nov	20 Nov	10 Dec	1 Jan	
Main crown	27.5	13.8	11.5	16.0	17.2^{NS}
Secondary crown	23.0	9.5	8.5	21.5	15.6
Time means	**25.3b**	**11.7a**	**10.0a**	**18.8b**	16.4

Notes : Data presented are means of 4 replicates of 100 pods; LSD (P=0.05) : Time means = 9.7; Position means = NS; Positions X times = NS

B. Percentage of pods bearing two seeds (%) :

Positions	Time of inflorescence emergence				Position means
	1 Nov	20 Nov	10 Dec	1 Jan	
Main crown	7.0	14.2	14.5	7.5	10.8^{NS}
Secondary crown	5.0	16.5	16.0	9.5	11.8
Time means	**6.5 b**	**15.4a**	**15.3a**	**8.5 b**	11.3

Notes : Data presented are means of 4 replicates of 100 pods; LSD (P=0.05) : Time means = 5.8; Position means = NS; Positions X times = NS.

C. Percentage of pods bearing one seed (%) :

Positions	Time of inflorescence emergence				Position means
	1 Nov	20 Nov	10 Dec	1 Jan	
Main crown	65.5	72.0	74.0	76.5	72.1^{NS}
Secondary crown	72.0	74.0	75.5	69.0	72.6
Time means$_{NS}$	68.8	73.0	74.8	72.8	72.4

Note : Data presented are means of 4 replicates of 100 pods.

D. Thousand seed weight (g, SMC 10%) :

Main crown	2.708	2.644	2.398	2.678	2.607 NS
Secondary crown	2.599	2.499	2.699	2.647	2.611
Time means NS	2.654	2.572	2.549	2.613	2.609

Note : Data presented are means of 4 replicates of 100 seeds.

E. Seeds per pod :

Main crown	0.8	0.9	1.0	0.9	0.9 NS
Secondary crown	0.9	1.1	1.1	0.9	1.0
Time means NS	0.9	1.0	1.1	0.9	1.0

Notes for A, B, C, D, and E : Data presented are means of 4 replicates of 100 pods randomly taken from 15 inflorescences/treatment. Values in time mean row or position mean column followed by the same letters are not significantly different according to LSD test (P=0.05); NS = no significant differences.

Plate 3.5. **General appearance of main crown inflorescences (A) and secondary crown inflorescences (B) at ripening stage**

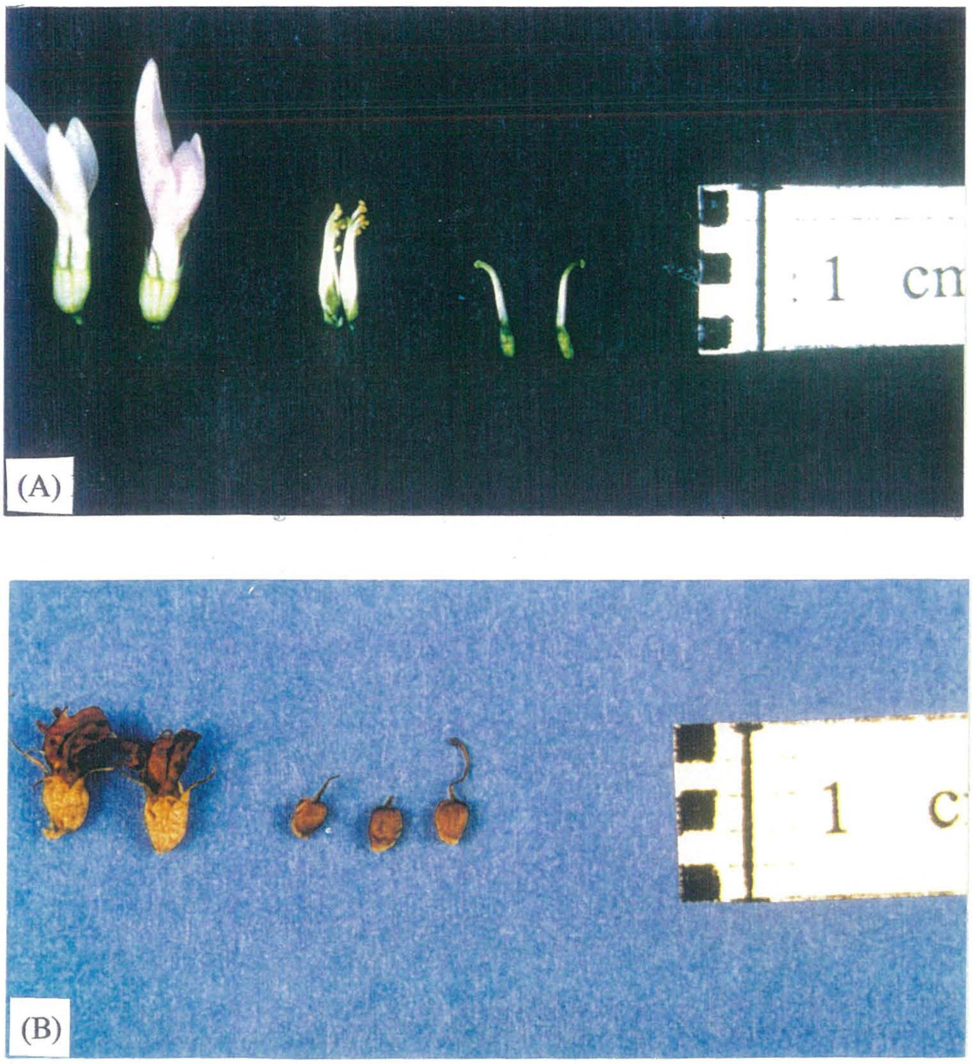


Plate 3.6. Morphology of florets and carpel containing two ovules at blooming stage (A) and florets at maturity with one seeded pods (B)

3.3.5. Seed yield and yield components

Visual assessment was used to decide harvest time in this study (about 34 days after peak flowering) and the data on seed yield and yield components at harvest are presented in **Table 3.4.** The flower composition at harvest was 78% ripe, and 32% unripe 10% of which was still in the bud stage (**Table 3.4**). From the dissection of ripe inflorescences, it was found that in *T. ambiguum* (cv. Monaro) on average 12% of total pods had two seeds, 79.5% had one seed and only 8.5% were without seed (**Table 3.4**). The average number of seeds per head was 110. *T. ambiguum* (cv. Monaro) had seed weight of 2.576 g/1000 seeds at 10% SMC. Seed yields were calculated in various ways and the results show that Monaro produced a potential seed yield of 53.6 g/1m row, equivalent to 893.3 kg/ha, while the calculated potential harvestable seed yield (PSHY) was 42.4 g/1m row (equivalent to 707 kg/ha). Actual seed yield resulted from hand threshed inflorescences was 25.6 g/1m row (426.7 kg/ha). The dry matter of vegetative matter at harvest was 167.4 g/1m row resulting in a calculated harvest index of 12.1% (**Table 3.4**).

Table 3.4. Vegetative dry matter, reproductive composition, seed yield components, potential seed yield (PSY), potential harvestable seed yield (PHSY), actual seed yield (ASY) and harvest index (HI) of Caucasian clover (cv. Monaro)

Parameters	Results	
1. Vegetative dry matters	167.4 ± 6.30** gr/1m row	
2. Reproductive distributions	Buds unripe ripe total	
	20 33 136 189 (±12.4) (±9.6) (±8.31) (±16.8)**	
3. Seed yield components :		
a. No. inflorescences/1m row	136 ± 8.31	
b. Percentage of seeds/pod	No seed one seed Two seeds	
	8.5 79.5 12 (±7.24) (±10.34) (±7.56)	
c. No. pods/inflorescence	110 ± 7.72	
d. No. seeds/inflorescence	104.7 ± 9.27	
e. T S W (g, 10%)	2.576 ± 0.007	
4. Estimated seed yield :	g /1m row	kg / ha
a. Potential seed yield (PSY)	53.6 ± 2.35	893.3 ± 39.17
b. Potential harvestable seed yield (PHSY)	42.4 ± 3.88	706.7 ± 64.67
c. Actual seed yield (ASY)	25.6 ± 4.72	426.7 ± 78.67
5. Harvest index (HI) (%)	12.1 %	

Note :
1. Data presented are means of 5 replicates.
2. Potential seed yield = P X E X N X S , Where;
   P = the number of inflorescences/1m row
   (PSY: total inflorescences; PHSY: total ripe inflorescences)
   E = the number of florets/inflorescences
   N = the number of seeds/floret
   S = seed weight
2. Harvest index (HI) = $\frac{\text{Actual seed yield (0\% SMC)}}{\text{Total dry matters (vegetative + seeds)}}$ X 100%
3. ** = Standard Error of means (±SE).

3.4. DISCUSSION

3.4.1. Shoot system and general plant growth

The morphological characteristics of *T. ambiguum* have been partially described in the botanical reviews of Komarov (1945), Hermann (1953), Hossan (1961), Zohary (1970) and Townsend (1974). However, a detailed study of the root and shoot system in relation to reproductive growth has never been undertaken. The description of plant structure in this study has shown the dominant role of reproductive shoots arising directly from the main crown, and to a lesser extent a smaller number of reproductive shoot arising from secondary crown as contributors to reproductive growth (**Table 3.1**).

The present results on dry matter distribution between reproductive growth and vegetative growth of the aerial components of the plant (Figure 3.2) agree with the results by previous workers in other legumes such as *Lotus corniculatus* (Li, 1989), *T. medium* (Robertson and Armstrong, 1964; Townsend, 1967), as well as in *T. ambiguum* (Stewart, 1979). These workers emphasized low assimilate partitioning to the reproductive growth as an important factor limiting the seed production of these legumes.

Results from this study also show that the decrease in total root dry weight following inter-row cultivation was associated with a linear increase in dry matter of aerial components during early regrowth. It appears that new leaf and existing tissue growth was obtained at the expense of organic reserves in the roots. The movement of organic compounds from the root region into formed leaves and shoots has been observed other legumes following defoliation (*e.g. T. repens, T. pratense* and *L.*

pedunculatus, Butler *et al.*, 1959) and in lucerne (Hodgkinson, 1969). Working with *T. subterraneum*, Small and Leonard (1969) estimated that 37% of carbon involved in shoot and leaf growth came from carbon compound re-exported from the root region.

The commencement of net root growth from 26 November was accompanied by a slowing down of dry matter accumulation in vegetative and reproductive parts over the same period, even though there seems to be a preferential translocation of assimilate from vegetative growth to reproductive growth, as shown by the fact that vegetative growth also slowed down. It is likely that such slowing down is mainly caused by the accumulation of assimilate in roots over the same period reducing dry matter accumulation in the above-ground components, especially reproductive parts. These results suggest that inter-row cultivation caused an increase in competition between root and reproductive parts resulting in detrimental effects on seed yields. Data taken at harvest time indicated that inter-row cultivation reduced total inflorescence numbers by around 45% compared to uncultivated crops (**Appendix 3.3**). However, as no detailed data on other seed yield components were observed, it is unclear whether this reduction is followed by changes in other components (*e.g.* seeds/inflorescence, seed weight, and seeds/pod). This needs further study in the future.

3.4.2. Shoot-root ratio

As discussed in the **section 2.6.3**, *T. ambiguum* has very extenssive root growth compared to shoot growth. The highest proportion of aerial components in *T. ambiguum* (cv. Monaro) was 32% which is similar to those reported by Spencer *et al.* (1975) in *T. ambiguum* (cvs. Summit and Treeline). As a comparison, the highest proportion of the aerial components in white clover (cv. Grassland Huia) is up to 90% (Spencer *et al.*, 1975).

3.4.3. Reproductive shoots and flowering patterns

Throughout this study, it was found that reproductive shoots produced inflorescences soon after emergence (often at the first node of the stem). Shoots emerging early, therefore contribute to the early part of the flowering sequence while shoots which emerge later are responsible for supporting later flowering causing variability in time of flower emergence. It is likely that continuing reproductive shoot production in combination with continuing inflorescence emergence on one stem is the main cause of the long flowering period.

The results from flowering pattern studies show that about 70% of inflorescences appear within a period of less than four weeks, resulting in a contracted peak of flowering within a protracted total flowering period (**Figure 3.6**). Although, reproductive shoots were produced for a long period (about five months), only reproductive shoots emerging in late October, November and December are substantial contributors to the total flower population (**Figures 3.5 and 3.6**). It appears that the first appearance of peak flowering was caused by the massive increase of reproductive shoots from 29 October to 12 November. Since no significant increase in net reproductive shoot numbers was observed from 12 November to 10 December and it took about 15 days for the second peak of flowering to occur, it is possible that this second peak may have been caused by a simultaneous flush of open inflorescences from later fromed nodes on the same reproductive shoots. Another possibility is that it may take a longer time for inflorescences from later nodes to develop as environmental conditions became unfavourable in the late season. The low inflorescence numbers during the early stages of flowering simply reflect the lack of reproductive shoots available at that time, with a decrease in the number of reproductive shoots being due to senescence resulting in low flower emergence during the late season (January and February).

Inter-row cultivation carried out on 15 October reduced the number of reproductive shoots from about 88 to 32 shoots/1m row, but, this number recovered rapidly within 8 weeks. The objective of this practice was to reduce competition between massive rhizomes and to provide enough space for main crowns to develop flowering sites and enough space for light penetration to the reproductive growing point to promote flower head development as occurred in white clover (Clifford, 1980; Thomas, 1980). The same effects might occur in *T. ambiguum*, however, it appears that cultivation had no effect on maximum reproductive shoot numbers compared to those produced before cultivation (**Figure 3.5**). As no detail data of uncultivated plants were gathered due to the amount of workload during this study, this aspect including time and method of cultivation needs further study.

The present study has highlighted the lengthy (12 weeks) flowering period in *T. ambiguum* (cv. Monaro). Experiments conducted by Stewart (1979) in cv. Treeline (hexaploid) have suggested that day length requirements are not the major factor determining floral initiation. He speculated, however, that plants observed during his study were too small and immature to flower when day lengths were condusive to flowering and could only flower when mature enough to do so. In addition, he found a highly significant correlation between mean monthly temperature and cumulative flowering percentage. It seems most likely that flowering is related to plant size or maturity and this is related to temperature and day length. Kannenberg and Elliot (1962) and Hely (1957) were able to initiate flowering of Caucasian clover in the glasshouse by subjecting plants to a 17 hour day length and a temperature range from 4.5 to 10°C at night to a daytime temperature of 21°C. The plants used in this study were from a four year established stand which was mature enough to fulfil such requirements (Stewart, 1979). This was supported by the facts that in Palmerston North during the period from the beginning of November to the beginning of February meets the day length requirement (Kannenberg and Elliot, 1962) for flower induction which agrees with the observed flowering pattern shown in Figure 3.6 where flowering began in November and finished in early February (see **Appendix 3.2**).

3.4.4. Potential seed yield components of main and secondary crowns

In this study, throughout the flowering season, the main crowns produced a significantly higher number of floret buds/inflorescence and eventually also had a significantly higher number of seed pods per inflorescence than those produced on secondary crowns. The possible explanation for this is that main crowns originate from the base of the plant which are more mature and have their own strong root system, while secondary crowns develope from rhizome nodes and do not have strong root systems. This suggestion is supported by Gavin *et al.* (**in press**) who found that seed yields in *T. ambiguum* (cv. Monaro) increased more than six fold (from 6 g/m^2 in the first year to 38 g/m^2 in the second year) as rhizomes increased plant size. They further suggested that plants in the first year did not attain enough size to produce high seed yield.

3.4.5. Likely causes of loss of potential seed yield

3.4.5.1. Bud abortion

Flower bud abortion was monitored in the present study. Results throughout the flowering season suggest that about 10% of buds produced from main crowns and secondary crowns consistently abort before flower opening (**Table 3.1 A**).The factors contributing to this phenomena are still unknown. Reproductive abortion seems to be a common feature in herbage legumes which occurs throughout reproductive development, but particularly during the very early stages of inflorescence development before flower opening. This has also been reported by many workers (*e.g.* in *Lotus corniculatus,* Giles, 1949; Bubar, 1958; Joffe, 1958; Seaney and Henson, 1970; Li, 1989; Supanjani, 1991). 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) studied the effects of possible causal factors,including boron treatment, photoperiod, and temperature, on the abortion of the flower buds of *L. corniculatus*. None of these factors were found to satisfactorily explain the cause of bud abortion. Stephenson (1984) suggested that the violability of maternal resources (assimilate supply) played a key role in regulating the abortion of inflorescences. The result of 90% of bud survival found in this study suggests that abortion prior to flower opening does not seem to be a major problem in reducing seed yields. Apart from the effects of any one of these causes, it is possible that bud abortion may be caused by a combination of some or all of these factors. Nevertheless this aspect still need clarification through further study.

3.4.5.2. Post pollination abortion

At other stages of floret development, the present study revealed that throughout the flowering season, about 70 % of the florets at the blooming stage developed live pods at maturity (about 30-35 days after open flower) but only one out of two ovules in an ovary developed seeds (**Tables 3.1E** and **3.2D**). Since *T. ambiguum* is a completely self-incompatible and self sterile plant (Bryant, 1974), it is possible that lack of pollination in some florets in the flower head may be the cause of this reduction. In *L. corniculatus*, incomplete self-incompatibility and self sterility have also been suggested to be major causes of a failure in ovule development (Silow, 1931; Seaney, 1964; Dobrofsky and Grant, 1980). They indicated that high levels of ovule abortion occur within the first 5 days following pollination suggesting that ovule degeneration can also be a possible source of seed loss. Beyond this point, relatively less seed abortion is observed. Li and Hill (1989) stated that lack of assimilate and intense competition among fertilized ovules might reduce the number of florets per inflorescence, while Pasumarty (1991) found in *T. repens* that 20% of unfertilized ovules degenerated immediately after pollination.

The percentage of floret bearing pods (70%) obtained in this study was relatively high compared to other legumes (*e.g.* 40 to 47% in Zigzag clover, Gurung, 1991) and comparable to those reported by Supanjani (1991) in *L. corniculatus* (75%). Despite the fact that the early flower group (1 November) bore more floret buds than later flowers, the results indicate that the early flower group produced significantly lower pod numbers (pod set of 51%) than those in the middle group (20 November with 70% pod set and 10 December with 85% pod set), with no differences from the latest flowers (1 January) (**Tables 3.1 D** and **E**). The high proportion of pods bearing seeds indicates that pollination does not seem to be a serious problem in *T.*

serious problem in *T. ambiguum* for high seed production. It was found during field observation that almost any types of pollinators (*e.g.* moths, butterflies, honey bees and bumble bees) visited the flowers. Unlike Zigzag clover which has a long corrolla tube (Robertson and Armstrong, 1964), *T. ambiguum* flowers have a short corolla tubes which are rich in nectar which make them very attractive to pollinators (Bryant, 1974). Therefore, inadequate pollination in this clover is unlikely to be a serious problem if the population of pollinators is high. The differences in pod set and seed set in different flower groups can be associated with pollinator activity which depends upon weather conditions during the critical flowering period. Usually warm, dry and sunny days favour bee visits to florets (Bryant, 1974; Stewart, 1979; Gurung, 1991). As the condition in the early flowering season (November) was cool (8.1 - 16.2°C; see **Appendix 3.2**), this may help to explain why pod set in early flowers was significantly lower than in later ones.

In this study, an average of 11.4% of pods containing seed were two seeded and about 16% of pods were without seed. Stephenson (1975) reported that even when each floret was outcrossed and pollination occurred, a proportion of florets still failed to develop pods and become aborted. It was found that from 100 florets observed, about 98% contains two ovules per ovary. Apparently, the second ovule was not fertilized or abortion follows soon after fertilisation as suggested by Townsend (1970). This suggests that other factors must be involved in the mechanism of abortion. One possible explanation is that, although the results suggest that lack of assimilate is the apparent cause of reproductive abortion as suggested by Li and Hill in *L. corniculatus* (1989), 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 in the early season has a major effect on abortion levels in early flowers (1 November) (**Table 3.1 D**). Similar results have been reported in *Lotus corniculatus* by Hansen (1953) and Seaney and Henson (1970) in the case of seed abortion by Stephenson *et al.* (1986; 1988) in the case of floret abortion.

3.4.7. Potential seed yield and actual seed yield

The values of calculated potential seed yield (893 kg/ha), harvestable seed yield (707 kg/ha) and hand harvested actual seed yield (427 kg/ha) reported in this study are relatively higher than those from other reports for this species (Kannenberg and Elliot, 1962; Hely, 1963; Townsend, 1970; Stewart, 1979; Stewart and Daly, 1980; Gurung, 1991). Mean actual seed yield in this clover is considerably higher than commercial machine harvested seeds recorded white clover (200-300 kg/ha) and red clover (180-250 kg/ha) (Clifford, 1985; Stewart, 1979). Even though hand threshing was carried out carefully to avoid seed loss, there was a 40% reduction in PHSY compared to ASY. This could be due to some seed loss during harvesting due to shattering problems or to the criteria used for visual assessment and calculation for ripe inflorescences may have been unavoidably overestimated. The visual assessment based on inflorescence colour is not an absolute indicator that flowers will produce mature seeds.

Potential harvestable seed yield was calculated based on the assumption that all ripe inflorescences are available and recovered during harvesting at one time. Such recovery is unlikely because of the protracted flowering behaviour and pod shattering nature of this clover. The difference between PSY and PHSY was that about 28% of total harvested inflorescences bore immature pods and seeds which were removed during processing. It seems likely in practice that only about half of the seeds produced are actually recoverable during a once over machine harvesting operation.

This idea is supported by the results of this study where only 60% of potential harvestable seed yield was recovered in hand harvested actual seed yield (427 kg/ha). Such an actual seed yield is likely to be obtainable in practise only if seed loss can be minimized during the harvesting and cleaning process. As other experiments were conducted in the same field, this limited the area for harvesting. As a result harvesting was carried out only at one time. It would be desirable, however, to assess the effects of different times of harvest on seed yield and yield components.

CHAPTER 4

SEED DEVELOPMENT STUDY IN CAUCASIAN CLOVER

4.1. INTRODUCTION

Seed recovery in clover species is often low and unreliable (Hare and Lucas, 1984**a**). The main problem occurs because of the extended flowering period which may extend over 5 months and because of problems of unpredictable pod shattering (Armstrong, 1974). Both of these problems cause subsequent difficulty in defining the most appropriate time for seed harvest as a result of varying proportions of flower buds, open flowers, immature pods, ripening pods and shattered pods on plants at harvest.

Seed development studies have been valuable in providing information to allow harvesting for both maximum quality and quantity of many different seed crops. *e.g.* White clover (Hyde *et al.*, 1959), Lotus corniculatus (Hare and Lucas, 1984**b**; Li, 1989; Li and Hill, 1989) and many other flower crops. Correct harvest timing is very important since delayed harvesting can result in much seed being lost because of shattering or shedding (Hawthorn and Pollard, 1954), while harvesting too early, particularly before seed reaches physiological maturity can reduce seed yield and quality and subsequent seed storage life (Harrington, 1972).

As in many of the traditional clovers previously mentioned, it may be difficult in Caucasian clover to determine the optimum seed harvesting time since flowering continues for a long time which in turn, resulting the presence of varying levels of seed maturity at harvest (**Chapter 3** and also Dear and Zorin, 1985; Gurung, 1991). The pattern of flower and seed development in *T. ambiguum* is not fully understood. This study investigated the sequence of seed development and changes in seed quality components in an attempt to define more closely the optimum time to harvest the crop to obtain maximum yield of high quality seeds.

4.2. MATERIALS AND METHODS

The experiment was carried out in the same field plot described in the previous experiment using four year established plants (**Section 3.2.1** and **Appendix 3.1**). This study involved investigations of the seed development sequence at two different times during the growing season. The sequence was measured by assessing changes in seed fresh and dry weight, seed viability, germinability and hardseededness using samples collected at different times after pollination. Details of prevailing daily temperature, daily rainfall, wind speed and relative humidity were also obtained from climatic data ex AgResearch Grassland Division, Aorangi, Palmerston North which were recorded at the trial area. The areas allocated for the seed development study in the experimental field are presented in **Appendix 3.1**.

4.2.1. Inflorescence tagging and sampling

The area was divided into four subplots according to the positions in the field (see **Appendix 3.1B**). On 18 November 1991 (early flowers) and 13 December 1991 (middle flowers), about 150 fully blooming pollinated inflorescences on each of the 4 subplots (total 600 inflorescences on each date) were tagged with fine plastic rings. Inflorescences were considered to have been pollinated when 50 to 90% of the florets were reflected and slightly wilted (evidence that florets have been visited by a pollinator, Hare and Lucas, 1984**a**).

4.2.2. Data collection

From 14 days after tagging (days after pollination, 10 tagged inflorescences/plot (total 4 X 10 inflorescences) were hand harvested every 4 days until 46 days after pollination and examined separately. Seeds were removed from the pods from half of each sample immediately after collection and the following measurements were made :

(1) Seed fresh weight was measured directly after dissection and dry weight and moisture percentage were determined by using the oven method at 130°C for 1 hour. All these measurements were carried out separately on each replicate by taking 100 seeds per subplot (total 4 X 100 seeds).

(2) Total viability, germination and hard seeds were also measured separately by using 100 seeds/subplot (total 4 X 100 seeds) which were germinated on top of blotting paper in a growth cabinet at 20°C without pre-chilling. Germination counts were made at 4 and 12 days with distinguishing between normal and abnormal, fresh ungerminated, hard and dead seeds. This classifications were made according to the International Seed Testing Association (ISTA) 1985. Viable seeds were referred to as the sum of normal seedlings, fresh ungerminated and hard seeds. The remaining inflorescences from each sampling date were kept in paper envelopes under ambient conditions for air drying. Germination test on fresh material were conducted at each harvest time and on air dried materials 7 weeks after the final harvest. Germination after scarification was carried out on a proportion of seeds from each air dried sample to examine its effectiveness in breaking hardseededness. Scarification was done by rubbing the seeds between the sand paper for one minute before germination testing.

(3) Shattered pod percentages per inflorescence were counted under field conditions using 4 X 10 inflorescences at each sampling date. Assessment was determined by counting the total number of split/open pods (with no seed) and/or the number of detached florets from peduncle of flower heads having mature pods inside per inflorescence, but still stuck to other florets in the flower heads. Visual assessment (qualitative measurement) was also carried out by estimating the proportion (percentage) of dropped florets in one flower head.

To avoid variation among seeds in one inflorescence, only the seeds from the middle and bottom of the inflorescence (approximately 75% of total seeds) were taken for seed development observations.

4.2.3. Data analysis

Statistical tests were done by determining the standard errors of individual means ($\pm$SE) and t-testing.

4.3. RESULTS

4.3.1. Growth and food reserve accumulation stage

Mean values of changes in physiological components (fresh weight, dry weight and moisture percentage) are presented in **Figures 4.1a, b** and **c**. The results show that in general, both in early and middle flowers follow a similar pattern of seed development.

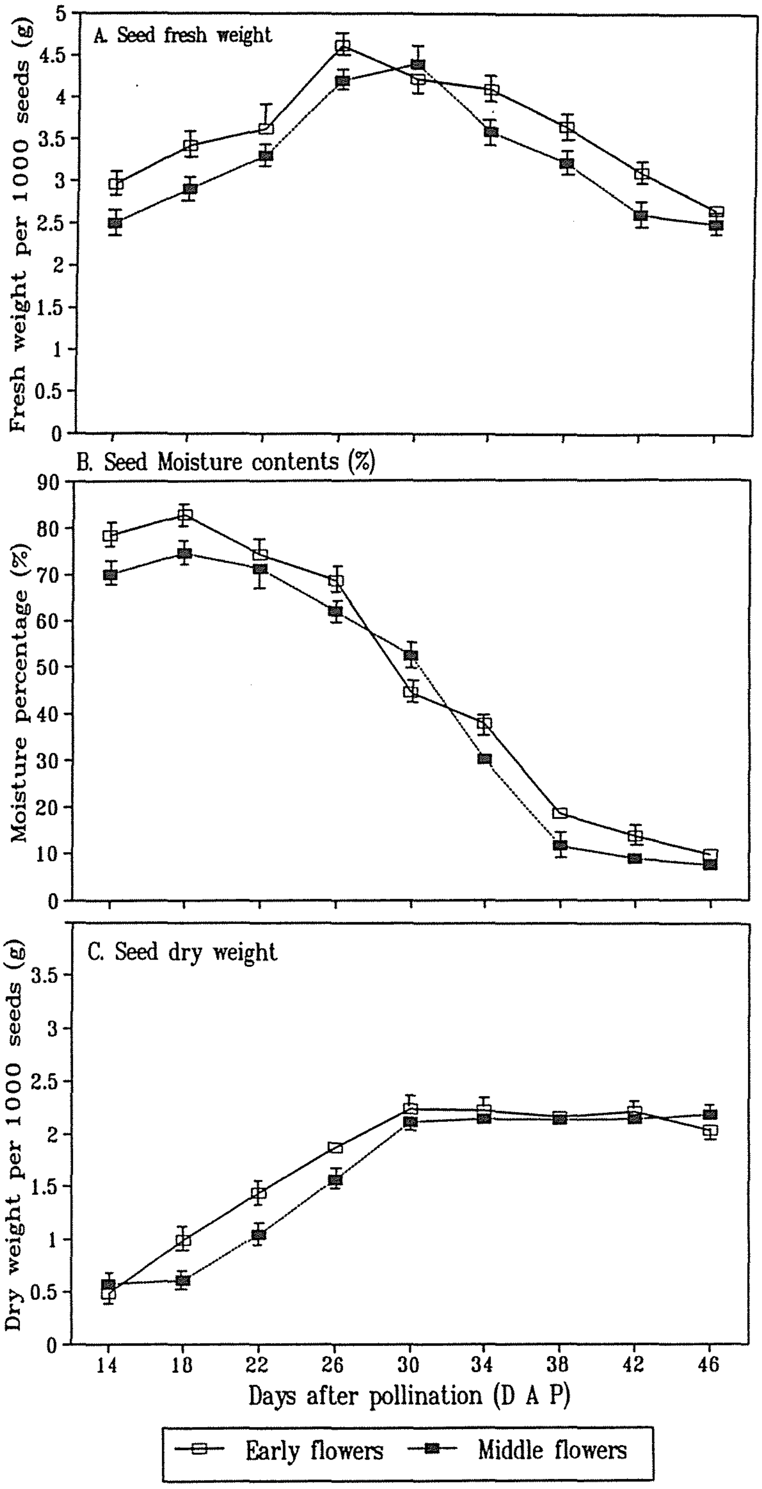


Figure 4.1. Thousand seed fresh weight (A), seed moisture percentage (B) and thousand seed dry weight (C) of Caucasian clover (cv. Monaro) from pollination. Data presented are means of 4 replications. Vertical bars represent SE's calculated for individual means and shown when larger than the symbols used

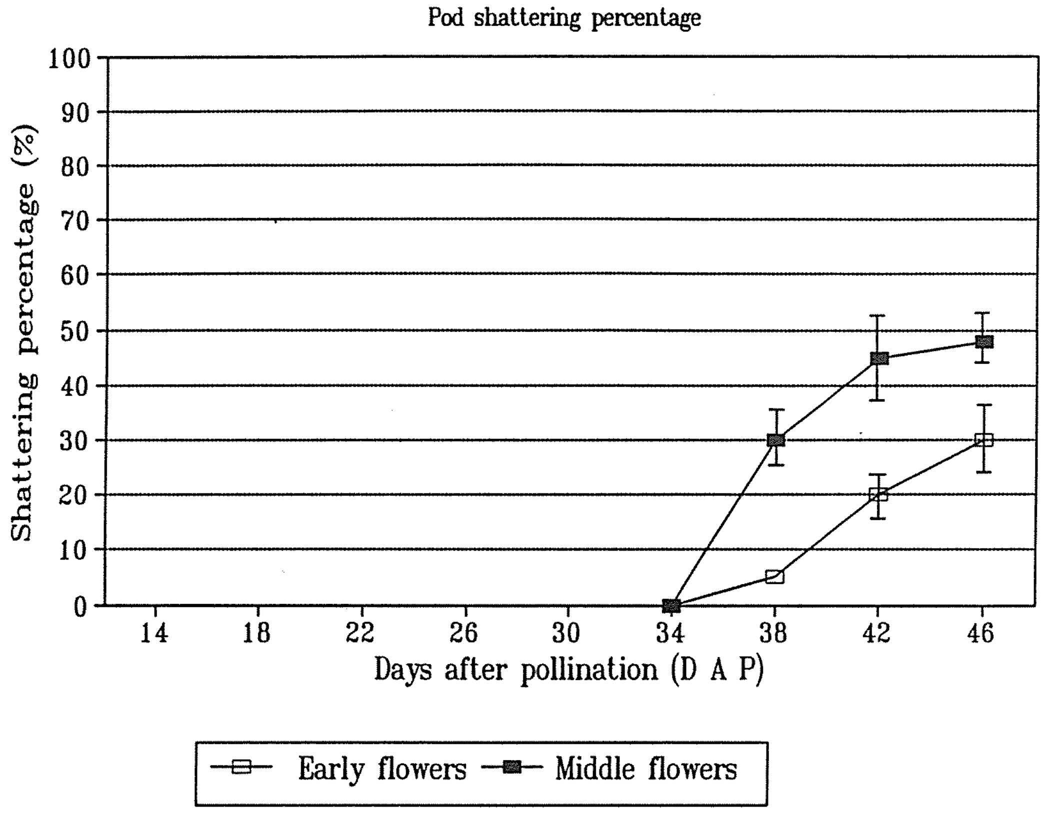


Figure 4.2. Pod shattering percentage of Caucasian clover (cv. Monaro) from pollination in freshly harvested seeds. Data presented are means of 4 replications. Vertical bars represent SE's calculated for individual means and shown when larger than the symbols used

In the first sampling date (14 days after pollination), the average fresh weight of thousand seeds was only 2.96 g and 2.5 g in early flowers and in middle flowers respectively, it is 64 % of its maximum value in early flowers and was about 57% of maximum fresh weight in middle flowers. From this point, seed fresh weight increased markedly during the period of 14 to 26 days after pollination (**Figure 4.1a**). In both early and middle flowers maximum fresh weight of Caucasian clover seeds was reached about 26 days after pollination (in middle flowers, fresh weight at day 26 was not significantly different with those at day 30) (**Figure 4.1A**). The maximum thousand seed fresh weight was about 4.62 g (68.6% SMC). In early flowers a rapid decline in fresh weight occurred from 34 to 42 days after pollination, while in middle flowers was from 30 days onward.

Both in the early and middle flower groups, increases in seed dry weight occurred up to 30 days after pollination before remaining constant throughout the remaining seed development sequence. However, in early flowers a linear increased in seed dry weight (stage II of seed development) had started by 14 days after pollination, while in middle flowers the linear phase of grain growth (stage II) began at 18 days following pollination. Maximum dry weight did not significantly differ between early and middle flower groups (about 2.24 g). In early flowers, the seed moisture percentage when seeds attained maximum dry weight was 44.6%, significantly lower (P,0.05) than those in middle flowers (52.4%) (**Figure 4.1C**). Both early and middle groups had a similar pattern of changes in seed moisture content and in both groups moisture content began to decline at ~ 18 DAP.

It was observed that pod colour progresses from green to yellowish brown on the surface when the moisture content of seeds falls to approximately 20 to 30%. maximum dry weight was reached in both flower groups when pods were

Plate 4.1. **Stages of seed development of *Trifolium ambiguum, M.Bieb.* from 0 - 46 days after pollination (DAP) after seed dried to ~ 8% SMC (A : Scale for actual seed size; B : scale for actual inflorescence size).**

yellowish brown and the fresh harvested seeds were yellowish green and became yellowish brown after air drying. In both flower groups, the onset of pod shattering appeared to be consistent with seed SMC's approaching 20% (**Figure 4.2**). Overall the shattering percentage in middle flowers was significantly higher than in the early ones. In early flowers pod shattering increased rapidly from 5% at day 38 to 20% at day 42, while in middle flowers, a shattering percentage of around 40% was reached at 38 days after pollination and remain relatively constant percentage until day 46 (**Figure 4.2**).

4.3.2. Viability and germination in fresh and dry seeds

At 14 days after pollination about 28% of seeds from early flowers, but only 11% in middle flower seeds were viable, although all these seeds were still ungerminable. In both early and middle flowers the maximum viability seed (98.5% and 96% respectively) occurred about four days before maximum dry weight when on average the seed moisture content was 65.4% (**Figure 4.3a**). At this stage the seed was 83.5% of its final dry weight in early flowers and 73.6% in middle flowers (**Figure 4.1**). Up to 26 days after pollination, as seed age increased the number of normal seedling increased. However, at 34 days after pollination the percentage of normal germination began decreasing as hardseededness appeared.

In freshly harvested seeds, hardseededness appeared as the moisture percentage fell below 40% in both early and middle flowers (**Figure 4.3b**) and in middle flowers over 48% of seeds were observed to be hard when the moisture percentage of seeds below 15%. In early flowers, 34% of seed were counted as hard at 20% SMC (**Figures 4.3b and 4.1b**). Overall, the rate of hard seed appearance was significantly higher in middle flowers than those in early flowers (**Figure 4.3b**).

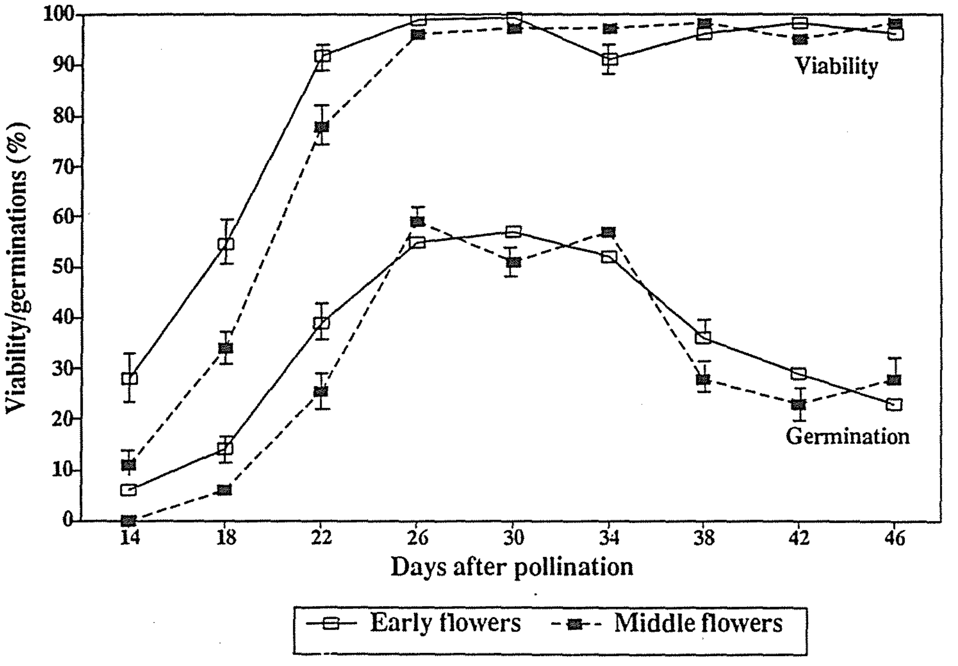


Figure 4.3a. Total viability and germination percentage of Caucasian clover (cv. Monaro) from pollination in freshly harvested seeds. Data presented are means of 4 replications. Vertical bars represent SE's calculated for individual means and shown when larger than the symbols used

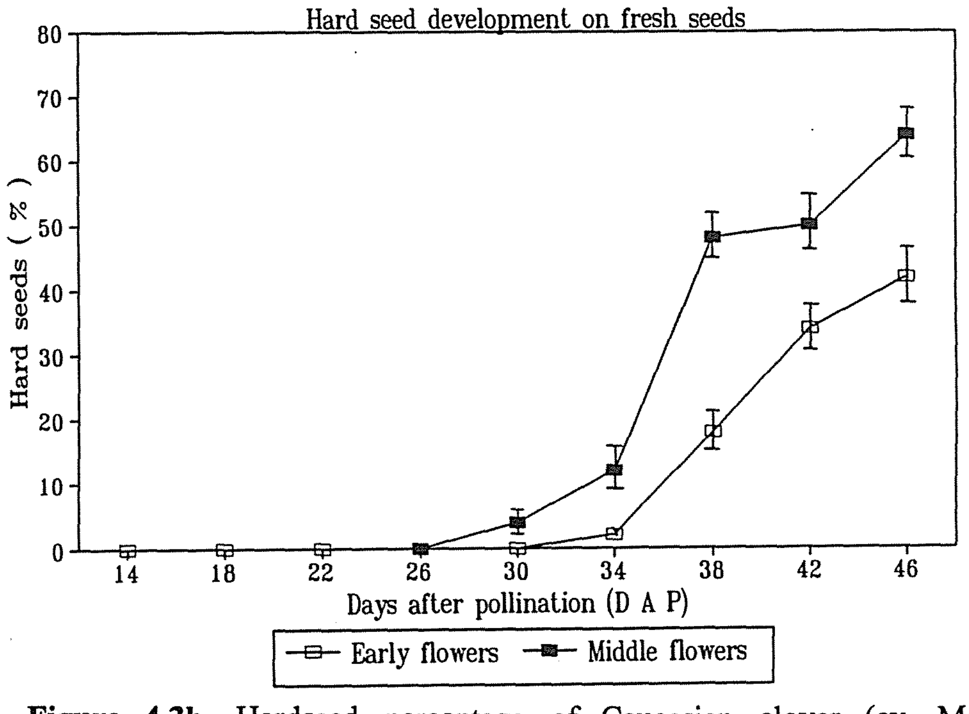


Figure 4.3b. Hardseed percentage of Caucasian clover (cv. Monaro) from pollination in freshly harvested seeds. Data presented are means of 4 replications. Vertical bars represent SE's calculated for individual means and shown when larger than the symbols used

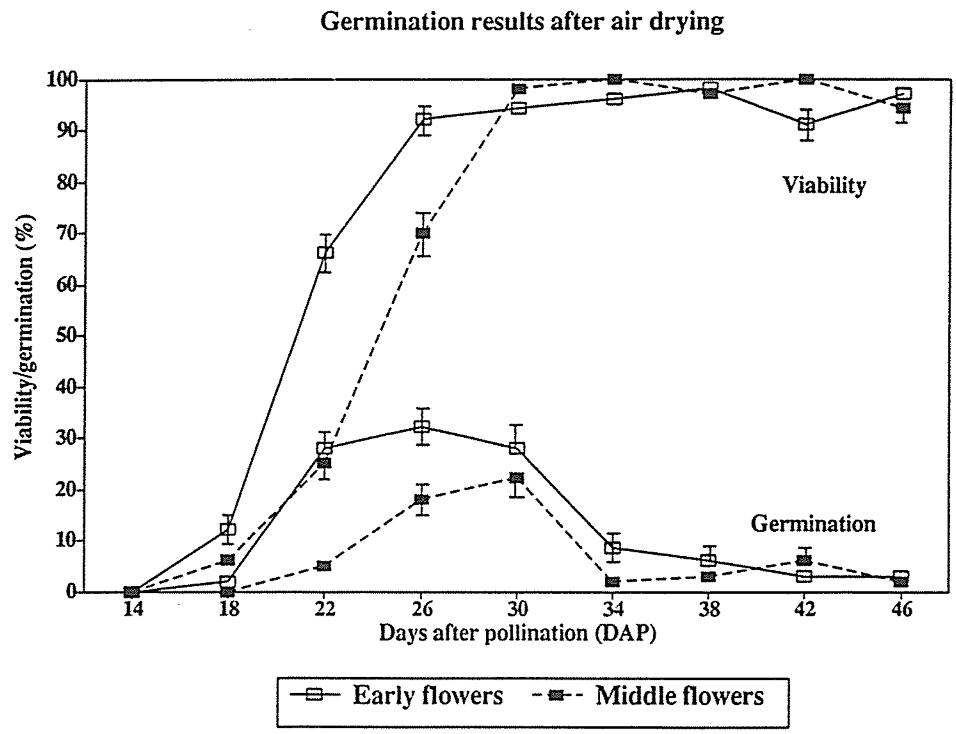


Figure 4.4a. Total viability and germination percentage of Caucasian clover (cv. Monaro) from pollination in air dried seeds. Data presented are means of 4 replications. Vertical bars represent SE's calculated for individual means and shown when larger than the symbols used

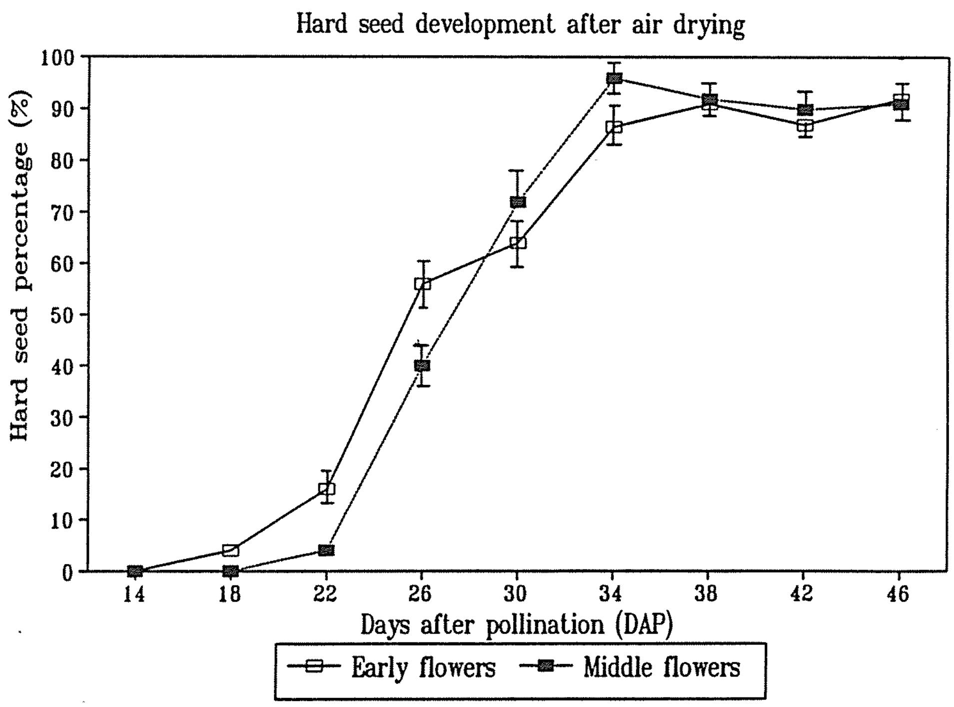


Figure 4.4b. Hard seed percentage of Caucasian clover (cv. Monaro) from pollination in air dried seeds. Data presented are means of 4 replications. Vertical bars represent SE's calculated for individual means and shown when larger than the symbols used

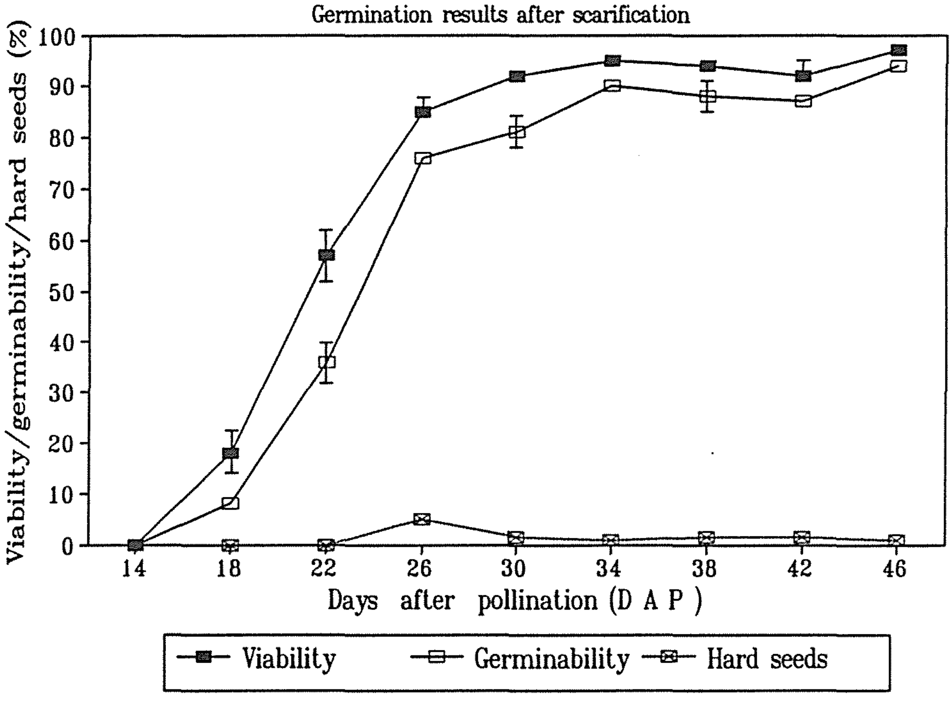


Figure 4.5. Total viability, germinability and hardseed percentage of early flowers of Caucasian clover (cv. Monaro) from pollination after scarification of air dried seeds. Data presented are means of 4 replications. Vertical bars represent SE's calculated for individual means and shown when larger than the symbols used

Germination tests were carried out again after seeds were left to dry at ambient temperature and humidity in the laboratory until the seed moisture content was around 8%. In both early and middle groups, after drying seeds samples taken from early harvest (~day 14 to 22) did not retain their viability on drying. Dried seeds began to retain viability (~90%) at about 26 DAP in early flowers, and at day 30 in middle flowers. Germinability, as shown by the production of normal seedlings, started at day 22 and reached a peak at about 26 DAP in the early flowers and 30 DAP in the middle flowers. After that period germinability of dried seeds begun to decrease as the levels of hard seeds increased (**Figure 4.4b**).

Figure 4.4a shows that after seed were dried to a moisture content of about 8% hardseededness appeared in sample taken at about 18 days after pollination (about 4%) in the early flower group. In the middle flower group, the first appearance of hardseededness was after 22 days from pollination (**Figure 4.4b**).

Light scarification was applied on air dried seeds of early flowers by scarifying between two layers of sand papers for 1 minute and the results are presented in **Figure 4.5**. This method was found to be effective enough to break dormancy in Caucasian clover seeds with only 5% of seeds remaining hard (**Figure 4.5**). As the availability of seed samples was limited, scarification tests were not done on seeds from the middle flowers.

4.4. DISCUSSION

Several studies have been undertaken to determine the stage of maturity and ripeness of different crops in order to guide seed growers in deciding when crops are at the correct stage for harvest. Such studies have been valuable in ensuring the harvesting of both the maximum quality and quantity of seed crops (Hyde, 1950; Anderson, 1954; Hyde *et al.*, 1959; Hill, 1971; Win Pe, 1978; Kowithayakorn and Hill, 1982; Juntakool, 1983; Waikakul, 1983; Hare and Lucas, 1984**a**; Witchwoot, 1987). However, the timing of physiological and harvest maturity of *T. ambiguum* seeds had not been reported.

Physiological changes during seed development in *T. ambiguum* appeared to follow three stages, the growth stage (stage I), the food reserve accumulation (stage II) and the ripening stage (III), similar to those described in other traditional herbage legumes.

4.4.1. Growth and food reserve accumulation stages

In *T. ambiguum* observed in this study, the growth stage appears to be the period of seed development up to 14 days in early flowers and up to 18 days after pollination in middle flower group. This stage is usually marked by a rapid increase in seed weight due to intense cell multiplication in both the embryo and cotyledons (Hyde *et al.,* 1950; Bewley and Black, 1978; Kowithayakorn and Hill, 1982). The seed moisture content during this stage was very high about 80% and 70% in early flowers and middle flowers respectively. In general, the seeds were non-viable, however, germination data indicated that at day 14, early flowers produced significantly more viable seeds than those in middle flowers suggesting that at this stage seed from early flowers were physiologically more mature. As no data was taken before day 14 it is possible that the start of stage II in early flowers might be earlier than day 14.

The duration of stage II in this experiment was shorter than the 22 days in

Medicago sativa (Kowithayakorn and Hill, 1978) and in *Lotus corniculatus* (Li and Hill, 1989) and the 19-23 days in *Lotus pedunculatus* (Hare and Lucas, 1984**a**), but longer than the 10 days growth stage found in white and red clover (Hyde *et al.*, 1959; Win Pe, 1978) . This is possibly related to species differences as well as environmental conditions during seed development. Differences in the onset of stage II and seed moisture content between early and middle flower groups may also be attributed to the weather conditions. The frequent and high total rainfall (41 mm; **Figure 4.7**) during the early stage of the development of early flowers (from day 14 to 18) may affect its fluctuation in moisture content. The average daily temperature during period from day 14 to 18 was 16.2°C and 22.°C in early flowers and middle flowers respectively. Working in several genotypes of cereals, Sayed and Gandallah (1983) suggested that at the end of the stage I, individual grain dry weight is a function of the grain filling rate (GFR) which is constant and is not sensitive to the changes in environmental conditions as well as the duration of the filling period which tends to be affected by environmental factors. However, whether the differences in environmental conditions during earlier development affects seed growth in clover species is unclear. A more comprehensive study is needed to understand this aspect.

Even though the timing of onset of stage II was different, seeds from both early and middle flowers reached maximum dry weight at 30 days after pollination (**Figure 4.1C**). This indicates that the grain filling rate (GFR) of early flowers is slightly lower (0.11 mg/day from day 14 to 30) than those in middle flowers (0.12 mg/day from day 18 to 30). Data on daily temperature and rainfall indicated that during this stage, early flowers were exposed to relatively lower average maximum temperature of 17.4°C (from day 14 to 30) compared to average of 20.5°C

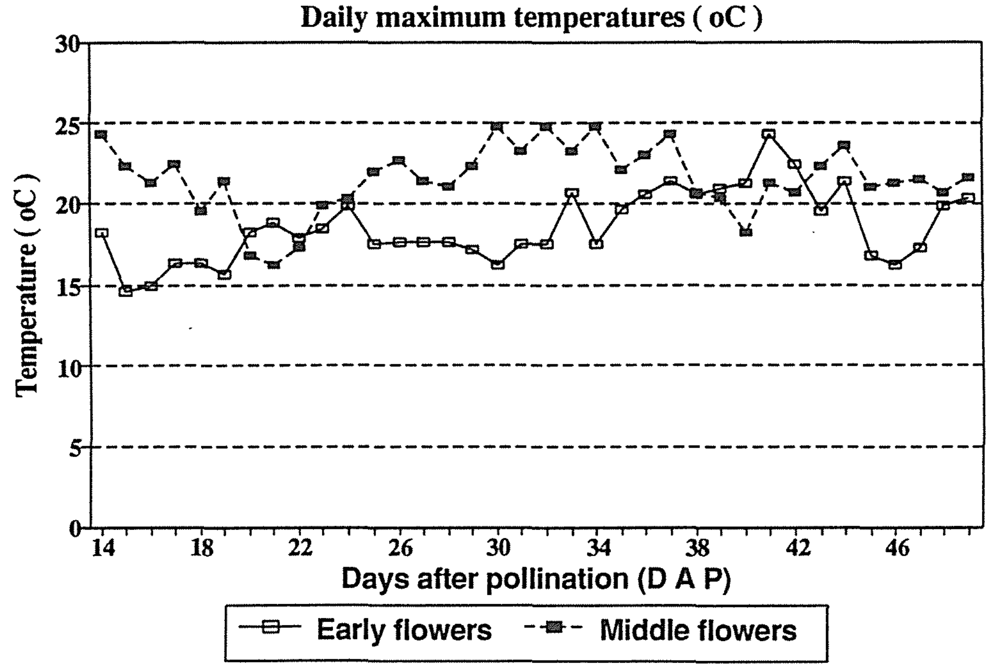


Figure 4.6. Maximum daily temperatures from 14 to 46 days after pollination both in early flowers and middle flowers

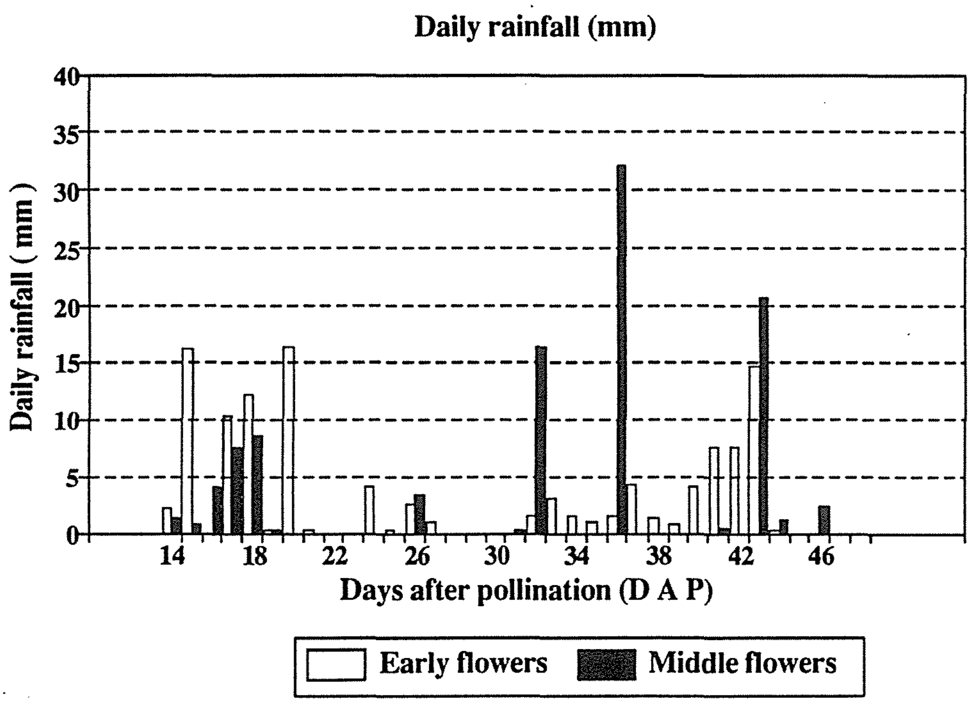


Figure 4.7. Daily rainfall from 14 to 46 days after pollination both in early flowers and middle flowers

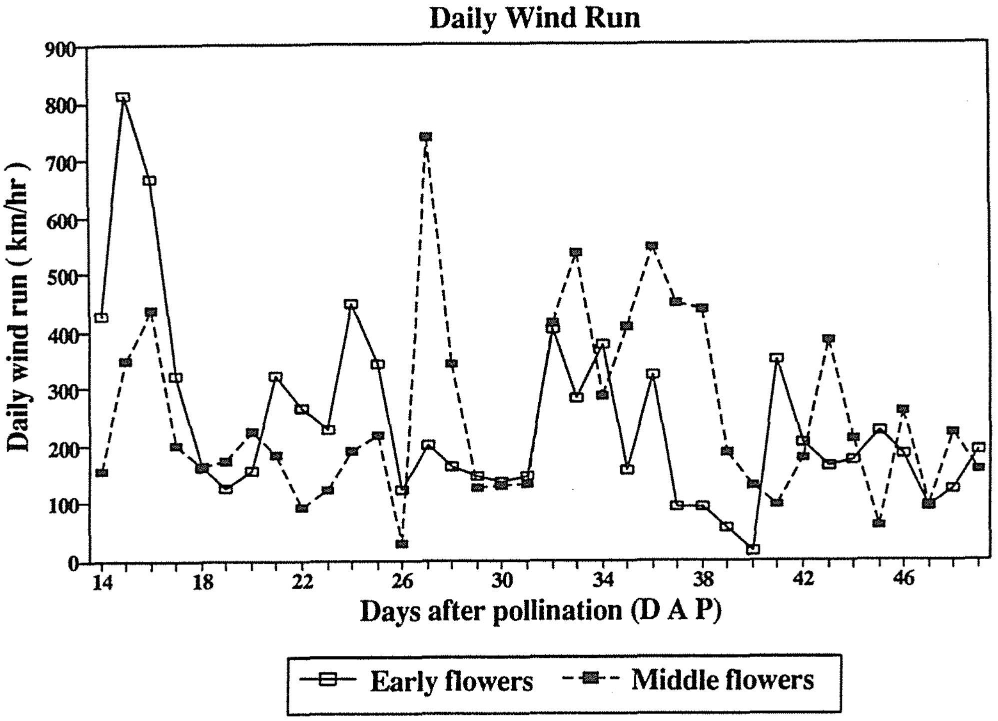


Figure 4.8. Daily wind run from 14 to 46 days after pollination both in early flowers and middle flowers

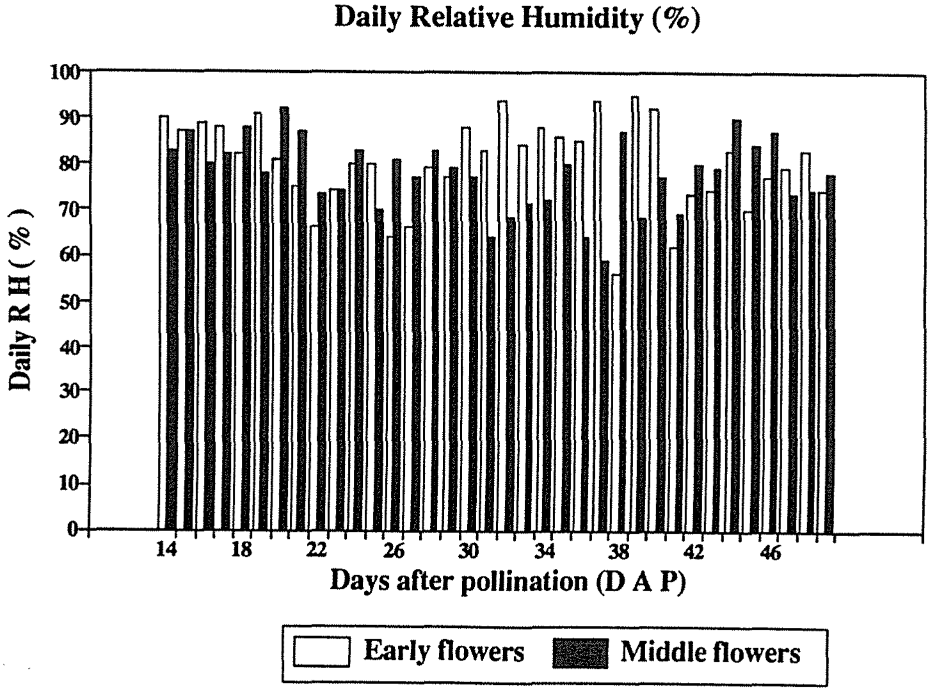


Figure 4.9. Daily relative humidity from 14 to 46 days after pollination both in early flowers and middle flowers (data were taken at 9.00 a.m.)

(from day 18 to 30) for middle flowers (**Figure 4.6**) and higher rainfall (in total of 65.6 mm compared to only 26 mm over the accumulation stage period. As the maximum dry weight was not affected, it is likely that these fluctuations affects both the rate of seed filling as well as filling duration. This results does not appear to be in agreement to those found in cereals by Sayed and Gandallah (1983) who suggested that only grain the filling period is sensitive to changes in environmental conditions. In addition to this, Weighand and Cueller (1981) have demonstrated that for wheat the period of grain filling may be reduced by three days for every 1°C rise in temperature. They concluded that temperature speed up the senescence of the plant. As the average daily maximum temperature was 3^0C higher in middle flowering than in early flowering, it is possible that temperature was responsible to the fluctuation in the duration of stage II. Also, it is likely that water stress experienced in the middle flowers, by restricting plant growth, also appeared to hasten the transfer and accumulation of food reserve to seeds, as also reported by Robins and Domingo (1956) in dry beans, in soybeans (Sionit and Kramer, 1977), and in siratro (Juntakool, 1983).

Early in this stage a high proportion of seeds (>50% in early flowers and >34% in middle flowers) became viable (**Figure 5.3**). The changes outlined for *T. ambiguum* during this stage have also been reported by other workers for other herbage legumes but with variation in the rate of development. Hyde *et al.* (1959) reported that the food reserve accumulation stage in white and red clovers took 10-14 days (similar to the 8-12 days in *Maku lotus*, Hare and Lucas (1984**a**) and in agreement with the results in *T. ambiguum*), but Win Pe (1978) with Pawera red clover, and Kowithayakorn and Hill (1982) with Lucerne, found that this stage took longer (16 to 17 days compared to only 12 to 16 days in *T. ambiguum*). Hare and Lucas (1984**a**) suggested that these differences may be attributed to weather conditions or species. However, they reported that the differences due to technique used affected their results from different seasons.

4.4.2. Maturation stage

22 to 30 days after pollination seems to be an important stage in *T. ambiguum* (**Figure 4.3**). It was found that important changes in each viability components took place in this stage. In freshly harvested seeds, high levels of normal seedlings appear in the germination test about this time. It was also found there was a higher percentage of abnormal seedling in this stage indicating that some seeds were intermediate in their development between non viable and viable seeds (**Appendices 4.1A and B**). The decrease in abnormal seedling percentage in germination tests on more mature seed lots indicates that seeds have completed their development and became normal seedlings or fresh ungerminated seed categories (**Appendices 4.1A** and **B**).

4.4.3. The effects of drying on seed maturation

A peak of seed germination and viability of *T. ambiguum* was reached during the food reserve accumulation stage 26 days after pollination, at about 4 days before maximum dry weight was reached in both flower groups (**Figure 4.3a**). Similar results were reported by Hyde *et al.* (1959) and Win Pe (1978) in white and red clover and by Hare and Lucas (1984**a**) in *Maku lotus*. By contrast in *Lotus corniculatus* maximum seed viability was found at or just after mass maturity (Anderson, 1955; McKersie, 1982). These differences may be due to the technique employed. Germination testing in *Maku lotus*, white and red clover was carried out on freshly harvested seed, but in *Lotus corniculatus* dry seed stored for several weeks was used.

Results in this study also indicated that after drying, seeds harvested early at day 14 to 22 did not retain their viability suggesting that these seeds was still immature and could not withstand desiccation. After day 30 the increase in the percentage of hard seed following drying was accompanied by a rapid decline in normal seedling numbers. Thus by the time the seeds was able to germinate and became tolerant to dessication, the development of hardseededness prevented them from germinating.

Results in **Figure 4.5** demonstrated the disappearance of dormant seeds after scarification. Satisfactory germination results (over 90%) were obtained when seeds were tested following mechanical scarification (rubbing between sand paper for 1 minute) implying that the hard seed condition in this cultivar is of a "shallow type" which is easily removed as suggested by Kowithayakorn and Hill (1981). This also indicates that harseededness may be the only dormancy mechanism involved in this seeds. The effects of drying on germination and hardseededness found in this study also supports Ellis and Filho's (1992) view that seed development is not necessarily completed when maximum dry weight is achieved.

4.4.4. The development of hardseededness and climatic conditions

Results in this study indicat that seeds from middle flowers exhibit higher levels of hardseededness than these in early flowers (**Figure 5.3b**). Studies in *Stylosanthes humilis*, by Cameron (1967) and Argel and Humpreys (1983) showed differing levels of hardseededness in lines with differing flowering times and in successive seasons. Further, they stated that hardseededness was positively related to the temperature regime during seed maturation. In fact, temperature acts as a modifying factor on the primary control of hardseededness development exerted by atmospheric humidity (Argel and Humpreys, 1983**a**). This study provides evidence that middle flowers were exposed to relatively lower levels of average relative humidity (71.3% compared to 84.7% in early flowers) recorded from day 30 to 38; **Figure 4.9**) during seed maturation which might have been associated with the high value for hardseededness in middle flowers. As seed moisture contents tend to equilibrate with the lowest relative humidity to which they have been exposed (Hyde, 1954), the humidity can therefore hasten the drying period, increasing the rate of hard seeds. Kowithayakorn and Hill (1982) have shown that a high percentage of hard seed (87%) was found in lucerne when the seed ripening period was hot and dry (ambient temperature ranged from 7 to 42°C). Conversely seed from plants where ripened during cool and wet weather (ambient temperature range from 2 to 25°C) showed the development of only 60% hard seed.

Daily rainfall data also indicated that from day 30 to 38 after pollination, the seeds of early flowers developed under relatively drier soil conditions than middle flowers (13 mm compared to 48.8 mm in 8 days during maturation) which may also have been related to its lower harseededness (**Figure 4.3b**). Even though the mechanism is unclear, this finding is also in agreement with the conclusion of Aitken (1939) and Quinlivan (1985) in *T. subterraneum* that plants maturing under conditions of soil moisture stress produce fewer hardseeds. The degree of seed dehydration seem to play a role in hard seed formation in this clover. A relatively lower soil moisture and higher relative humidity appears to cause a more gradual decline of seed moisture content (**Figure 4.1**) resulting in a lower hardseed level as reported in siratro by Juntakool (1983).

4.4.5. Seed shattering

Even though the onset of pod shattering was similar in both early and middle flowers (about 8 days following mass maturity), pod shattering was much more severe in the middle flower group than those in early flowers. The conditions between day 30 and day 38 in which middle flowers were exposed to higher rainfall (48.6 mm compared to 14.4 mm in early flowers; **Figure 4.7.**) in combination with a generally stronger daily wind run (128 to 544.6 km/hr compared to 93 to 403.5 km for early flowers; **Figure 4.8**) may have affected the high rate of pod shattering observed in this groups. Another possible factor is that the generally lower humidity experienced by the middle flowers during this period (from day 30 to 38; **Figure 4.9**) may enhance seed's vulnerability to the physical forces of rain or wind. Under the conditions of this experiment, the period between seed mass maturity the onset of pod shattering both in early and middle flowers was about 8 days. Similar results were reported by Hare and Lucas (1984**a**) in *Lotus corniculatus.*

4.4.6. Harvest maturity

In *T. ambiguum* the ripening stage (stage III) took 8 to 12 days from mass maturity. Seed ripeness (harvest maturity) was considered to be achieved when the seeds have dried to a moisture content in equilibrium with the surrounding atmosphere (Hyde, 1950). According to Hill (1971) seed ripeness is the condition in which the seeds in the field have dried to a moisture content suitable for harvesting. In early and middle flowers the equilibrium was attained when the seeds contained 9.6% and 7.9% respectively. Even though harvest maturity may differ from season to season, in most traditional herbage legumes (*e.g. Maku lotus,* *T. repens, T. pratense* and *Lotus pedanculatus*), moisture content of 15% or lower were considered to be suitable for harvest (Hare and Lucas, 1984**a**). The ripening stage of development observed in this study was longer than that for red and white clover (3-7 days), as reported by Hyde *et al.* (1959). Win Pe (1978) and Hare and Lucas (1984**a**) found that a 10 to 14 days ripening period was required for red clover (cv. Pawera), which was similar to the 12 days *T. ambiguum* ripened in this study. The between-flower group variation in seed moisture content found in this study during the maturation stage (at day 26 to 38 in the early flowers and day 30 to 38 in the middle flowers) (**Figure 4.1B**) further stresses the influence of prevailing weather conditions on the rate and extent of natural drying in the field.

The apparent increase in drying rate in the early flowers from day 26 to 30 (**Figure 4.1B**) may have been associated with the generally lower relative humidity (~60%; **Figure 4.9**) and higher wind speed (**Figure 4.8**) in the early flowers compared to those in the middle flowers during this period. The decrease in drying rate from day 30 to 34 for early flowers may have been affected by the facts that the average relative humidity over this period was higher than those in the middle flowers. The relatively lower relative humidity in middle flowers from day 30 to 40 (~64%; **Figure 4.9**) apparently allowed seeds of this group to increase in drying rate at day 30 onward. The similar association was reported by Hare and Lucas (1984**a**) in *L. pedunculatus*, that if strong winds are blowing and the humidity is low (dry) then seeds will ripen and dry more rapidly.

4.4.7 Predicting the optimum time for harvest

A prediction of the optimum time for harvesting *T. ambiguum* for 1991/1992 season, can be done by superimposing **Figures 4.1 A, B** and **4.2** upon one and another as described by Hare and Lucas (1984**a**) and the result is presented in **Figure 4.10**. The judgement of this estimation (**Figure 4.10**) is based on the condition that the seeds have reached mass maturity (constant dry weight), moisture content is safe enough to allow harvest without damage and minimal pod shattering have occurred. The prediction was carried out only on the middle flowers as this group contributed about 70% to total inflorscences. Mowing or chemical desiccation may be employed as the first stage of harvesting to reduce the risk of seed losses from pod shatter and enable the crop to dry more rapidly in the field before combine harvesting. Hare and Lucas (1984**a**) suggested that a seed moisture content of 35% is considered to be safe for such activities. This would be on day 34 in this study (see Figure 4.10). This stage can be recognised in the field by the dark brown flower color, yellowish brown pod colour and light yellow seed colour (SMC about 30 to 40%). The time taken to dry the sward in the windrow after mowing or in the stand after chemical desiccation may vary depending on the weather conditions.

The results of this experiment suggest that among all stages throughout seed development, the ripening stage from mass maturity toward the onset of shattering and harvest maturity may be the most sensitive stage to changes in weather conditions. The time occupied by each stage of seed development, especially stage III (maturation stage) is apparently influence by the environmental conditions. Weather conditions may vary from year to year and the duration of each stage may slightly changes. It is likely that the time to reach harvest maturity will be slightly altered. It is recommended, therefore, that after about 26 days from peak flowering, swards must be inspected daily and the weather monitored. Seeds will ripen and dry rapidly under strong dry winds and high temperatures (more than 20°C) and heavy rainfall can be detrimental to mature pods (Lucas and Hare, 1983**a**). It may need several seasons of experiment before the consistency of the relationship between external appearance of pods/seeds and level of maturity can be fully determined.

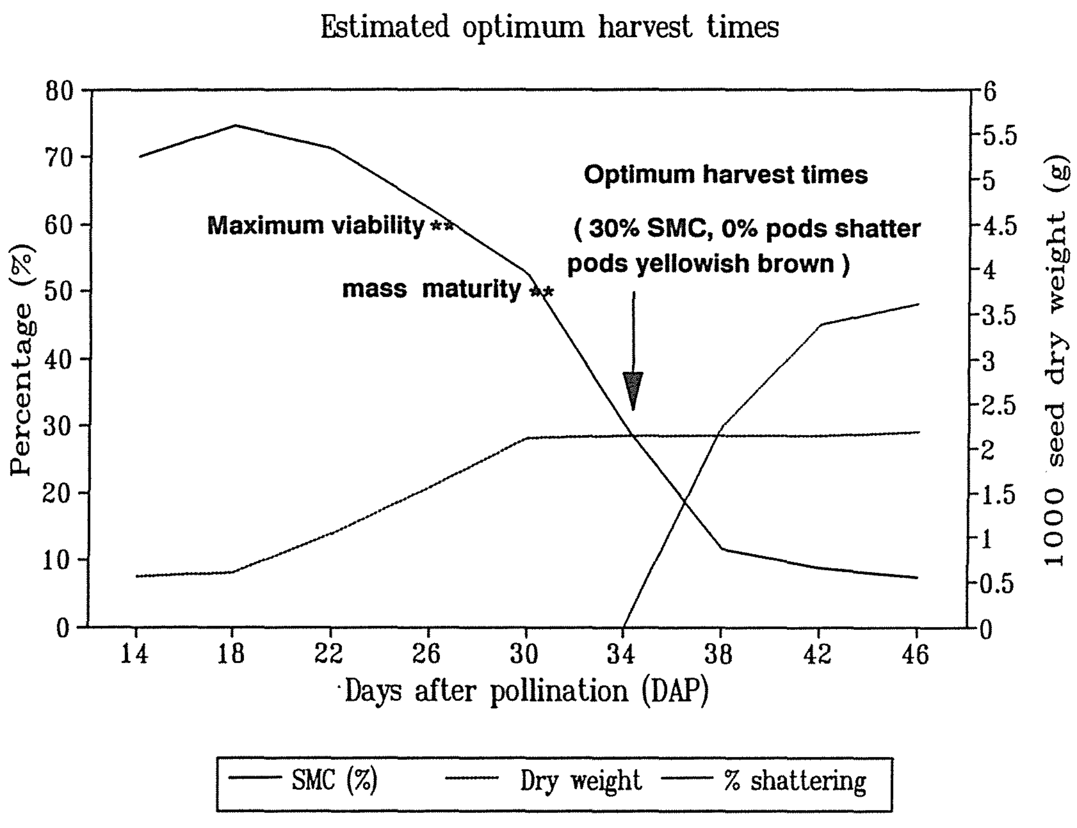


Figure 4.10. Estimated optimum harvest times in 1991/1992 according to changes in seed moisture percentage, pod shattering percentage, and 1000 seed dry weight.

CHAPTER 5
THE EFFECTS OF DEFOLIATION DATES ON VEGETATIVE AND REPRODUCTIVE GROWTH OF CAUCASIAN CLOVER.

5.1. INTRODUCTION

The onset of reproductive growth in species with a determinate growth pattern usually marks the end of active vegetative growth. In most clovers, however, the indeterminate plant growth habit results in active vegetative growth continuing during reproductive development. This resulting competition between vegetative and reproductive growth complicates management decisions for clover seed production.

As discussed in previous sections (see **Section 2.9.2.2**), seed yield and seed yield components are known to be affected by closing time related to the developmental stages of the plants. Defoliation prior to flowering has been reported to increase seed yield in some clovers. These increases are usually due an increase in the number of flower heads. In Zigzag clover, Townsend *et al.* (1968) reported that defoliation prior to flowering resulted a much earlier flower appearance than cutting at flowering. Working with *Trifolium medium*, an approximate 30% increase in seed yield over uncut plants was achieved by Robertson and Armstrong (1964) in earlier cut plants. There have been few published results on the response of *Trifolium ambiguum* to common cultural practices used to improve seed production in clovers.

Some other beneficial effects of defoliation have been recognized such as that, under the appropriate environmental conditions flowering is slightly delayed as a result of defoliation until ambient temperature are more conducive to pollinator activity; the ratio of reproductive to vegetative growth is increased; the possibility to compress the long flowering period (Robertson, 1983); new leaves are produced which are not infected with early spring foliar leaf diseases; and the life cycles of pests such as the clover seed weevil (*Tychius picirostis*) and ladino clover midge (*Dasineura gentreri*) are disrupted (Steiner, **in press**).

However, the detrimental effects to seed yield due to late defoliation (at flowering stage) have also been reported in many clovers (Anderson and Metcalfe, 1957; Bader and Anderson, 1962; Sheath, 1978; Maldonado, 1985). This might be caused by a delay in subsequent flowering period into the time of decreasing day length which therefore, reduces floral initiation and cause a considerable reduction in most components of seed yield (Thomas, 1961; Clifford, 1979**a**). Robertson and Armstrong (1964) reported that no flowering occurred after late defoliation. In white clover (cv. Huia) a ~50% reduction in seed yield was reported for a seed crop which was closed after flowering (Clifford, 1979**a**). However, in red clover (cv. Pawera), late defoliation in December increased seed yield relative to early defoliation in October and November (Clifford, 1979**b**). These reports suggest that the optimum time for defoliation for maximum seed production may vary widely from season to season and from species to species. The objectives of the present study, therefore, were to study the effect of two defoliation dates on the vegetative and reproductive growth of Caucasian clover *(Trifolium ambiguum, M.Bieb).*

5.2. MATERIALS AND METHODS

5.2.1. Experimental site and preparation

This experiment was carried out in a specially allocated area in the same field and season used in previous experiments from September 1991 to April 1992 (see **Appendix 3.1**). At the beginning of the experiment (15 October 1991), the swards were inter-row cultivated with a 60 cm spacing between rows (see **Plate 3.1**). Sampling procedures and general management, unless otherwise stated, were the same as described previously in **Chapter 3.**

5.2.2. Experimental design

The design used for the present experiment was Randomized Complete Block Design (RCBD) with five replicates of 3 levels of cutting treatments. Thus, there were 15 plots in total. Details of experimental layout are presented in **Appendix 3.1.** The treatments were three cutting times as follows :

1. C = Control (uncut)
2. O = October cutting (21-10-1991)
3. N = November cutting (20-11-1991)

The crops were cut at different dates as mentioned above by cutting to ground level (**Plate 5.1**) by using a hand mower machine. To ensure that cutting was carried out evenly to ground level, this was repeated by using hand clippers.

Plate 5.1. Cutting at ground level on October and November using hand mower.

5.2.3. Field observations and measurements

5.2.3.1. Aerial component determinations

All plot areas were also divided into two subplot areas for destructive and non-destructive sampling areas as described previously (see **sections 3.2.2** and **Appendix 3.1**). During the regrowth cycle after cutting covering the period of September 1991 to March 1992, a grid pattern of 0.25 m^2 was randomly allocated in the row on each plot every two weeks to provide materials for aerial component analysis. Samples were cut to ground level and then each component was separated into different categories of vegetative and reproductive parts such as the number of trifoliate leaves, leaf area indices (LAI), the number of reproductive shoots, and dry matter of each of the aerial components.

All measurements were carried out using the same methods described in the previous sections (see **section 3.2.2**). Leaf area indices (LAI ; m^2 leaf/m^2 ground area) was measured by counting total number of open photosynthetic leaves of the cut sample and then a sub-sample of 40 trifoliate leaves were taken and individually plucked from stem and leaf area were determined on a "Hayashi" photocell planimeter. From these measurements total green leaf area of the cut sample and hence leaf area indices were measured.

5.2.3.2. Dry weight of underground components

On each subplot in the same grid positions where aerial components were taken, sod samples of 25 x 50 x 20 cm were dug after each cut to provide material for underground plant component analyses (total 3 X 5 sods). Each sod was individually soaked in water for at least 30 minutes, washed free of soil and then disentangled. Underground parts consist of the primary crown and taproot,rhizome, secondary crown and taproot, and fibrous root. To minimize possible sampling effects on the remaining plant growth, sod samples were taken alternately (detailed strategy see **Appendix 3.1**).

Drying of all aerial and underground parts for dry weight determinations were also conducted in air drying ovens at 80°C for 24 hours. All data were collected every two weeks for all treatments. Unless specifically stated, plant component data were considered on a per row basis.

5.2.3.3. Reproductive growth determination

(1) Flowering pattern

The start of flowering, flowering duration, peak flowering and completion of flowering were observed on permanent quadrats of 2 X 1.5m rows in all subplot of cutting treatments. Observations were carried out using the same method described in the previous section (see **section 3.2.2.3**).

(2) Seed yield and seed yield components.

Measurement of yield components, potential seed yield (PSY) and potential harvestable seed yield (PHSY) and actual seed yield (ASY) were carried out from inflorescences obtained at harvest time. Visual estimations were made to determine the percentage of mature inflorescences in each treated plot. Mature inflorescences were identified by the brown colour of the pedicles/seed heads and plants were harvested when more than 90% of inflorescences were in this category. From these assessments, uncut control, October cutting and November cutting were harvested on 9 January 1992 (about 34 days after peak flowering), 15 January 1992 (about 32 days from peak flowering), and 20 January 1992 (32 days from peak flowering) respectively. In each harvesting date, a quadrat of $0.25m^2$ per plot (total 2 X 3 X 5 = 30 quadrats) were taken for determinations of seed yield per 1m row. The number of inflorescences obtained were separated into two groups *i.e.* ripe and unripe inflorescences (brown, pink/white and green inflorescence buds). Only ripe inflorescences were used to calculate potential harvestable seed yield.

After harvesting samples were kept in the paper bags and dried under room temperature for 8 weeks. The number of seeds per pod was counted from 100 pods dissected from these flowers. The remaining harvested inflorescences then hand threshed and total actual seed yield per $0.25m^2$ was measured. Samples of 1000 seeds from each plot were counted and weighed. Means of five replicates were calculated to obtain an average of seeds per pod, 1000 seed weight and seed yield for each of cutting treatment. Thousand seed weight and seed yields were expressed at adjusted moisture content of 10% for every treatment.

5.2.3.3. Statistical analysis

Data for most characters were analyzed according to a randomized complete block design (RCBD) by the use of analysis of variance using SAS programme (1991). Treatment mean comparisons were performed by using Least Significant Differences (LSD) at a 5% level of probability.

5.3. RESULTS

5.3.1. Effects of cutting on aerial components

The results presented in **Figure 5.1** shows means of the changes in total dry weight aerial components per 1m row at two weekly intervals. In general, the aerial growth of *T. ambiguum* following defoliation comprises two phases in all cutting times. In plots defoliated in October, dry matter accumulation of tops increased over the first 4 weeks (29 October to 26 November). Subsequently, growth rate entered a second phase characterized by reduced dry matter accumulation. Similarly, plants defoliated in November also showed a sharp rise in aerial dry weight for 4 weeks (26 November to 24 December), followed by a more or less constant increase in weight toward the end of the experiment. Untreated control continued to show a relatively constant in dry weight accumulation after recovering from inter-row cultivation (from 26 November until mid of January and then started to decrease toward the end of the experiment) (**Figure 5.1**).

It is also important to note that cut plants reached their maximum dry weight by February 4th 1992, with the possible exception of that vegetative dry matters at harvesting of seed yield (control : 9 January; October cut: 15 January; November cut: 20 January 1992) was significantly reduced ($\pm$ 29.5 %; **Table 5.3**) by October and November cutting compared to control with no differences between these two treatments (**Table 3.3** and **Figure 5.1**). These reduction was mainly associated with the low proportion of reproductive shoot production (**Figure 5.4**).

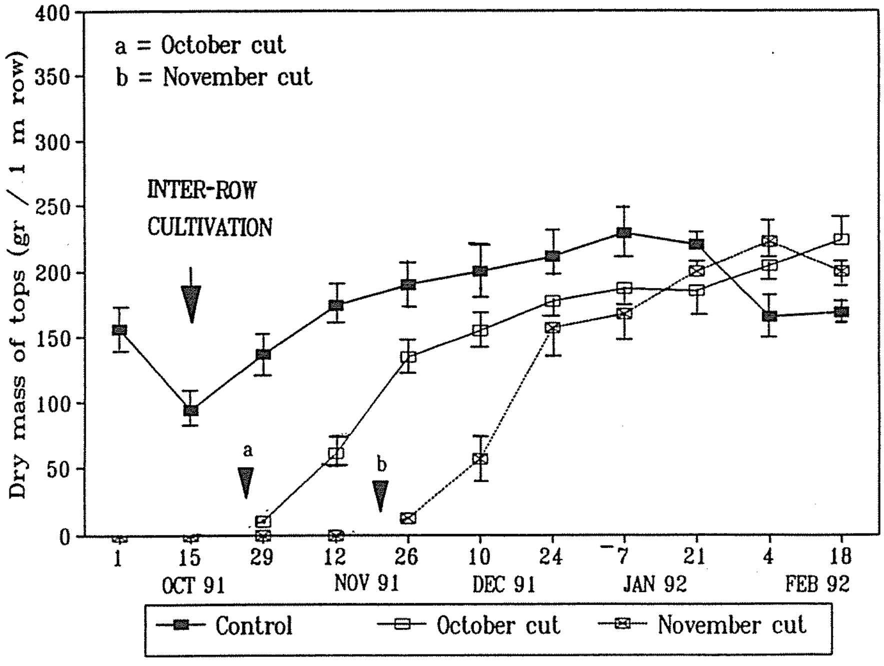


Figure 5.1. Effects of cutting dates on dry matter accumulation in aerial components for each sample date of caucasian clover (cv. Monaro). Data presented are means of 5 replications. Vertical bars represent SE's calculated for individual means and shown when larger than the symbols used

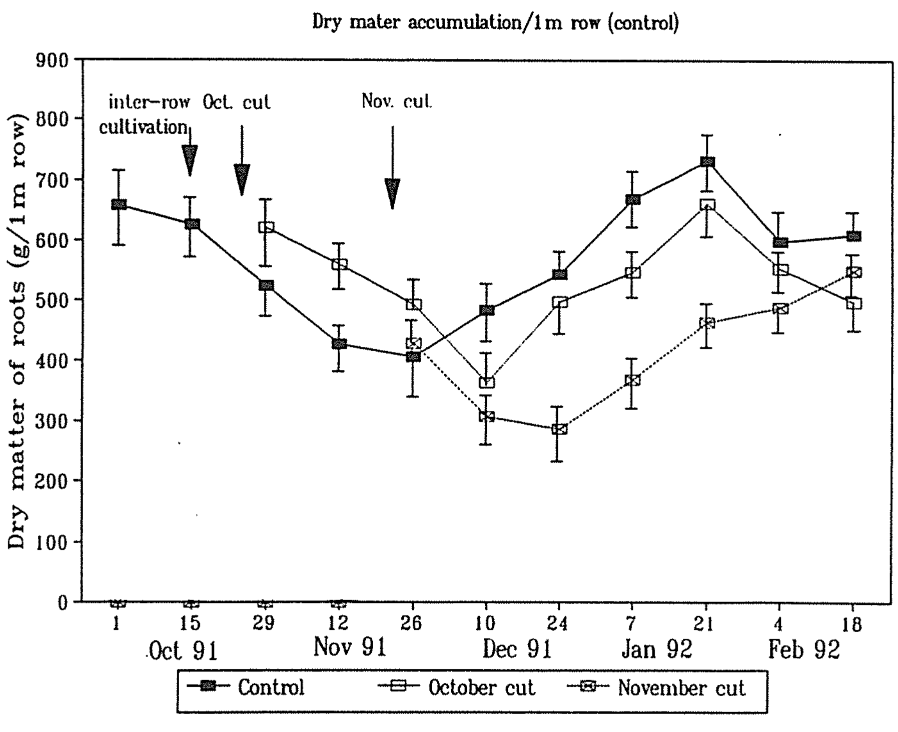


Figure 5.2. Effects of cutting dates on dry matter accumulation in underground components for each sample date of caucasian clover (cv. Monaro). Data presented are means of 5 replications. Vertical bars represent SE's calculated for individual means

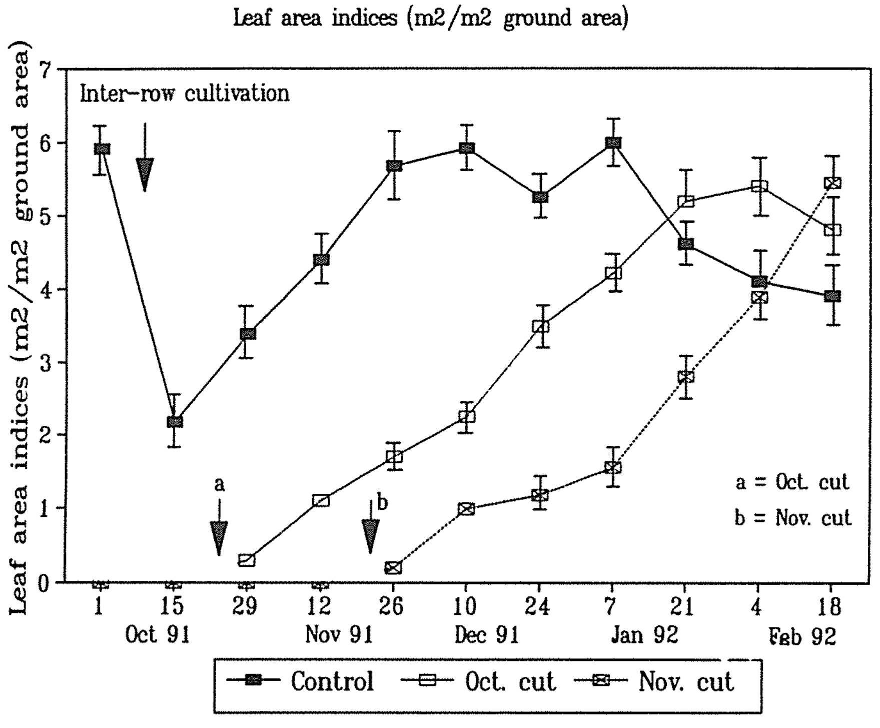


Figure 5.3. Effects of cutting times on leaf area indices (LAI) for each sample date of Caucasian clover (cv. Monaro). Data presented are means of 5 replications. Vertical bars represent S.E.'s calculated for individual means and are shown when larger than the symbols used.

5.3.2. Leaf area indices (LAI)

The effects of cutting treatments on leaf area indices (LAI) of each sampling date are presented in **Figures 5.3**. There were no differences in ceiling values (maximum LAI) between October cut, November cut and control. However, for October and November cut, the ceiling values were reached at about 13 weeks after defoliation date. The rate of change of LAI before reaching maximum value in control was 0.58/week (6 weeks following inter-row cultivation), 0.42 and 0.43/week (12 weeks after cutting both in October and November) respectively. Although the production of new leaves were evident, there was a significant leaf loss as well as the decline in LAI from early January to the end of the season in the untreated control.

5.3.3. Effects of cutting on underground components

The crowns, taproots, rhizomes and fibrous root system were collectively considered as forming total underground plant growth. The general trend of cutting influence on dry weight per 1m row of this total growth is shown in **Figure 5.2**. Following the October and November cutting, weight decreases were recorded (from 29 October to 10 December in October cut and from 26 November to 24 December in November cut). In the October cutting, root growth follows the pattern of the uncut control but in two weeks delayed. After 11 weeks from cutting, November cut reached the same dry weight to those in uncut control. Over the period from 24 December to 21 January, the November cut also resulted in an increase in dry weight accumulation with similar pattern compared to those in October cut (**Figure 5.2**) with 2 weeks earlier. In all treatments, total dry weight of underground components was restored to original value by the end of the season.

5.3.4. The number of reproductive shoots and flowering pattern

The effects of cutting times on reproductive shoot numbers produced during the period of four months of the growing season from 15 October 1991 to 18 February 1992 are presented in **Figure 5.4**. Cutting treatments resulted in the number of reproduction shoots being significantly reduced compared to control throughout the experiment. Visual assessment indicated that there seem to be no visible differences in the individual maximum reproductive shoot sizes between defoliated plants and the intact control.

The progress of flowering was observed to determine the effectiveness of cutting treatments on both flowering rate and the flowering duration (**Figure 5.5**). It was found that the cutting treatment both on October and November neither increased the rate of flower appearance and nor compress the flowering duration (**Figure 5.5**). The significant reduction in flower emergence appeared to be associated with the low emergence of reproductive shoots (**Figures 5.4** and **5.5**). There seems to be more than one peak flowering after both October and November cutting treatments. As no significant increase in reproductive shoots observed over the flowering period, it appears that the second peak flowering came from the flowers emerging from the later nodes of the same reproductive shoots (**Figures 5.4** and **5.5**).

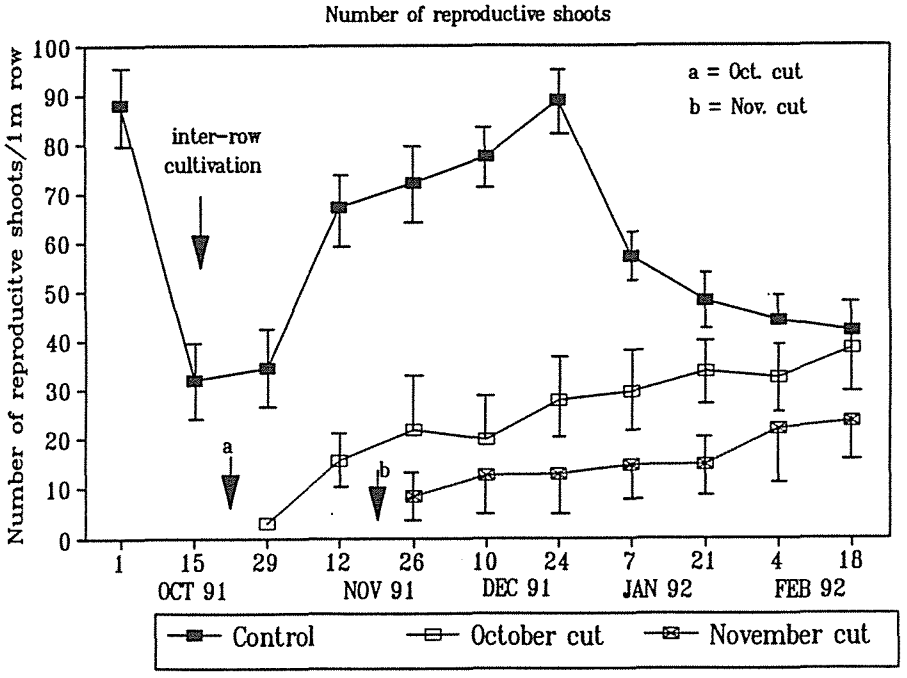


Figure 5.4. Effects of cutting dates on reproductive shoot numbers for each sample date of caucasian clover (cv. Monaro). Data presented are means of 5 replications. Vertical bars represent SE's calculated for individual means and shown when larger than the symbols used

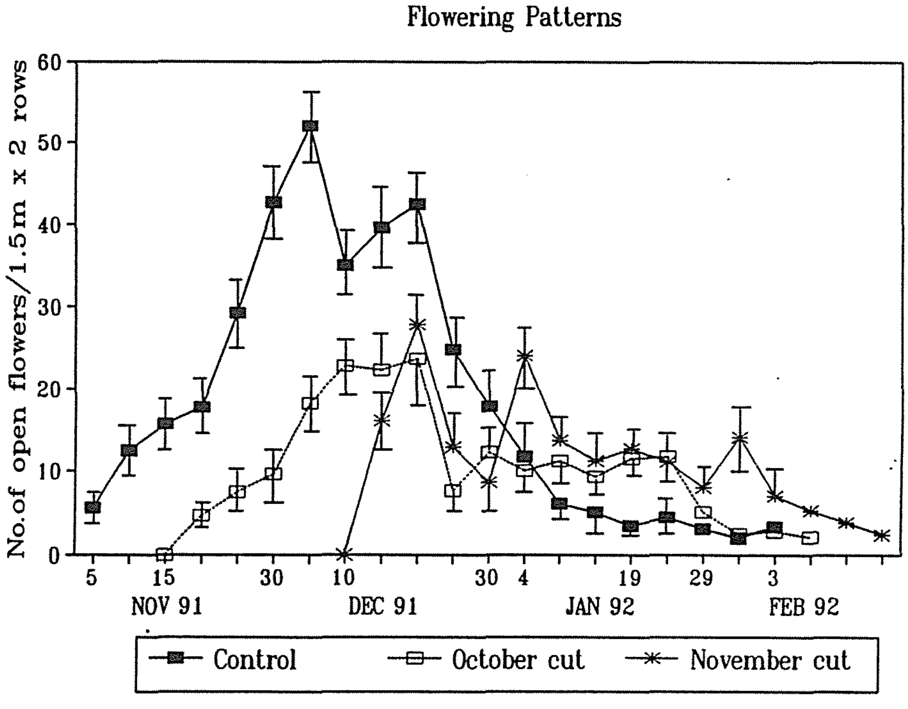


Figure 5.5. Effects of cutting dates on flowering patterns of Caucasian clover (cv. Monaro). Data presented are means of 5 replications. Vertical bars represent SE's calculated for individual means and shown when larger than the symbols used

5.3.5. Seed yield and seed yield components

Table 5.3 shows that a significantly higher potential seed yield (PSY), potential harvestable seed yield (PHSY) and actual seed yield (ASY) g/1m was obtained in the untreated control rather than the cutting treatments. Cutting on October and November resulted in very similar sharp decline in seed yield (between 60 to 70% in all values). Seed yield reduction in cutting treatments were mainly due to the decrease in the numbers of total inflorescences produced (~70%) (**Tables 5.1** and **5.2**). The percentage of ripe and unripe inflorescences at harvest was 22/78% in uncut control and there were no differences between October and November cut (~50% unripe, 50% ripe) (**Table 5.1**).

Except for the number of inflorescences, cutting at different times had no significant effects on other seed yield components (*e.g.* pods/ inflorescence, seeds/pod and seed dry weight) (**Table 5.2**). Cutting reduced vegetative dry matter at by 30% as well as decreasing harvest indices (**Table 5.3**).

Table 5.1. The effects of defoliation times on reproductive composition at harvest of *Trifolium ambiguum, M.Bieb.* (cv. Monaro).

Treatments	Inflorescence numbers/1m row			
	buds	open	brown/ripe	Total
Control	29.6**a**	39.2**a**	142.0**a**	210.8**a**
Oct. cut	21.4**b**	18.4**b**	42.8**b**	82.6**b**
Nov. cut	19.8**b**	22.4**b**	35.6**b**	77.8**b**
LSD (P=0.05)	5.72	8.2	16.7	22.3
C V (%)	14.6	54.6	42.2	28.5

Note : Values in the same column followed by the same letter are not significantly different according to LSD test at P=0.05.

Table 5.2. The effects of defoliation times on seed yield components at harvest of *Trifolium ambiguum, M.Bieb.* (cv. Monaro)

Treatments	Inflorescences/1m row	Florets/inflorescence	Seeds/pod	T S W (g)
Control	142.4**a**	105.8	1.02	2.576
October cut	42.8**b**	102.3	1.07	2.589
Nov. cut	35.6**b**	99.8	1.04	2.608
LSD(P=0.05)	16.7	N S	N S	N S
C V (%)	34.4	11	6	8

Note : Values in the same column followed by the same letters are not significantly different according to LSD test at P=0.05

Table 5.3. Effects of defoliation times on vegetative dry matter, potential seed yield (PSY), potential harvestable seed yield (PHSY), actual seed yield (ASY) and harvest index (g/1m row).

Treatments	Vegetative dry matters	PSY	PHSY	ASY	HI
Control	187.4**a**	58.6**a**	39.5**a**	29.6**a**	13.6**a**
October cut	132.2**b**	23.4**b**	12.1**b**	9.8**b**	6.9**b**
November cut	128.8**b**	21.1**b**	9.6**b**	8.3**b**	6.1**b**
LSD (P=0.05)	19.82	18.46	9.66	5.49	5.4
C V (%)	12	38	34	23	37

Notes :

1. Potential seed yield = P X E X N X S
   Where;
   P = the number of inflorescence/1 m row
   (PSY : total inflorescence; PHSY : total ripe inflorescence)
   E = the number of florets/inflorescence
   N = the number of seeds/floret
   S = seed weight
2. Harvest index (HI) = actual seed yield : total dry matter (vegetative+seeds) X 100%
3. Values in the same column followed by the same letters are not significantly different according to LSD test at P=0.05

Plate 5.2. **Differences in inflorescence density between untreated control (A), October cutting (B) and November cutting (C)**

5.4. DISCUSSION

There have been few published reports on how Caucasian clover grown for seed responds to cultural management practices commonly applied in other traditional forage legume seed production systems. Results in this study indicate the harmful effects of late cutting treatments being used as a strategy for improving seed production in *Trifolium ambiguum*.

In this study, late cutting caused a significant reduction in the number of inflorescences per 1m row. Steiner (**in press**) suggested that the reproductive morphology of *T. ambiguum* limits its ability to recover from defoliation when grown for seeds. *T. ambiguum* only produces leave and petiole growth above the ground until reproductive stems/shoots are initiated and elongate in Spring. Although these stems are indeterminate and are capable of producing one to five peduncles (Speer and Allinson, 1985; Stewart, 1979; Steiner, **in press**), it was found in this study that they are unable to regrow from the ground following defoliation. Unlike in white clover which has several vegetative buds between successive inflorescences in one stolon (Thomas, 1987; Budhianto, pers. commn.), in *T. ambiguum* no vegetative buds were observed along the reproductive stems. Therefore, these stems have no vegetative buds for survival (lateral growth) after defoliation. Thus to re-established inflorescences for seed production, new reproductive stems must be produced after cutting. This finding is contrary to Bryant's (1974) speculation that an early season seed harvest may allow a second season seed crop to be produced. In agreement with Steiner's (**in press**) finding, therefore, *T. ambiguum* should not be hayed if it is grown for seed or if cutting is applied this should be carried out before the appearance of reproductive stems by removing the vegetative shoots only during very early vegetative stage.

An increase of 40% due to cutting at the vegetative stage has also been reported by Gurung in 3 year stands of *T. ambiguum* (1991). Further he stated that this improvement was attributed to the increase in inflorescence numbers, however he did not mention whether this was associated with the increase in reproductive shoot number or the increase in inflorescence number per reproductive shoot. However, Gurung (1991) also reported that later cutting (after the appearance of inflorescence) significantly reduced seed yield.

In this study seed yield of *T. ambiguum* (cv. Monaro) was significantly reduced by cutting treatments regardless of the time of cutting (October/November). Average actual seed yields were reduced more than 66% in defoliated plants compared to the uncut control. This was mainly attributed to the reduction in inflorescence production. October cutting was carried out when a high rate of flower appearances had been achieved, while the November cutting was applied at about 13 days before first peak flowering was detected (**Figure 5.6**).

The reduction in seed yields due to the delay in the date of closing, especially after the appearance of flower buds, have also been reported in other traditional herbage legumes, *e.g. Lotus corniculatus, L* (Bader and Anderson, 1962; Li, 1989), *Lotus pedunculatus* (Hare, 1985), *T. subterraneum* (Rossiter, 1961; Collin, 1978), *Trifolium pratense* (Dade, 1966), *T. repens* (Clifford, 1985), *Medicago sativa* (Kowithayakorn and Hill, 1982), and in *T. ambiguum,M.Bieb* (cv. Monaro and Alpine) (Gurung, 1991). Nevertheless, the reduction in actual seed yield found in this study (66%) was lower than those achieved by Gurung (1991) in the same cultivar (about 88%) after late cutting at reproductive stage. The possible explanation for this is that this experiment was carried on a more mature sward (4 year established stand) than those used by Gurung (1991) (3 year stands). This might affect the plant's resistance to severe defoliation as the stands may have more root and rhizome reserves for recovery growth. These may also have been variations in soil fertility and in seasonal climatic conditions during regrowth. Temperature records (**Appendix 3.2**) shows that the 1988/1989 growing season at Lincoln University Research Farm was drier and hotter than at the AgResearch Grassland Research Unit, Aorangi, Palmerston North. During the 1988/89 season in Lincoln , the monthly mean temperatures ranged from 21 - 24°C, compared to 16 to 18.1°C at Aorangi, Palmerston North. In addition, average monthly rainfall during the same period was only 50 mm in Lincoln compared to 179.8 mm.

CHAPTER 6
GENERAL DISCUSSION AND CONCLUSION

6.1. GENERAL DISCUSSION

Caucasian clover (*Trifolium ambiguum,M.Bieb*) has been trialled in many countries including the USA, Australia and New Zealand. This clover is considered to be important due to its versatility and wide environmental adaptation (Bryant, 1974). In New Zealand, especially in high country environments, this clover has exhibited several characteristics which show it to be a promising plant for soil conservation as well as a herbage producer (Stewart and Daly, 1980). However, further acceptance and wider use by enthusiasts is currently restricted by the lack of reliable supplies of high quality seeds. Suitable management to improve seed production is directly related to an understanding of the morphological and physiological processes which control seed yield.

The protracted flowering characteristics of Caucasian clover result from its the indeterminate growth habit and recent studie have confirmed that flowering in this clover occurs over a period of more than 8 weeks, an extended period comparable to that described previously by other research reports (Stewart, 1979; Dear and Zorin, 1985; Gurung, 1991). Even though detailed data were not taken, results on reproductive shoot numbers and flowering pattern suggest that a long flowering period in *T. ambiguum* appeared to be sustained by a continuing reproductive shoot production rather than by a continuation of flowering on one reproductive shoot. This observation seems to be similar to those reported in *Lotus corniculatus,* by Li (1989). He reported that in *L. corniculatus*, stems do not produce vegetative nodes between successive inflorescences and each shoot bears only a limited number of inflorescences (usually 3-5; in this study around 2 to 5 were found in *T. ambiguum*), all of which open within a comparatively short period (in *T. ambiguum* about 7 to 15 days from the first bud stage). In *T. repens,* the period is longer because this clover continuously forms flowers along fertile stolons and there can be a variable delay in the number of vegetative nodes between any two successive flower buds extending flowering pattern (Thomas, 1987). Since *T. ambiguum* stems do not produce vegetative nodes in this way, all inflorescences in one reproductive shoot tend to open in a relatively shorter period than those in *T, repens.*

In this study an attempt was made to assess the importance of different seed yield components originating from different times and positions (*e.g.* main crowns *vs.* secondary crowns) in determining final seed yield (*i.e.* inflorescence numbers, numbers of florets, numbers of pods per inflorescence, numbers of seeds per pod and seed weight). As has been discussed in **Section 3.4.4**, it was found that inflorescences originating from main crowns bore more buds, florets and seeds per inflorescence than those originating from smaller crowns (secondary crowns). The age (maturity) of the crown (as well as its food reserves) appears to be associated with its ability to produce a high number of inflorescences. The same conclusion has been reported by Gavin *et al.* (**in press**) that seed yield of *T. ambiguum* increased with age as rhizomes increase plant expansion. Another possible explanation for this is that the main crowns originate from the base of the plant which are more mature and have their own strong root system, while secondary crowns are developed from rhizome nodes and do not have strong root systems. Apparently the plants in the first year do not attain enough size to produce high seed yield. It is also possible that plants with different levels of maturity may also differ in their sensitivity to external conditions related to the requirements for flowering (floral stimuli *e.g.* day length and/or temperature) with more mature plants being more sensitive to those stimuli, but this aspect needs further investigation in the future

Reproductive abortion from the bud stage to open flower as well as seed maturity is apparently one of the causes for the reduction in seed yield potential in *T. ambiguum*. Results throughout the flowering season suggest that flower buds consistently abort about 10% of floret buds before the flowers open, a common feature in Leguminosae with a mechanism which is not fully understood, even though this has also been reported in *Lotus spp.* by several workers (Giles, 1949; Bubar, 1958; Joffe, 1958; Seaney and Henson, 1970). The data on florets bearing pods (pod set) suggests that soon after flower opening, floret abortion may also become a major cause of potential loss in this species. The present study also revealed that throughout the flowering season only one out of two ovules in an ovary developed into seeds. Apparently the second ovule is either not fertilised or abortion follows soon after fertilisation (Townsend, 1970). On average 69 % of the florets in an open inflorescence develop into live pods at maturity. In this regard there were no apparent effects from position of flower production. Lower numbers of floret bearing pods and pods bearing seed (seed set) in early flowers compared to middle flower groups may be associated with pollinator activity as suggested by Robertson and Armstrong (1964). However, in general, pollination does not seem to be a serious problem causing low seed yield in *T. ambiguum* (cv. Monaro), as the overall mean of floret bearing pod (pod bearing seed) (69%) was relatively higher than other clovers (*c.f.* 40 to 47% in Zigzag clover, Gurung, 1991). It can be speculated that during the critical period for pollination of middle flowers, environmental conditions might be conducive to pollinator activities resulting in an increase in seed set.

Since all of the inflorescences emerge from the reproductive shoots/stems, the total number of inflorescences produced is dependent to the total number of reproductive shoots per unit area. To improve seed productivity, the manipulation of shoot status to accommodate inflorescence formation seems to be a realistic approach. However, the results of this experiment have shown that ground level cutting at the flowering stage resulted in a significant reduction in inflorescence production and a corresponding decrease in the number of reproductive stems during the same period. This response might be due to the time of cutting as well as the growth habit of the stem *e.g.* no vegetative buds (for branching) were found along the reproductive shoots, thus they are unable to regrow following defoliation (Steiner, **in press**), In addition, the environmental conditions may become unfavourable to recovery or floral development. Apparently, following late defoliation or inter-row cultivation, the growth of newly developed vegetative parts competes with the growth of reproductive components resulting in lower seed yields since these practices were carried out after the appearance of flowers. To reduce the pressure to reproductive development, therefore defoliation might be better carried out at the vegetative stage (refer to **Section 3.4.1** and **5.3**).

The results of this experiment have highlighted the high seeding potential of *T. ambiguum* (cv. Monaro). The potential seed yield of 893 kg/ha, potential harvestable seed yield of 707 kg/ha and actual hand harvested seed yield of 427 kg/ha achieved in this study is higher than the average commercial seed yield value of 153 kg/ha in White clover (cv. Grassland Huia, Hampton and Scott, 1983) and the value of 200-350 kg/ha in *Lotus corniculatus* as cited by Li (1989) and Supanjani (1991). Among these yield parameters, potential harvestable seed yield is considered to be close to maximum achievable seed yield considering density of inflorescences, number of ripe inflorescences at harvest together with seed setting ability. The proportion of potential seed harvested is dependent on pollinator activity at the critical period for pollination and the recovery of seed heads at harvest (Evans *et al.*, 1986). Even though the weather condition during this study were relatively cooler than the 60 year average (**Appendix 3.2**), the average temperatures of 16 to 24°C throughout this study and the sunshine hours in the critical period between October to December (161 to 193) hours can be suggested to be favourable for pollinator activity resulting in successful pollination and hence high seed yield.

The results of this experiment indicated that 30 days from pollination was required for seeds to attain maximum dry wight (mass maturity) as well as substantial viability. The results also suggest that among all stages throughout seed development, the ripening stage from mass maturity toward the onset of shattering and harvest maturity may be the most sensitive stage to changes in weather conditions (**Section 4.4**).

For seed crop management, decisions on optimum harvesting date of *T. ambiguum* are made difficult by the variable frequency of flowering and the appearance of a second peak of flowering. Although there were no differences in seeds per pod and thousand seed dry weight between different times of flower production, the middle flowering group contributed the highest number of inflorescences (70%) to total inflorescences compared to earlier or later groups. The number of inflorescences per unit area was the major yield component affecting seed yield. This agrees with the results found by Gurung (1991) in the same species and similar findings reported by several workers in *Lotus corniculatus* (Albrechten, 1966; Mos, 1983; Stephenson, 1984; McGraw *et al.*, 1986) and in *T. repens* (Maldonado, 1985; Budhianto, 1993). As flowering pattern data indicated that the middle flower group contributed the 70% to total inflorescences, for maximum recovery of high quantity and quality of seeds, therefore, special attention should be paid to middle flowering groups in deciding the optimum time for harvesting the crop by monitoring changes in seed moisture content and dry weight. Under field conditions pod colour can be a reliable indicator for predicting harvest time.

6.2. CONCLUSION

The following conclusion can be drawn from this study :

(1) Inflorescences originating from the main crown produced higher numbers of floret buds and seeds per inflorescence than those originating from secondary crowns.

(2) Throughout the flowering season, *T. ambiguum* consistently aborts about 10% of flower buds before the flowers open. On average 60% of the florets in an open inflorescence develop into live pods at maturity, but only one from two ovules in an ovary usually develop into seeds.

(3) The results of this experiment have confirmed the high seeding potential of *T. ambiguum* (cv. Monaro). The potential seed yield of 890 kg/ha, potential harvestable seed yield of 707 kg/ha and actual seed yield of 427 kg/ha (hand harvested) suggest that *T. ambiguum* (cv. Monaro) has a high capacity for seed production and this should facilitate the production of adequate seed supplies of this cultivar. This high production appeares to be associated with the crops age (4 year established stand).

(4) In the development of seeds of *T. ambiguum* (cv. Monaro), three common features and three developmental phases can be recognised in each case. The first phase last about 14 and 18 days in early and middle flowers respectively. Both flower groups attained maximum dry weight within 30 days after pollination. At this time the pods are yellowish brown in colour.

(5) Hardseededness first occurs in freshly harvested seed after maximum dry weight is reached when seed moisture content percentage close to 20%. Inflorescences set later in the season produced more hard seeds than inflorescences set early in the season (middle flowers) due to climatic conditions. Hot and dry weather conditions during the maturation stage appear to hasten the drying rate and increase hardseededness.

(6) Seed losses due to dehiscence of ripe pods were higher in inflorescence set in the middle of the season than those in early flowers due to both heavy rainfall and strong wind run as well as low relative humidity over the ripening period.

(7) Based on the results of seed development, though some alteration due to weather conditions may occur, harvesting should not be carried out before 30 days after pollination, but should not be left any longer than 38 days, as a high rate of pod shattering began only 8 days after reaching maximum dry weight. Therefore, 34 days from pollination is considered to be an optimum time at which some of the earlier inflorescences have reached maximum dry weight and shattering in middle flowers is minimised. At this stage the colour of flower heads is brown with yellowish brown pod colour. It is recommended that the crops and the weather must be monitored from day

26 after pollination. If a strong winds are blowing and the humidity is low then seeds will ripen and dry rapidly increasing the risk of pod shattering. Heavy rainfall can also enhance the rate of pod shattering.

(8) Cutting the crop at the reproductive stage significantly reduces actual seed yield. This reduction in seed yield is mainly attributed to the low numbers of new reproductive shoots and those the low number inflorescences produced after cutting. It is recommended that cutting should not be done after flower appearance.

6.3. SCOPE FOR FUTURE WORK

The results achieved in this study identify some areas where there are insufficient data in this clover. There are aspects of management and cultural practices which are obvious targets for continued research on Caucasian clover seed production. For example, as discussed in **Section 2.9.1** and **3.2.2**, during preparation of this experiment, the sward was inter-row cultivated. *T. ambiguum* is a rhizomatous legume which have produced very dense rhizomes over four years. However, the assessment of the effects of inter-row cultivation on seed yield and yield components has not been carried out in any detail. The decision not to take any detailed data on the effects of this cultural practice on components seed yield and final yield was made on the grounds of spread workload as the other experiments described in the previous chapter were also being carried out in the same season. It is desirable, therefore, to conduct more study in this aspect in relation to the age/maturity of the swards in the future.

Another interesting area of research would be the determination of the cause of variation in seed set per floret and seed abortion and to study the mechanism controlling these phenomena. If seed set (pod bearing two seeds) per floret could be increased, this would to be advantageous. The role of pollinators and efficiency of fertilisation in improving seed set would seem to be rewarding areas of future investigation.

As defoliation seems impractical for improving seed production, other approaches need to be investigated. One is to use chemicals (*e.g.* PP333) for shoot manipulation during vegetative stage (pre-flowering). This chemical appears to be very successful in improving seed yield in sward situations through its effectiveness in removing apical dominance and hence promoting lateral branching on main crowns in *L. corniculatus* (Li, 1989) and in white clover (Marshall and Hides, 1987). Seed yield of *T. ambiguum* may be increased directly by encouraging greater reproductive shoot production and therefore increasing the potential sites for inflorescence production.

The number of inflorescences per reproductive shoot originating at different times have not been investigated in any detail in the current study. This information would be advantageous for evaluating its response to different treatment such as inter-row cultivation, fertilizer regimes and plant growth regulators (PGR).

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Appendix 2.1. MORPHOLOGICAL DESCRIPTION OF *Trifolium ambiguum* IN GENERAL (Komarov, 1945)

Perennial, 8-6 cm high; taproot straight, woody, multi-capital; stems often short, rarely branched, ribbed, glabrous or slightly pubescent in upper part; leaves mostly radical; stipules lanceolate, broadly scarious-margined; petioles glabrous, the lower 3-2 cm long, the upper shorter; leaflets on short crisp-hairy petioles, lanceolate, glabrous, with an arrow-shaped spot, 1-5 (7) cm long and 0.8 - 3.5 cm broad, broadest somewhat below the middle, the numerous and very prominent veins thickened toward the serrate-denticulate margin; heads terminal, solitary, rarely 2 or 3, at first globose, finally oblong ovoid, 2.5 - 4 cm long, ribbed, glabrous or slightly hairy; bracteoles linear-subulates, scarious, three to six times as long as pedicle, these 1-2 mm long, recurved in fruit, glabrous or sparsely hairy; flowers 12-16 mm long; calyx 5 mm long, the pale tube distinctly nerved, glabrous or in upper part sparsely covered with soft crisp hairs; teeth less than half length of calyx, subulate from broadly lanceolate base, with white scarious margin; corolla white, finally reddish; standard oblong, 12-15 mm long and 5-6 mm broad, narrowly lanceolate; keel 5-6 mm long, lanceolate, broader; ovary sessile, lanceolate, glabrous, 2 ovuled; pod containing 1-2 seed. Fl. June-July. Fr. July-August.

Steppe depressions, forest margin and glades, mountain meadow up to the subalpine and even the alpine zone; in S. Transc. - to 3,000 m above sea level. - European part: Bes. M. Dnp. (SW), Bl., L. Don (SW), Crim.; Caucasus: all regions except Asia Minor (Turkish Armenia-Kars, Melyazgert, Artwin, Ardahan). Described from the Crimea.

Economic importance. A very valuable forage plant, worthy of introduction into cultivation for cutting in perennial pastures; it also holds promise for the Southern parts of the forest zone.

Appendix 2.2. Distinguishing characteristics of species related to *Trifolium ambiguum, M.Bieb.* (Bryant, 1974)

Charac ters	*T. ambiguum*	*T. montanum*	*T. hybridum*	*T. repens*
Inflores cence	capitae, many flowered, oblong	capitae,many flowered, globose or ovate	umbellate, 12-2- flowered	umbellate, 20-40 flowered
Bracts	Linear, lanceolate (c.1mm) not embracing calyx	Broadly lanceolate (c.2mm) embracing calyx tube	-	-
Pedicle	Short (up to 1/3 length of calyx tube)	Short (up to 1/3 length calyx tube)	long	long
Flowers	White tinged, pink, rose pink after anthesis	White, yellowish (rarely pink) yellowish brown after anthesis	Purple, white or pink	yellow, white or pinkish, light brown and strongly deflexed after anthesis
Calyx	10-nerved	10-nerved	5-nerved	10-nerved
Corolla	10-15 mm	12-17 mm	7-9 mm	4-12 mm
Leaflets	Elliptical obovate	Elliptical, leathery	Obovate to rhombic	broadly obovate, rounded at apex
Habit	Rhizomatous	-	-	-
Seed	Legume, 1-2 seeded (c. 3 mm)	Legume usually 2- seeded (c. 6mm)	Legume 2-4 seeded	Legume, usually 3- 4 seeded
Somatic number	2N = 16	2N = 16	2N = 16	2N = 32.16

Note : There are 3 levels of ploidy number in *T. ambiguum* : diploid (2N= 16), tetraploid (4N= 32) and hexaploid (6N=48).

Appendix 3.1. OVERALL EXPERIMENTAL LAYOUT

A. Study of vegetative and reproductive morphology (Chapter III) :

subplot

	I	II	III	IV	V
Plot :	C1	C2	C3	C4	C5

B. Seed development study (Chapter IV) :

Subplot area :

	Group I	Group II	Group III	Group IV
	No. flowers : Early : 150 Middle : 150	No. flowers : Early : 150 Middle : 150	No. flowers : Early : 150 Middle : 150	No. flowers : Early : 150 Middle : 150
Plots :	C1	C2	C3	C4

Notes :1. From each group of flowers, from 14 to 46 days after pollination, 10 inflorescences/group were taken every 4 days.

2. Flowers were randomly tagged every tagging date in the specially allocated area in uncut control plots (C) (see plot layout).

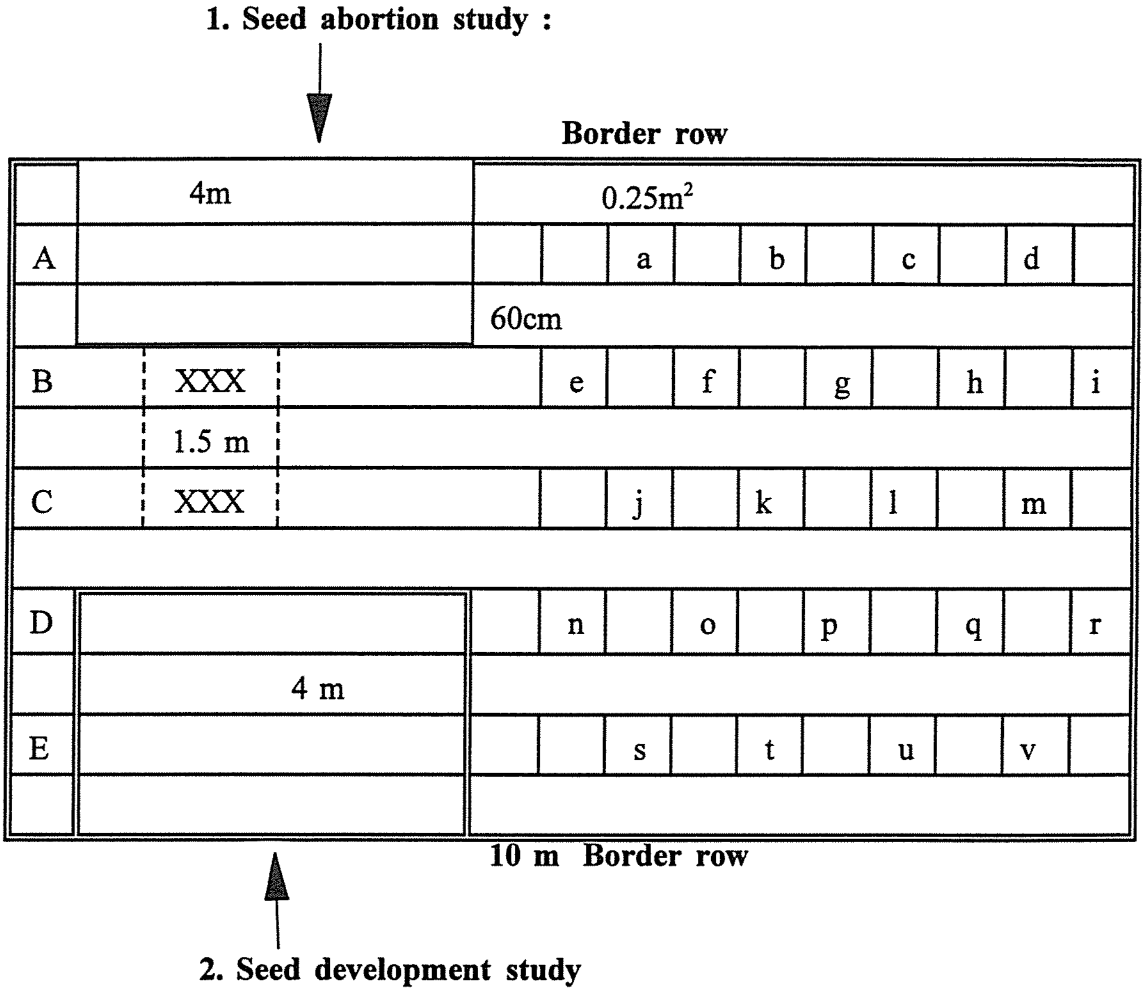
C. Cutting treatments (Chapter V) :

I	II	III	IV	V
O1	**C2**	**N3**	**O4**	**N5**
N1	**O2**	**C3**	**N4**	**C5**
C1	**N2**	**O3**	**C4**	**O5**

Notes :1. **C** : Control (uncut); **O** : October cut (23-10-1991)
N : November cut (22-11-1991); Total plot size : 62 X 20 m

2. Experiments I and II were conducted on specially allocated area on uncut control plots (C) (see **plot layout**)

D. PLOT LAYOUT AND SAMPLING AREAS IN THE PLOT :



Notes :

1. **A,B,C,D,E** : plant rows with 60 cm row spacing.
2. **a to v** : allocated destructive sampling areas (total 22 quadrats of 0.25 m^2) for vegetative and reproductive analysis taken every two weeks, the remaining were used for seed yield analysis at harvest.
3. **XXX** : permanent quadrant for flowering patterns observation (1.5m x 2 rows)

Appendix 3.2. Sixty year average for temperature (minimum and maximum), sunshine hours, day length and rainfall at Aorangi, Palmerston North, and deviations from these average during 1991/1992*

	August		September		October		November		December		January		February		March	
	Temperature (°C)															
	min	max	min	max	min	max	min	max	min	max	min	max	min	max	min	max
Ave. 60 yrs	5.0	13.1	6.6	14.7	8.3	16.6	9.8	18.5	11.6	20.6	12.8	21.9	12.8	22.3	11.7	20.9
1991/1992	1.7	1.2	0.7	1.1	-0.2	-0.6	-1.5	-2.3	-0.3	-1.3	0.1	0.0	-0.4	-1.4	-2.2	-2.3
	Number of Sunshine Hours															
Ave. 60 yrs	132.0		133.0		158.0		177.0		193.0		209.0		186.0		170.0	
1991/1992	-34.4		-7.0		0.9		-42.2		-74.4		-36.6		-18.8		-33.7	
	Rainfall (mm)															
Ave. 60yrs	89.0		75.0		88.0		78.0		94.0		79.0		67.0		69.0	
1991/1992	24.4		-2.4		-11.4		11.0		12.4		-2.8		88.2		19.9	
	Day length (hours, minutes)															
1991/1992	10.30		11.41		13.07		14.25		15.13		14.47		13.55		12.55	

Note * : data (obtained from AgResearch Grasslands) were recorded at the trial area Aorangi, Palmerston North.

Appendix 3.3. The effects of inter-row cultivation on the number of reproductive shoots and inflorescences at harvest (1m row)

Treatments	Flower distributions			Total inflorescences	No. reproductive shoots
	Bud	Open	Ripe		
1. Inter -row cultivated	20 (±12.4)	33 (±9.6)	136 (±16.8)	189 (±16.8)	88 (± 8.6)
2. Uncultivated	50.5 (± 6.87)	99 (±11.2)	236 (± 31.81)	384.6 (± 28.67)	148.8 (±13.42)

Note : Data presented area means of five replications. In the brackets are the standard errors of individual means (± SE)

Appendix 4.1A. Germination results in freshly harvested seed of early group inflorescences from pollination of *Trifolium ambiguum, M.Bieb.* (cv. Monaro)

DAP	Germination (%)					
	NS	AS	HS	FUS	DS	TV
14	6 (±1.2)	9 (±1.8)	0 (±0.0)	13 (±2.1)	72 (±2.7)	28 (±4.7)
18	14 (±2.6)	14.5 (±4.1)	0 (±0.0)	26 (±4.7)	45.5 (±4.2)	54 (±4.3)
22	39 (±2.7)	16.5 (±7.7)	0 (±0.0)	36 (±3.9)	8.5 (±1.5)	91.5 (2.4)
26	55 (±1.2)	3.5 (±1.9)	0 (±0.0)	40 (±3.9)	1.5 (±0.5)	98.5 (±0.5)
30	57 (±1.2)	4 (±1.5)	0 (±0.0)	38 (±4.6)	2 (±0.5)	99 (±0.5)
34	52 (±1.5)	4 (±0.5)	2 (±0.5)	32 (±3.1)	1 (±0.5)	91 (±2.6)
38	36 (±3.4)	6 (±1.2)	18 (±3.4)	34 (±2.4)	2 (±0.5)	96 (±1.4)
42	29 (±1.4)	4 (±0.8)	34 (±3.8)	30 (±3.9)	1 (±0.5)	98 (±0.5)
46	23 (±1.4)	3 (±0.5)	42 (±4.5)	26 (±5.5)	2 (±0.5)	96 (±1.7)

Notes :

Data presented are means of 4 replications, individual SE's are shown in brackets;
DAP : Days after pollination ;
NS : normal seedlings;
AS : abnormal seedlings;
HS : hard seeds;
FUS : fresh ungerminated seeds;
DS : dead seeds;
TV : total viability;
Type of seedling abnormalities : seedling with weak or unbalanced development *e.g.* retarded primary roots with no secondary root and root hairs, short and thick hypocotyl/epicotyl, and mouldy seedlings.

Appendix 4.1B. Germination results in freshly harvested seed from pollination of middle inflorescences of *Trifolium ambiguum, M.Bieb.* (cv. Monaro)

DAP	Germination (%)					
	NS	AS	HS	FUS	DS	TV
14	0 (±0.0)	3 (±0.5)	0 (±0.0)	8 (±4.7)	87 (±8.4)	11 (±2.6)
18	6 (±2.7)	11 (±3.9)	0 (±0.0)	17 (±5.9)	63 (±4.7)	34 (±2.8)
22	25.5 (±3.8)	15 (±6.9)	0 (±0.0)	39.5 (±4.4)	12 (±4.2)	78 (±3.8)
26	59 (±2.8)	6 (±1.2)	0 (±0.0)	31 (±5.0)	1 (±0.5)	96 (±1.2)
30	51 (±1.2)	4 (±0.5)	4 (±2.5)	36 (±3.2)	2 (±0.5)	97 (±0.5)
34	57 (±0.8)	3 (±0.8)	12 (±3.8)	24 (±3.6)	1 (±0.5)	97 (±0.6)
38	28 (±2.8)	4 (±0.6)	48 (±4.0)	16 (±3.0)	2 (±0.5)	98 (±0.6)
42	23 (±3.4)	6 (±1.2)	50 (±4.4)	16 (±1.8)	0 (±0.0)	95 (±0.8)
46	28 (±1.2)	2 (±0.5)	64 (±4.8)	3 (±4.8)	1 (±0.5)	98 (±0.5)

Notes :
Data presented are means of 4 replications, individual SE's are shown in brackets;
DAP : Days after pollination;
NS : normal seedlings;
AS : abnormal seedlings;
HS : hard seeds;
FUS : fresh ungerminated seeds;
DS : dead seeds;
TV : total viability;
Type of seedling abnormalities : seedling with weak or unbalanced development *e.g.* retarded primary roots with no secondary root and root hairs, short and thick hypocotyl/epicotyl, mouldy/decayed seedlings.

Appendix 4.2A. Germination results of seeds from pollination after air drying of early group inflorescences of *Trifolium ambiguum, M.Bieb.* (cv. Monaro)

DAP	Germination (%)					
	NS	AS	HS	FUS	DS	TV
14	0 (±0.0)	0 (±0.0)	0 (±0.0)	0 (±0.0)	96 (±1.8)	0 (±0.0)
18	2 (±0.5)	4 (±0.8)	4 (±0.5)	2 (±0.5)	88 (±6.9)	12 (±0.6)
22	28 (±3.2)	14 (±5.6)	16 (±3.8)	8 (±1.8)	34 (±5.8)	66 (±4.2)
26	32 (±3.5)	2 (±0.5)	56 (±5.2)	2 (±0.6)	4 (±1.8)	92 (±2.2)
30	28 (±3.9)	2 (±0.6)	64 (±5.4)	0 (±0.0)	3 (±0.6)	94 (±1.6)
34	8.5 (±2.4)	1.5 (±0.8)	86.5 (±4.4)	0 (±0.0)	2 (±0.5)	99 (0.5)
38	6 (±1.6)	1 (0.5)	91 (±2.6)	0 (±0.0)	1 (±0.0)	98 (±0.4)
42	3 (±0.8)	1 (±0.0)	87 (±3.2)	0 (±0.0)	1 (0.5)	91 (±2.4)
46	3 (±1.2)	2 (±1.0)	92 (±0.8)	0 (±0.0)	1 (±0.5)	97 (±2.0)

Notes :
Data presented are means of 4 replications, individual SE's are shown in brackets;
DAP : Days after pollination ;
NS : normal seedlings;
AS : abnormal seedlings;
HS : hard seeds;
FUS : fresh ungerminated seeds;
DS : dead seeds;
TV : total viability;
Type of seedling abnormalities : seedling with weak or unbalanced development *e.g.* retarded primary roots with no secondary root and root hairs, short and thick hypocotyl/epicotyl, and mouldy seedlings.

Appendix 4.2B. Germination of seeds after air-drying of middle inflorescences of *Trifolium ambiguum, M.Bieb.* (cv. Monaro)

DAP	Germination (%)					
	NS	AS	HS	FUS	DS	TV
14	0 (±0.0)	0 (±0.0)	0 (±0.0)	0 (±0.0)	98 (±0.8)	0 (±0.0)
18	0 (±0.0)	0 (±0.0)	0 (±0.0)	6 (±1.8)	94 (±1.3)	6 (±0.6)
22	5 (±	10 (±4.2)	4 (0.8)	11 (±4.8)	58 (±3.5)	25 (3.4)
26	18 (±2.9)	6 (±2.2)	40 (±4.2)	6 (±1.2)	30 (±4.5)	70 (±4.2)
30	22 (±3.8)	4 (±0.6)	72 (±5.8)	0 (±0.0)	4 (±0.6)	98 (±0.8)
34	2 (±0.5)	2 (±0.5)	95 (±2.4)	0 (±0.0)	0 (±0.0)	99 (0.5)
38	2 (±1.0)	2 (±1.0)	92 (±2.4)	0 (±0.0)	3 (±0.5)	97 (±1.3)
42	5 (±0.6)	4 (±0.8)	90 (±2.7)	0 (±0.0)	0 (±0.0)	99 (±0.5)
46	2 (±1.0)	1 (±0.0)	91 (±0.6)	0 (±0.0)	6 (±2.2)	94 (±0.5)

Notes :
Data presented are means of four replications, individual SE's are shown in brackets;
DAP : Days after pollination;
NS : normal seedlings;
AS : abnormal seedlings;
HS : hard seeds;
FUS : fresh ungerminated seeds;
DS : dead seeds;
TV : total viability;
Type of seedling abnormalities : seedling with weak or unbalanced development *e.g.* retarded primary roots with no secondary root and root hairs, short and thick hypocotyl/epicotyl, and mouldy/decayed seedlings.

Appendix 4.3. Germination results of seeds after scarification in air-dried seeds of early inflorescences

DAP	Germination (%)					
	NS	AS	HS	FUS	DS	TV
14	0 (±0.0)	2 (±1.0)	0 (±0.0)	0 (±0.0)	98 (±0.5)	2 (±0.5)
18	8 (±0.8)	10 (±4.2)	0 (±0.0)	0 (±0.0)	88 (±6.8)	18 (±4.2)
22	36 (±4.2)	21 (±6.6)	0 (±0.0)	2 (±1.0)	41 (±6.4)	59 (±5.8)
26	74 (±2.7)	5 (±2.0)	5 (±2.2)	2 (±0.5)	14 (±1.3)	86 (±2.8)
30	81 (±2.9)	11 (±1.8)	2 (±1.0)	0 (±0.0)	7 (±2.7)	93 (±1.5)
34	90 (±1.4)	3 (0.8)	2 (±0.5)	0 (±0.0)	5 (±1.0)	95 (±0.4)
38	88 (±2.7)	5 (±2.2)	2 (±1.0)	0 (±0.0)	4 (±2.0)	95 (±2.6)
42	87 (±1.7)	3 (±1.3)	2 (±1.0)	0 (±0.0)	8 (±1.9)	92 (±2.4)
46	94 (±0.8)	2 (±0.5)	1 (±0.0)	0 (±0.0)	3 (±1.0)	97 (±2.0)

Notes :
Data presented are means of four replications, individual SE's are shown in brackets;
DAP : Days after pollination;
NS : normal seedlings;
AS : abnormal seedlings;
HS : hard seeds;
FUS : fresh ungerminated seeds;
DS : dead seeds;
TV : total viability;
Type of seedling abnormalities : seedling with weak or unbalanced development *e.g.* retarded primary roots with no secondary root and root hairs, short and thick hypocotyl/epicotyl, seedlings with broken roots.