SEED PRODUCTION IN CAUCASIAN CLOVER

(*Trifolium ambiguum* Bieb.) CV. MONARO

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1998

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A thesis presented in partial fulfilment
of the requirements for the degree of
DOCTOR OF PHILOSOPHY
in the Institute of Natural Resources
of Massey University
Palmerston North
New Zealand

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1998

ABSTRACT

A number of factors which may affect seed production in Caucasian clover (*Trifolium ambiguum* Bieb.) cv. Monaro were investigated *viz.* root system development and its relationship with seed yield; morphological and reproductive characteristics of different genotypes from within the cultivar; the effects of plant density on seed yield; and, the effects of insect pests on seed yield and quality.

Caucasian clover is a rhizomatous legume pasture plant with a large root system. A sand bed was used to investigate root system development and the relationship between the root system and seed yield. During vegetative growth (September to November) root dry matter (DM) accumulated more rapidly than shoot DM, and the root to shoot ratio was 2.45 by mid November. However as reproductive growth accelerated the rate of root DM accumulation decreased and by early February the root to shoot ratio was 0.65. Plants developed primary, secondary and tertiary crowns in this first growing season, with both primary and secondary crowns producing reproductive shoots. The protracted flowering period (four months) was a result of an initial production of reproductive shoots from primary crowns, and an extended production of reproductive shoots from secondary crowns when primary crown shoot production had ceased. There were therefore two flowering peaks. A single primary crown produced an average seed yield of 3.8 g compared with 0.81 g seed from an average secondary crown. However as there was only one primary crown but 26 secondary crowns per plant, the total seed yield from secondary crowns reached 21.1 g per plant. Root DM was highly correlated (R^2 = 0.80-0.97) with the number of leaves, reproductive shoots and inflorescences, as well as the number of secondary crowns. Seed yield per plant was almost entirely dominated by the number and size of the secondary crowns, which in turn depended on the size of the root system prior to reproductive development.

The seed production potential of three genotypes selected from within cv. Monaro was investigated. The genotypes differed significantly in their morphological and reproductive characteristics, including the number of reproductive stems and inflorescences produced. Genotypes 2 and 12 had seed yields of 3.65 g and 2.99 g per plant respectively, which genotype 9 had a seed yield of only 0.18 g per plant, primarily because it produced very few inflorescences. Poor inflorescence production by some genotypes within a cultivar will limit seed yield and may alter the genotypic composition of the cultivar as seed multiplication progresses.

A radial trial was used to determine the optimal density for seed production of the cultivar. Plant density significantly affected leaf number, reproductive stems, inflorescences number and seed yield per plant in that plants at the highest density (38.2 m^{-2}) produced about 7 times fewer reproductive stems and over 15 times fewer inflorescences per plant compared with those in the lowest density (3.1 m^{-2}). Inflorescence number increased as plant density decreased (R^2 = 0.82-0.89). The lowest density plants had the greatest reproductive growth per plant but were not capable of creating the highest reproductive production per unit area. Cultivar Monaro showed a parabolic relationship for reproductive production per unit area with plant density, both for inflorescence number and seed yield m^{-2}. Plants grown at the density of 10.8 plants m^{-2} produced the highest inflorescence number and seed yield per unit area. The number of inflorescences per plant was the most important seed yield component determining final seed yield in cv. Monaro (R^2 = 0.95). Plant density affected seed yield only through reducing inflorescence number; it had no effect on thousand seed weight, or germination and hard seed percentage. It is probable that plant density affects reproductive development through its effects on root system development in Caucasian clover.

A semi-radial trial was also used to examine the effects of plant density on seed production of twelve genotypes from within cv. Monaro. These genotypes demonstrated significant differences in reproductive capability. Genotypes 1, 2, 4 and 12 represented a high or normal reproductive capability group compared with others and these genotypes produced inflorescences across all five densities. Genotypes 3, 5, 8, 9, 13 represented another group which either failed to produce or produced only a small number of inflorescences even at the lowest density. Genotypes 6, 7 and 10 were intermediate. Seed yield per plant at the 10.8 plants m^{-2} density ranged from 0 g for genotypes 5, 8, 9 and 13 to 0.89 g for genotype 1. Reproductive response to plant density did not differ from that when seeds of the cultivar were sown in the full radial trial.

Insect pests, particularly thrips (Thripidae) may reduce seed yield and quality. The insecticide taufluvalinate (Mavrik) was applied at 150 ml ha^{-1} either once (just prior to peak flowering) or every 14 days until harvest, beginning at first inflorescence appearance. Both adults and larvae of onion thrips (*Thrips tabaci*) and red clover thrips (*Haplothrips niger*) were detected in inflorescences during peak flowering and seed development. The multiple insecticide applications significantly increased seed number per inflorescence and thousand seed weight, therefore increasing seed yield. However the single application had no effect on seed yield or quality.

ACKNOWLEDGMENTS

I wish to express my deepest and sincere thanks to **Professor Murray J. Hill** (chief supervisor, 1993-1997; co-supervisor 1998) and **Professor John G. Hampton** (chief supervisor, 1998; co-supervisor, 1993-1997) for their valuable guidance, helpfulness and support in many ways throughout my study, particularly for their constructive criticism in the planning and writing of this thesis. Enthusiastic encouragement from them has made this work possible.

My sincere gratitude is also extended to the following:

Mrs. Karen Hill for her advice on conducting the germination test and for her assistance in usage of facilities; the other staff of the Seed Technology Centre: Mr. Craig McGill, Mr. Robert Southward, Ms. Collette O'Neil and Ms. Ruth G Morrison, as well as Dr. Ian Gordon and Mr. James Millner for the provision of facilities and assistance in different ways; Dr. Fuyuan Liu, Dr. Guangdi Li, Dr. Zhongnan Nie, Dr. Yuxi Wang, Mr. Tao Zhang, Mr. Xirimenfu and all the postgraduate students at the Seed Technology Centre for their friendship and support.

Mr. Peter T. P. Clifford for his valuable suggestions on my study; Dr. M. P. Rolston, Dr. K. H. Widdup and Dr. S. V. Pasumarty for sharing information with me.

Dr. W. D. Pearson for his very important support in identification of insect pests on Caucasian clover, and Dr. Philippa Stevens for her useful suggestion in the same area.

Professor Roderick G. Thomas for his advice and the supply of the material used in one of my experiments; Dr. Kerry C. Harrington for his assistance with weed identification.

Professor John Hodgson and his wife Mrs. Ruth Hodgson, and Professor Kim Jin Key for their encouragement for my study and their friendship during the study.

The New Zealand Government through the Ministry of External Relations and Trade for financing part of my study in New Zealand; Massey University for awarding me the Helen E. Akers Scholarship for 1993, 1994 and 1995; The Miss E L Hellaby Indigenous Grasslands Research Trust for financial support in 1994.

Mr. L. Gou, Dr. M. Lu, Ms. J. Wang, Ms. J. Yang, Dr. H. Chen, Mr. D. Moar, Mr. W. Bayan, Ms. J. Shen, Ms. Q. Hai, Miss. W. Luo, Mr Xiaoping Li, Ms. Q. Su, Mr. Q. Su, Dr. F. Yu, Dr. C. Cheng and Mr. Z. Li for their friendship and support in different ways.

Finally, I would like to express my deepest gratitude to my parents, brother and sister of my Chinese family, and to my New Zealand family Professor Maurice and Dorothy Alley, my sisters Petina and the late Julie and my brother Malcolm for their love, encouragement, support and understanding throughout the completion of my study. The wonderful time I had with you will be remembered forever.

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Chapter 1

GENERAL INTRODUCTION

Caucasian clover (*Trifolium ambiguum* Bieb.) has a potentially wide range of adaptation throughout high mountain environments in middle latitudes including cold, temperate, intermontane areas, as well as continental rangelands and steppes (Bryant, 1974). It is believed that the clover originated and was distributed in southern Russia, Crimea, the Caucasian region and Asia Minor (Kannenberg and Elliott, 1962).

Caucasian clover had been evaluated for agricultural purposes in the former USSR, Czechoslovakia (Kannenberg and Elliott, 1962), Canada, USA (Agababyan, 1960,1966; Duke *et al*., 1981), Australia (Bryant, 1974) and New Zealand (White, 1995). In northern United States and southern Canada it is used for grazing (Duke *et al*., 1981). It was introduced to the USA in 1911 and Pellet (1946) suggested it had four major advantages: firstly, once established, it provides highly productive and long-lived permanent pasture for hay, forage and silage; secondly, it is profusely flowering with nectar readily available; thirdly, its habit of spreading from underground rhizomes ensures an increase in the stand and fourthly, its extensive root system makes it a good proposition for oversowing on highly eroded sites for soil conservation. 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 the species as a promising plant for eroded slopes as well as a valuable pasture clover for high country environments (Efendi, 1993).

Caucasian clover is a rhizomatous, highly persistent perennial (Speer and Allinson, 1985; Hill and Mulcahy, 1993). It can persist under heavy grazing

(Agababyan, 1960), spreads well over 20 years and is capable of maintaining production for several years without phosphate application (Yates, 1993). It resists insect pests (Norton and Isely, 1967; Stewart, 1979; Lucas *et al.* 1981; Watson *et al.* 1996, 1997) and the viruses common to other clover species (Barnett and Gibson, 1975; Jones *et al.*, 1981; Alconero and Sherring, 1986). It is considered a valuable pasture legume at it produces nutritious biomass (Duke *et al.*, 1981) and is palatable for animals (Kannenberg and Elliott, 1962; Carlson *et al.*, 1985). In addition, Caucasian clover is also being increasingly used for soil conservation due to its deep rooted-rhizome system.

Seed production of Caucasian clover has not satisfied researchers for a long time. Difficulty in achieving high seed yields is frequently reported as a major factor limiting its more widespread use in pasture systems (Widdup *et al.* 1996). Seed supplies at present are very limited and erratic. Seed yields in the first year after planting are often as low as 70 kg ha^{-1}. Seed yields in later years are reported to range from 100 - 600 kg ha^{-1} (Bryan, 1974; Voloshenko *et al.*, 1979; Stewart and Daly, 1980; Steiner, 1992). The factors contributing to this erratic seed yield have been suggested to be associated with the inherently poor flowering of the older cultivars, such as Monaro, and the lack of application of appropriate seed production technology such as appropriate row spacing, sowing rate, weed control, use of the correct inoculants and water management (Hampton *et al.* 1990; Hill and Loch, 1993; Guy, 1996).

The present study was aimed at investigating the role of morphological and reproductive characteristics that may limit full seed yield exploitation within Caucasian clover cv. Monaro, and the effects of plant density and possible insect pests on seed production. The study consisted of several experiments, each emphasising one particular aspect. It begins with a review of the species and its agricultural value, plant growth and development, variation within species and cultivar, effects of plant density and effects of insect pests on seed production, seed production and management which is presented in Chapter 2. Root system development and its contribution to aerial components and seed yield is reported in Chapter 3. Morphological and reproductive characteristics (genetic differences) among different genotypes and their effects on seed production are investigated in Chapter 4 and part of Chapter 5. Chapter 5 mainly focuses on stand establishment, the effects of plant density on vegetative and reproductive growth of Caucasian clover cv. Monaro and the relationship between plant density and seed yield and its components. Insect pests and their effects on seed yield and seed quality are investigated in Chapter 6. The last Chapter (Chapter 7) in this study comprises a general discussion and conclusions, and recommendations for future research.

Chapter 2

LITERATURE REVIEW

Legumes used for temperate pasture purposes include species belonging to the genera *Trifolium, Medicago, Vicia, Lespedeza* and *Lotus.* Of these, the genus *Trifolium* L. contributes the largest number of species widely grown on a commercial basis (Speer and Allinson, 1985). However, only 10 species in the genus are considered to be agriculturally important. Of these, five are annuals: Persian clover (*T. resuponatum* L.), crimson clover (*T. incarnatum* L.), Egyptian clover (*T. alexandrinum* L.), subterranean clover (*T. subterraneum* L.) and yellow suckling clover (*T. dubium* Sibth.); while five are perennials: white clover (*T. repens* L.), red clover (*T. pratense* L.), alsike clover (*T. hybridum* L.), strawberry clover (*T. fragiferum* L.) and Caucasian clover (*T. ambiguum* Bieb.) (Evans, 1976; Speer and Allinson, 1985).

In New Zealand, clovers play an essential role as forage in most pasture farming systems. However, under the harsh conditions of New Zealand hill and high country, there are few species that are able to persist and make a significant contribution to pasture (Scott and Charlton, 1983). Caucasian clover is one legume that has demonstrated an ability to perform in these New Zealand conditions. It grows and persists on low-fertility and acidic soils, tolerates winter coldness and resists some clover pests and diseases, especially grass grub (*Costelytra zealandica* White) (Stewart and Daly, 1980). With the release of hexaploid cultivars such as Monaro which were specifically bred for pastoral use (Dear and Zorin, 1985), increased research in New Zealand has concentrated on Caucasian clover as a grazing plant (White, 1995).

2.1. CAUCASIAN CLOVER (*Trifolium ambiguum* Bieb.) AND ITS AGRICULTURAL VALUE

2.1.1 Species description

Caucasian clover (*Trifolium ambiguum* Bieb.), also known as Kura, Honey and Pellet clover in the USA (Keim, 1954; Kannenberg and Elliott, 1962), is a rhizomatous perennial legume species (Bryant, 1974). Marshall L.B.F.von Bieberstein first mentioned Caucasian clover in 1808 (Speer and Allinson, 1985).

Komarov (1945) described it as a perennial, 8-60 cm high; taproot straight, woody, multi-capital; stems often short, rarely branched, ribbed, glabrous or slightly pubescent in the upper part; leaves mostly radical; stipules lanceolate, broadly scarious-margined; petioles glabrous, the lower 3-20 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 towards 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 pedicles, 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 seeds. Flowering in June-July. Fruiting in July-August in Northern Hemisphere (Figure 2-1).

Zohary (1970) described it as: procumbent to ascending perennial 8-35 cm. Leaflets (1) 1.5-3.5 cm, elliptic to obovate; stipules broadly ovate, adnate to the petiole, the free part lanceolate-subulate. Inflorescence capitate, ovoid, later elongating, 1-1.5 cm wide,

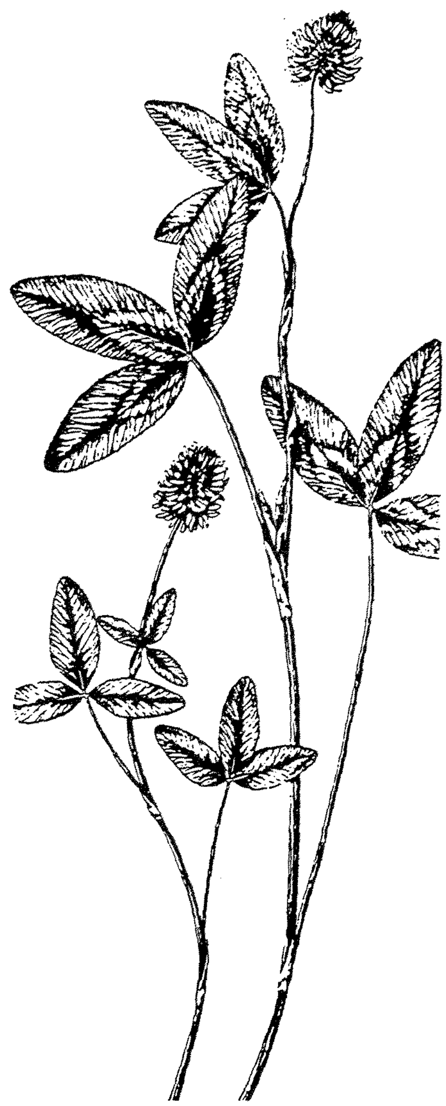


Figure 2-1. Caucasian clover (*Trifolium ambiguum* Bieb.) (From Handbook of Legumes of World Economic Importance. James A. Duke, 1981).

peduncles 4-10 cm. Bracts narrowly lanceolate-subulate, shorter than calyx tube. Flowers white, turning pink, the pedicels at most as long as the calyx, deflexing later. Calyx 4-5 mm, campanulate, whitish, 10-nerved, teeth subequal. Corolla 1.0-1.2 cm, standard longer than keel. Legume 0.3 mm, beaked, 2-seeded.

Taxonomically, *T. ambiguum* is related to the now cosmopolitan *T. repens*, and to *T. hybridum* and *T. montanum*. A number of annuals (*T. retusum* L., *T. balansae* Boiss, *T. badium* Schreb., *T. ochroleucum* Huds.) also appear to be related geographically (Bryant, 1974).

2.1.2 Origin, distribution and habitat

It is believed that Caucasian clover is indigenous to the Crimea, Caucasian region (Azerbaydzhan, Armenia, Georgia) of the former USSR, Eastern Turkey and Northern Iran (Zohary, 1970; Bryant, 1974). This clover is a species adapted to mountain slopes, valleys and screes, drier grassy steppes, edges of forests and fields etc. at 1700-2750 m elevations (Zohary, 1970; Bryant, 1974), glades, mountain meadows up to subalpine regions, and even in mountain alpine regions, up to 3,000 m elevation (Duke *et al*., 1981). A high altitude ecotype has been referred to as *T. ambiguum* var. *alpinum* (Vacek and Ded, 1957).

2.1.3 Growth habit and adaptability

Caucasian clover is a relatively low-growing, rhizomatous perennial herb that possesses a multi-branched deep taproot. Plant height is variable. Initially, stems are frequently procumbent. As development proceeds, stems become ascending, reaching heights of 10-50 cm. There is substantial variation among individual plants, ranging from those that are very erect to those that are prostrate (Speer and Allinson, 1985).

This clover is reported to tolerate annual precipitation of 490-1160 mm, annual mean temperatures of 8.4-12.5° C, pH range of 4.5-7.3, and is tolerant to disease, drought, frost and insects (Duke *et al.* 1981). The species, which has a very broad ecological range, grows in the moister lowlands of the chernozem steppes in South Ukraine, while its var. *alpinum* is found up to 3200 m.a.s.l. on Caucasian meadows and on rocky debris. It can endure inundation and a high level of underground water (Vacek and Ded, 1957).

Caucasian clover is a drought resistant, summer-growing legume that can tolerate low winter temperatures and heavy frosts (Dear and Zorin, 1985). Yates (1993) reported that the clover is unaffected by snow cover or freezing of the surface soil, being completely dormant during winter in high country. It exhibits tolerance to extended dry periods because of its deep and extensive root system (Allinson *et al.*, 1985). The proportion of total biomass contributed by rhizomes and roots increases as soil and climate conditions become more severe. This appears to be an adaptation for survival in cold, infertile environments (Daly and Mason, 1987).

2.1.4 Productivity

Caucasian clover forage yields are initially considerably less than those of lucerne, cicer milkvetch, or birdsfoot trefoil. However its superior persistence under a diversity of cutting schedules and high forage quality indicates its potential as a forage legume for northern environments in USA (Sheaffer and Marten, 1991). Peterson *et al.* (1994) showed that in their clipping and grazing trials conducted on a 5 year old stand of Caucasian clover cv. Rhizo for 3 years, total season yield (mean of 8.6 t DM ha^{-1}) was unaffected by clipping frequency until the third year, when five and six cuttings per season produced 80 and 70% as much forage, respectively, as either three or four cuttings. The forage yield of Caucasian clover was greatest in spring. Allinson *et al.* (1985) also reported that lucerne and Caucasian clover produced 15.1 and 10.7 t DM ha^{-1}, respectively over the 1980-1981 period in U.S.A.

Dear and Zorin (1985) found that under higher rainfall conditions in Australia, productivity of Caucasian clover could be expected to be considerably greater, as was observed in 1981 when, with consistent rainfall in spring, cv. Monaro produced the equivalent of 5500 kg DM ha^{-1} in 4 months. Virgona and Dear (1996) examined the productivity, composition and quality of plots containing white clover cv. Haifa, Caucasian clover cv. Monaro and cv. Apline, and subterranean clover cv. Woogenellup 11 years after establishment in the snowy mountains of New South Wales. When averaged across harvests, Monaro produced more DM at high-fertiliser (280 kg ha^{-1} of Mo superphosphate) and had a significantly higher sown-legume content (75%) than Alpine and Woogenellup. With no fertiliser, total DM production was similar among cultivars but Monaro had a significantly higher legume content (22%).

Caucasian clover is very productive in fertile lowland soils, moderately productive on dry hill country and less so in an infertile high country environment (Daly and Mason, 1987). On the extensive terrace country of Mesopotamia Station (New Zealand) at an altitude of 700 m and with a mean annual rainfall of 1000 mm, cultivar Prairie establishment was sparse and yields were low at first, but after five years it had spread by rhizome growth to form a moderately productive sward (Lucas *et al.*, 1981). Under favourable conditions in New Zealand, the cultivar Treeline yielded up to 12100 kg DM ha^{-1} (Stewart and Daly, 1980; Dear and Zorin, 1985). At Hunua in a drought-prone, medium fertility Haldon soil in New Zealand, the species was twice as productive as Huia white clover (Daly and Mason, 1987).

The role of Caucasian clover as a spring/early summer producer suitable for low fertility high country soils (Scott *et al.*, 1985) can be extended to soils of moderate natural fertility. On such soils, 6-8 t DM ha^{-1} per year could be expected from this rhizomatous clover with infrequent fertiliser application (Daly and Mason, 1987). White (1995) concluded that Caucasian clover shows most promise as a low-input legume for the tussock grasslands, because of its ability to develop a large rhizome/root system, which allows it to persist and produce for many years under a range of grazing management and irregular topdressing.

2.1.5 Nutrient composition and palatability

Caucasian clover is a good pasture legume for animals due to its very nutritious biomass. Green forage of Caucasian clover is reported to contain 79.5% moisture, 4.1% protein, 0.8% fat, and 7.4% fibre. Hay of the clover contains 15.5% moisture, 16.7% protein, 2.8% fat, and 30.3% fibre (Duke *et al.*, 1981). Allinson *et al.*, (1985) showed that the mean *in vitro* dry-matter disappearance (IVDMD) value of Caucasian clover and white clover, averaged over 1981 harvests, was 15 units higher than the mean of other legume species (luceme, crownvetch, cicer milkvetch and birdsfoot trefoil). The data reported in this study indicate that Caucasian clover possesses a high nutritive value. Digestibility, as predicted by IVDMD values, appears to be greater than many other commonly grown leguminous forages. In this regard Caucasian clover appears to have nutritional characteristics similar to that of white clover (Allinson *et al.*, 1985). Certainly, Caucasian clover compares favourably in feeding value with other traditional legumes (Speer and Allinson, 1985). Its non-oestrogenic nature (Anonymous, 1977), its high concentration of nitrogen and minerals, and its high digestibility (FitzGerald, 1980; Davis, 1981; Allinson *et al.*, 1983) are valuable characters which may make this clover superior to other traditional legumes in many circumstances.

In Caucasian clover, the quality of harvested and grazed forage is excellent because of high leafiness throughout the growing season (Peterson *et al.*, 1994). Lambs grazing Caucasian clover and birdsfoot trefoil had similar average daily weight gains due to their high herbage availability and very high forage quality (Sheaffer *et al.*, 1992).

The long-term persistence of *T. ambiguum* in "Tasmanian high country" in Australia is considered outstanding. Yates (1993) reported that cv. Summit, established over 20 years previously, showed no signs of deterioration and had spread well beyond the original plot boundaries It is also capable of maintaining production for several years after phosphate application has ceased, and appears to perform better than subclover in this regard (Yates, 1993). The main reason for Caucasian clover persistence and dominance without maintenance fertiliser is likely to be the ability of the plant to store and reutilise nutrients from its below ground biomass. This large underground root system acts as an important nutrient store that can be remobilised for growth in the absence of regular topdressing. Also, after a period of years without fertiliser, Caucasian clover is capable of responding quickly and strongly to topdressing (Virgona and Dear, 1996) a valuable attribute under low-input systems on high country farms (White, 1995).

Caucasian clover is able to persist under heavy seasonal grazing because of its vigorous rhizome development (Agababyan, 1960) and its many growing points which are below ground level (White, 1995). Under continuous grazing, Caucasian clover will continue to spread laterally. White (1995) noted that after 20 years rhizomes had spread up to 2-3 m outside the original plots at Mesopotamia station (NZ) under set stocking by deer, while new plants had spread from seed dispersal at Mt Possession station (NZ). Although both Caucasian clover and birdsfoot trefoil are well adapted to grazing, Caucasian clover persistence and forage production following three years of grazing with high herbage allowance was superior to that of birdsfoot trefoil. Caucasian clover provided lamb gains per hectare and animal grazing days per hectare equal to or greater than birdsfoot trefoil when the herbage allowance was high (Sheaffer *et al.*, 1992).

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* is restricted (Sinclair and McIntosh, 1983; Scott and Charlton, 1983; Chapman and Macfarlane, 1985). However, Caucasian clover has shown superior persistence under these conditions (Spencer *et al.*, 1975; Dear and Zorin, 1985).

With the demand to develop alternative productive pastures in marginally productive areas, the potential uses of Caucasian clover as a forage legume have become well recognised. Caucasian clover persists during drought (Duke *et al.*, 1981), and can also recover quickly after drought (Dear and Zorin, 1985). However, it is likely that Caucasian clover will be superior only where white clover persistence is poor (Dear and Zorin, 1985).

2.1.7 Pest and disease resistance

Caucasian clover usually resists grassgrub attack (Lucas *et al.*, 1981; Watson *et al.* 1996, 1997). Stewart (1979) observed that Caucasian clover persisted in an area, which was heavily infested with grassgrub where both white clover and alsike clover were killed. The larvae eat the finer roots but the larger taproots and rhizomes survive (White, 1995). Caucasian clover is a host for clover cyst nematode (*Heterodera trifolii* Goffart), but has been demonstrated to be much more resistant to it than white clover (Norton and Iselly, 1967). However, *Epyaxa rosearia* was found to be a significant defoliating insect on Caucasian clover (Watson *et al.* 1996).

Caucasian clover is resistant to many of the viruses common to other temperate clovers, including lucerne mosaic, bean yellow mosaic, clover yellow mosaic, clover yellow vein, pea stunt, white and red clover mosaic viruses (Barnett and Gibson, 1975; Jones *et al.*, 1981; Alconero *et. al.*, 1986). Accessions of Caucasian clover tested were least affected by virus infection, and persisted better than other clover species (*T. alpestre, T. hybridum, T. pratense*, and *T. repens*) (Alconero *et al*., 1986).

2.1.8 Soil conservation and other uses

The deep rooted-rhizome system that Caucasian clover possesses makes it attractive for soil conservation purposes. In New Zealand and Australia this aspect of usage has received some attention. It has been suggested that Caucasian clover has a place in the stabilisation and revegetation of montane, subalpine, and alpine areas, for erosion control in special situations such as waterways, dams, and diversion ditches, and in areas where soil fertility is low and mid-summer droughts is the norm. These are the type of situations in which other legumes, such as white clover, fail to persist (Bryant, 1974; Spencer *et al*., 1975; Speer and Allinson, 1985).

Apiarists consider Caucasian clover to be a good source of nectar (Carlson *et al*., 1985). It has been used by apiarists in the United States (hence the common name - honey clover) (Speer and Allinson, 1985). The sugar content of the nectar is very high, ranging from 42-58% (Pellett, 1945,1948; Speer and Allinson, 1985).

Because of its early seasonal production, persistence under heavy seasonal grazing, its vigorous rhizome development, and its adaptability for growth and persistence in low pH (<5) soils where other clovers (e.g. white clover) fail, Bryant (1974) concluded that Caucasian clover has considerable potential in the Australian high country, both for soil conservation purposes and in pastures, The work of Dear and Zorin (1985) and others supports these conclusions. Most research with Caucasian clover in New Zealand has been in the South Island high country on low fertility soils and in drought-prone climates (Moss *et al*., 1996). The species has shown promise in New Zealand and also in USA (Yates, 1993).

Despite its promising characteristics, however, this clover has not been fully developed commercially and has found only limited use outside its indigenous habitat (Townsend, 1970; Hely, 1971) principally because of its slow establishment and low, unreliable seed yield that includes poor pollination, erratic and protracted flowering, and poor seed set (Hampton *et al.*, 1990).

2.2 CAUCASIAN CLOVER GROWTH AND DEVELOPMENT

The development of a plant can be regarded as having two phases - vegetative and reproductive. In the vegetative phase the plant produces stems and branches, leaves and roots, increasing their numbers and enlarging those that already exist. In the reproductive phase, the plant produces inflorescences and flowers, and pollination takes place leading to the formation of fruits and seeds. These two phases are to some extent antagonistic; when a plant switches from the vegetative to the reproductive phase, vegetative growth becomes less luxuriant and may even stop altogether (Thomson, 1979).

2.2.1 Establishment

In Caucasian clover, establishment from seed is very slow. Caucasian clover seed may have a protracted germination period due to the prevalence of hard seed. The percentage of hard seed in seed lots of Caucasian clover can be over 90% (Khoroshailov and Fedorenko, 1973), but after mechanical scarification most hard seeds germinate. Scarification dramatically increased germination by 40-50% (Bryant, 1974). Aveyard (1970) at Scone, N.S.W. defined the optimum mechanical scarification treatment for seed of *T. ambiguum* cv. Summit, finding that a shaft speed of 2,375 r.p.m. of a standard drum-type scarifier lined with garnet paper (50El grit) gave the highest germination with the least breakage of seed. The 86% clean whole seed obtained by this treatment had a mean germination of 94% (Bryant, 1974).

Germination rate can be affected by temperature. A preliminary examination of the effect of temperature on germination of CPI 2264 Caucasian clover indicated that significant germination occurs at a constant temperature as low as 4° C, exceeded 50% after 10 days at 7° C, and with maximum germination percentages being reached at about 15° C. When germination temperature exceeds 23° C germination is markedly decreased (Bryant, 1974).

The slow establishment of Caucasian clover is usually measured in terms of its production of leaf and stem tissue. Seedling growth is characterised by better dry matter and leaf production from Caucasian clover at 15/10°. When seedling growth of Caucasian clover was compared to that of other forage legumes, Caucasian clover produced less leaf and stem growth after 45 days than white clover, red clover, birdsfoot trefoil, crownvetch and lucerne. However, production of root and rhizome biomass is considerable during the establishment phase (Speer and Allinson, 1985; Hill and Luck, 1991). Spencer *et al.* (1982) showed that although Caucasian clover produced little top growth during the first year, root production was three times that of white clover. Early in the second season of growth, plants have produced multi-branched roots that consistently exceed 60 cm in depth (Speer and Allinson, 1985). Although Caucasian clover may initially produce more belowground tissue than other species, the lack of herbage production during the seedling stage reduces its competitiveness with other species for light (Sheaffer *et al.*, 1992).

In extremely infertile soils, Hely (1963) found that seedling establishment and nodulation was enhanced by applying nitrogenous fertiliser. The nitrogen increased the proportion of late nodulating plants that survived, and subsequently fixed nitrogen, by preventing the onset of symptoms of early nitrogen deficiency.

Good establishment of Caucasian clover in monoculture can be achieved (Speer and Allinson, 1985). This may be partly attributed to inoculation with appropriate *Rhizobium* and the development of effective nodules (determined by visual observation of nodule size and colour), lack of which has been reported to limit Caucasian clover establishment at many sites (Bryant, 1974; Speer and Allinson, 1985; Sheaffer *et al.*, 1992). This will be discussed in the following section.

2.2.2 Root system development and nodulation

Root system development

A comprehensive review by Troughton (1957) has shown that in a wide range of plant species a high proportion of the total root mass is usually present in the surface layers of soil. Chemical analysis of samples from the soil profile show an accumulation of plant nutrients in the top 10 cm. Hence the proliferation of roots in this layer may have been stimulated (Millar 1925; Duncan and Ohlrogge, 1958; Wiersum 1958; Ozanne *et al.* 1965).

In a range of adapted legumes, all of which were capable of achieving similar biological or grain yield, wide variation exists in root characteristics. Thus differences in rooting depth, density and distribution can all influence water use and long-term productivity in a potentially 'high risk' environment subject to drought, secondary salinity and low inherent fertility (Hamblin and Hamblin, 1985).

Unlike other traditional herbage legumes, the structure of the Caucasian clover root system is complicated. Peterson *et al.* (1994a) separated the root system into eight fractions and described them as follows:

1. **Primary crowns.** The dominant crowns in a multi-crowned plant, primary crowns are assumed to be original plants, often composed of several short, woody branches giving rise to aerial shoots.
2. **Primary taproots.** Thick, woody, sometimes branched roots attached to primary crowns.

3. **Rhizomes.** Horizontally growing stems that arise from primary crowns, other rhizomes, and secondary crowns. Rhizomes are present in the upper 5 cm of soil.
4. **Secondary crowns.** Thickened, woody structures ranging from 0.5 to 4 cm in length and 0.3 to 1 cm in width, secondary crowns are produced at the end of rhizomes, giving rise to aerial shoots and additional rhizomes.
5. **Secondary taproots.** Roots > 0.3 cm in diameter at their point of attachment, secondary taproots arise from rhizome nodes and secondary crowns.
6. **Fibrous roots.** Roots < 0.3 cm in diameter at their point of attachment, fibrous roots arise from primary and secondary crowns and rhizomes.
7. **Rhizome shoots.** Unthickened regions that produce aerial shoots at ends of rhizomes and at rhizome nodes.
8. **Rhizome initials.** Tender, white, new rhizome growth, rhizome initials are the sites of rhizome extension and rhizome shoot and secondary crown development.

Rhizome development of Caucasian clover is extensive and top growth poor during the year of establishment, but excellent top growth can be produced and plants spreaded considerably in the following years (Townsend, 1970). Caucasian clover apparently continues to spread by making substantial underground growth because the top growth recovered rapidly when good rains came (Spencer *et al.*, 1975). Caucasian clover produced a strong taproot system comprising 4 to 6 thick main roots to 30-40 cm. All diploid and tetraploid lines spread to a similar extent but hexaploids can be more variable in this respect (Dear and Zorin, 1985; Gurung, 1991).

Under clipping and grazing conditions, Peterson *et al.* (1994b) showed that crowns, rhizomes, and roots from a 5 year old stand comprised about 25, 45 and 30%, respectively, of the total below ground mass sampled to a 15 cm depth, which averaged 6600 kg ha^{-1}. Primary crowns decreased from 95 to 55 m^2 during two years of grazing, whereas secondary crowns produced by rhizomes averaged 1340 m^2 and dominated aerial shoot production.

Strachan *et al.* (1994) reported that Caucasian clover cv. Prairie (hexaploid) persisted and dominated in high country plots which received more than 100 kg P ha^{-1} at establishment. After 13 years the biomass of coarse roots (rhizomes and taproots) amounted to 20 t ha^{-1}. Nutrient storage, as a function of underground biomass amounted to up to 58 kg P ha^{-1}, 455 kg N ha^{-1} and 20 kg S ha^{-1}. This amount of phosphate equates to 650 kg ha^{-1} superphosphate. The ability to store and remobilise these nutrients over the growing season is a valuable attribute of this plant in the high country where, in the absence of regular topdressing, other legumes have failed to persist.

Plants adapted to persistence under cold winter environments need to have the ability to withstand frost heave, and to survive many months without photosynthesis. The extensive and long-lived root system of Caucasian clover confers this species with these abilities. Because the rate of uptake of nutrients from cool soils is slow, it is likely that in the spring, nutrients are mobilised from the large reserves in roots and rhizomes in order to match the increased demand resulting from the increase in the rate of production of herbage at this time. In this way, the large nutrient content in roots and rhizomes may be functionally significant and so it can provide an explanation for the dominance of Caucasian clover in such extreme environments (Strachan *et al.* 1994).

Nodulation

The successful formation of nodules on legume roots relies on a complex sequence of physiological processes, many of which involve interaction between bacterium and host. Failure may occur throughout this sequence, from infection failure through to poor symbiotic fixation where nodules provide insufficient nitrogen for the host plant to achieve maximum growth. Any attempt to examine, or to improve, the nodulation of legumes must involve both bacterium and host (Gibson, 1980).

Nodulation is more variable in out-crossing than in self-pollinated species. This variability has been exploited in the selection of plants for early nodulation, increased nodule volume, sparseness or abundance of nodulation, and for symbiotic effectiveness. One of the more successful breeding programmes has been with Caucasian clover, which seldom nodulate, and then often very slowly and with poor effectiveness, even when inoculated with Rhizobium strains from the host region of origin (Gibson, 1980).

Caucasian clover is known to persist and produce under drought and low input systems (Woodman *et al.*, 1992; Yates, 1993) but is considered difficult to establish (Lucas *et al.*, 1981). Substandard inoculation is one possible reason for this problem as strains of rhizobia nodulating white clover either do not form nodules or form nodules that do not fix nitrogen on Caucasian clover (Parker and Allan, 1952). Inoculation with an effective strain of rhizobia is considered to be essential (Patrick *et al.*, 1994).

Evans and Jones (1966) showed that like other legumes, Caucasian clover responds to inoculation with an effective strain of *Rhizobium* both in the field and in the glasshouse. In earlier work, Erdman and Means (1956) reported dry matter and nitrogen yield increases in Caucasian clover after inoculation with *Rhizobial* strains from the Turkish clovers *T. spadiceum* L. and *T. ochroleucum* Huds. and from soils in which Caucasian clover occurs naturally (Bryant, 1974).

Evans and Jones (1966) demonstrated consistent differences between the responses of three chromosome races to inoculation, with diploids responding much later than tetraploids or hexaploids. This confirms observations by Hely (1957) who also found that diploid Caucasian clover was slow to nodulate and that consequently the benefits of more effective nodulation did not appear until much later in the growth of the plant.

It was reported that strains CC231a, CC283b, and CC286a of *Rhizobium trifolii* have been shown to be effective in inoculating a wide range of Caucasian clover ecotypes (Zorin *et al.* 1976a, 1976b; Dear *et al.* 1985). Using these rhizobial strains, Caucasian clover remained well nodulated in the field and had acceptable herbage nitrogen contents (2.3-3.4% N) (Zorin *et al.* 1976a, 1976b; Dear *et al.* 1985).

Levels of seedling nodulation in Caucasian clover cv. Monaro oversown on a range of tussock grassland sites were similar to white clover when seed was inoculated at a high rate (>3000 rhizobia/seed at sowing; Lowther and Patrick, 1992). Patrick *et al.* (1994) also demonstrated that nodulation effectiveness of oversown Caucasian clover cv. Monaro was very responsive to inoculation level. Inoculant was applied to seed at a range of rates, in a gum arabic/lime pellet, and when sown one day after inoculation the number of rhizobia ranged from 200 to 260,000 seed^{-1}. The percentage of seedlings which nodulated progressively increased from 5% up to 66% as the number of rhizobia/seed increased. The number of seedlings established at the end of the first season showed a similar response, illustrating the importance of maximising seedling nodulation.

The strain ICMP4073b of rhizobia was reported to be suitable for use under oversowing, although the minimum population of rhizobia needed for satisfactory nodulation was not defined (Patrick *et al.* 1994). The superiority of ICMP4073b in effectively nodulating oversown seedlings, coupled with effective symbiotic nitrogen fixation, indicates that this strain should be used to inoculate tetraploid cultivars of Caucasian clover, such as Treeline, when seed is oversown in New Zealand. The use of the same strain (ICMP4073b) also simplifies inoculant production and use for Caucasian clovers (Patrick *et al.* 1994).

2.2.3 Vegetative growth, reproductive growth and root/shoot ratio

Vegetative growth

Speer and Allinson (1985) observed that rhizome development began approximately three months after sowing. Daughter plants may have generally developed within 45 days after planting single-node rhizome segments. Spaced plants of Caucasian clover, when started from rhizome segments, produced approximately 19 daughter plants for each rhizome planted, after a five month growth period (Pellett, 1945).

After about a year of mostly favourable moisture conditions in an Australian montane environment, Caucasian clover plants spread by rhizomes, filling in spaces between the original plants by the production of daughter plants (Spencer *et al.*, 1975). The extent of this type of stand formation, as the result of vegetative spread, is quite variable. In some cases the mother plant develops to an appreciable size by the continued development of the main crown composed of many petioles. In these cases, few daughter plants develop. Other Caucasian clover plants initiate vigorous rhizome and daughter-plant development, ultimately resulting in a compact stand. A third type of propagation results in a wide area being colonised by relatively few daughter plants. These more open stands may be 1-1.5 m in radius, with the mother plant (primary crown) at the centre (Speer and Allinson, 1985).

Vegetative growth of pasture plants occurs until they are ready to flower. At this time, certain external stimuli can trigger floral induction, a physiological change that permits the development of reproductive primordia. This change may precede actual flowering by several days, weeks or even months (Hampton, 1994). The change from purely vegetative growth to flower head initiation is under the control of the environment, the two most important controlling factors being day length and temperature (Hampton, 1994). Thomson (1979) stated that the change occurs only at the season when the days are of a particular length and, if the plants are kept in the wrong day-length, they remain vegetative indefinitely. Some crop species and cultivars have a critical temperature requirement. Flowering may not follow immediately after exposure to this temperature, but some time later.

Having an indigenous habitat in high elevation areas and regions with cold winters, it seems that Caucasian clover appears to have strong photoperiod and vernalisation requirement for commencing reproductive growth (Meares, 1975). Caucasian clover is regarded as flowering under a long-day photoperiod. Kannenberg and Elliott (1962) obtained flowers when Caucasian clover was grown in the greenhouse using a 17-h photoperiod (Speer and Allinson, 1985).

Reproductive growth

Morphologically, Caucasian clover produces leaf and petiole growth above ground until reproductive stems are initiated and elongate in spring. These stems are multi-nodal and are capable of producing one or more peduncles which bear ovoid flower heads (Steiner, 1992). Flower heads can be lateral or terminal (Duke, 1981). The reproductive stems are indeterminate (Speer and Allinson, 1985).

Inflorescence emergence and growth, inflorescence and floret size, and the abortion of developing inflorescences and florets are strongly affected by environmental factors. Both temperature and day length strongly affect the rate of inflorescence emergence and growth (Thomas, 1987c). 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 suggests 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; Townsend, 1970; Meares, 1975). In Iowa

USA the principal period of flowering was from early June to mid July (Pellett, 1945). However, flowering in Connecticut USA can be in mid-May and continues through June, when herbage is harvested. Regrowth flowers in mid- to late summer and after another harvest in August, occasional flowers are produced in mid-September (Speer and Allinson, 1985).

Townsend (1970) reported that there was a highly variability in flowering dates within ploidy levels, with diploids earlier than tetraploids which were also earlier than hexaploids. Under field conditions in Michigan, diploid collections flowered earliest and most profusely, reaching their flowering peak on May 20 and diploids also flowered earliest in Colorado. However, there was an overlap in flowering dates among all ploidy levels (Speer and Allinson, 1985).

In Australia, the first flower buds can usually be seen in mid-October with peak flowering in November (Dear and Zorin, 1985). The flowering of the diploid cultivar, Summit, occurs in early October, and seed maturation and harvest in mid January at Cooma, Australia. Immediate post-harvest mowing or grazing may result in a second flowering and seed harvest in March-April at least in the Cooma environment (Bryant, 1974). In the North Island of New Zealand the flowering of the hexaploid cultivar Monaro begins in early November with peak flowering in early December. Mature seed can be harvested by mid January (Coolbear *et al.*, 1994). Caucasian clover has a characteristic protracted flowering. This largely results from the continuous production of reproductive shoots from crowns. Two classes of crowns can be identified, the large, main crowns producing an average of 7.4 shoots per crown at peak flowering compared to smaller, secondary crowns producing only 1.7 shoots (Coolbear *et al.*, 1994).

Pollination is an important procedure during flowering in Caucasian clover because it is a cross-pollinated species. In legumes, pollination is a two-stage process. Tripping, which involves releasing the pistil and staminal column from the fused and clasping keel petals of the flower, is then followed by the transfer of pollen, preferentially from a different plant, to the stigma (Fairey, 1993). In most temperate forage legumes, insect pollinators play a pivotal role in seed set. Honey bees (*Apis* spp.), leafcutter bees (*Megachile rotundata* Fab.), Alkali bees (*Nomia melanderi* Cockerell) and bumble bees (*Bombus* spp.) are the predominant pollinators (Fairey, 1993). The foraging behaviour of bees is influenced by weather conditions and the attraction of nectar secretion (which is also influenced by environment). During unfavourable weather (wet, cool and windy) bees tend to forage nearby, or remain in the hive, while warm and dry weather favours bee foraging (Free, 1970). Nectar secretion is greater on a sunny than a dull day, reflecting the fact that the nectar sugars are products of photosynthesis, which in turn is influenced by sunlight (Shuel, 1952). Once a floret has been pollinated, compatible pollen grains germinate quickly on the stigma. The tube nucleus and generative cell then pass into the growing pollen tube where the latter divides to give rise to two male gametes (Chubirko, 1965). Temperature has a strong influence on pollen germination and pollen tube growth, which are faster at high temperatures than low temperatures (Chen and Gibson, 1973).

Caucasian clover has a high degree of self-incompatibility. Complete self-incompatibility has been noted by Guravich, (1949), Keim (1954) and Kannenberg and Elliott (1962). However, some self-compatibility often exists, although this is often less than 1% (Hely, 1957). Townsend (1970) showed that 56% of a population (465 plants representing 51 populations) was completely self-incompatible, while 36% set only 1-4 seeds per head. In a freely flowering, open-pollinated diploid (CPI. 2264), however 100-130 seeds matured per head (Bryant, 1974).

Root-shoot ratio

Caucasian clover is a species closely related to white clover (Zohary, 1970) but is more perennial, with prominent and persistent semi-woody, often branching taproots and having its runners (rhizomes) produced underground (Donskova, 1968, Spencer *et al.*,

Table 2-1. Root distribution of Caucasian and white clover 17 months after sowing.

Clover and cultivar	DM of roots 0-10 cm	DM of roots 10-20 cm	Yield of foliage	Root / shoot ratio
	(kg ha^{-1}*)		(kg ha^{-1}*)	
T. ambiguum				
Summit	3270	600	1640	2.36
CP150329	4350	780	1640	3.13
T. repens				
NZ. Huia	920	0	6060	0.15
Tasmania	1010	0	5640	0.18

* Means of 2 sites and 5 phosphorus treatments. (From Spencer *et al.*, 1975)

Table 2-2. Root/rhizome: shoot ratio of Caucasian clover.

Location	Ratio	Plant age	Source
Victorian Mts., Aust.	3.13	17 months	Spencer *et al.* (1975)
Victorian Mts., Aust.	5.73	12 months	Spencer and Hely (1982)
Mesopotamia, NZ	2.73	9 years	Daly and Mason (1987)
Mesopotamia, NZ	3.15 sod-seeded	6 months	Moorhead *et al.* (1994)
	3.36 strip seeded		
Mt. Possession, NZ	4.60	13 years	Strachan *et al.* (1994)

(From White, 1995)

1975). Well established Caucasian clover, compared with white clover, has a root system which extends to a greater depth and which is substantially more fibrous and stouter than that of white clover. Some four times as much underground plant material was recovered from Caucasian clover plants than from white clover (Table 2-1) (Spencer *et al.*, 1975).

The contrast between the root distribution of these two clovers was striking when root/shoot ratios were calculated. The ratio was some 16 times greater in Caucasian clover. By far the greatest part of the plant weight is present above ground in white clover, whereas most is below ground in Caucasian clover (Spencer *et al.*, 1975).

White (1995) stated that the underground biomass of rhizomes and roots increases over time in Caucasian clover. At three weeks of age 30-35% of the dry matter of seedlings can be underground biomass (Paljor, 1973), with the proportion increasing to 50-60% after 3 months (Meares, 1975) and 70-80% in 17 months (Spencer, 1975). Coolbear *et al.* (1994) showed that the highest proportion of aerial components observed in Caucasian clover cv. Monaro was 32% which was similar to data reported by Spencer *et al.* (1975) for other cultivars. In general, mature plants or mature swards commonly have a rhizome and root / top ratio of 3 or higher (Table 2-2) (Daly and Mason, 1987; White, 1995).

2.2.4 Seed development

Studies on seed development in several traditional herbage legumes have been carried out by many workers (Hyde, 1950; Hyde *et al.*, 1959; Win Pe, 1978; Kowithayakorn and Hill, 1982; Hare and Lucas, 1984). However, seed development studies in Caucasian clover are very limited. Information on herbage legume seed development (e.g. white clover) can perhaps be used as comparable to the situation that occurs in Caucasian clover.

Under natural conditions, the development of an ovule into a mature seed following pollination and fertilisation comprises three stages (Hyde, 1950):

a. The growth stage (Stage I):

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

b. The stage of food reserve accumulation (Stage II):

This stage takes 10-14 days following the first stage or 20-24 days after pollination. It is characterised by a constant rate of growth and is presumably determined by the rate at which food reserves can be transferred from the parent plant to the seed. The dry weight of the seed during this stage increases about three times, reaching a maximum at the end of the period. The actual amount of water in the seed decreases slightly, but seed moisture content changes from 79% to 63%. At the end of this stage the seed is structurally complete and attains total viability and vigour, being physiologically mature.

c. The ripening stage (Stage III).

This stage lasts from three to seven days from the completion of the second stage. During this period the seed dries out rapidly and shrinks in size. The dry weight changes very little, but the fresh weight decreases by over a half as the moisture content decreases from 63% to 10%. The seed moisture content finally reaches equilibrium with the relative humidity of the surrounding environment.

Efendi (1993) has reported that in seed development of Caucasian clover cv. Monaro the same three developmental phases can be recognised. The growth stage lasted about 14 to 18 days and the seed moisture content during this stage was very high at 70% - 80% depending on flower position. In general, such seeds are non-viable. Even though the timing of the onset of food reserve accumulation stage differed, a peak of seed germination and viability was reached during this stage some 26 days after pollination. Maximum dry weight was reached by 30 days after pollination, at which time the seed moisture content had fallen to 30 to 40% and, the pods were yellowish brown in colour. To ensure the production of good quality seed combined with high yield in Caucasian clover it is necessary, therefore, to wait until at least 34 days after pollination before harvesting the seeds.

Efendi (1993) demonstrated that hard seed development in Caucasian clover began 30 days after pollination. However, satisfactory germination results (over 90%) were obtained when seeds were tested following mechanical scarification (rubbing between sand paper for one minute) implying that the hard seed condition in cv. Monaro is of a "shallow type' which is easily removed, as suggested in lucerne by Kowithayakorn and Hill (1981). It also indicated that hardseedness may be the only dormancy mechanism involved in the seeds of the cultivar (Efendi, 1993). Moreover, hard seed development means that seed development is not necessarily completed when maximum dry weight is achieved (Ellis and Filho, 1992).

Kannenberg and Elliott (1962) reported that 35-145 and 43-133 seeds per head can be obtained from diploid and hexaploid cultivars of Caucasian clover, respectively. The 1000 seed weight ranges from 1.0 to 3.2 g (Khoroshailov and Fedorenko, 1973).

In many flowering plants, only a small proportion of ovules initiated result in the formation of mature seeds (Stephenson, 1984; Bawa and Webb, 1984; Lee, 1985). Flowers and fruits are aborted at various stages of development and, in fruits that are matured, not all ovules give rise to seeds (Hossaert and Valero, 1988). The abortion of some developing seeds might arise as a result of competition for nutrients within the inflorescence (Atwood, 1940). In Caucasian clover, there is no information available on this aspect. It needs to be investigated in future work

2.3 GENOTYPE (Variation within Species and Cultivar)

Caucasian clover is regarded as being a considerable genetic mixture. It is one of a few species in the genus *Trifolium* L. exhibiting natural polyploidy (Kannenberg and Elliott, 1962). By cytological examination three naturally occurring chromosome races of diploid (2n = 16), tetraploid (2n = 32) and hexaploid (2n = 48) with a basic somatic number of 2n = 16 have been confirmed (Hely, 1957; Kannenberg and Elliott, 1962).

Khoroshailov *et al.* (1987) reported that of 116 seed samples collected from all areas of distribution of the species in the USSR, the most widespread cytotype proved to be hexaploid (2n = 48). Tetraploids were a half and diploids a third as frequent. Diploids have so far been found only in the Caucasus, where the whole range of cytotypes can be found, including an octoploid (2n = 64) reported for the first time from Armenia (Khoroshailov *et al.*, 1987).

Diploid, tetraploid and hexaploid cultivars (2n = 16, 32, 48) have been developed in Australia (Duke *et al.*, 1981). The most common of these cultivars under field assessment in New Zealand are Summit (2n), Forest (2n), Treeline (4n), and Prairie (6n), and the most recently produced Australian cultivars Alpine (2n) and Monaro (6n). Morphological and agronomic characteristics of these cultivars have been described by Bamard (1972) and Gurung (1991).

Morphologically, Caucasian clover is a very diverse species (Townsend, 1974). In general, plant height, leaf size, petiole length, rhizome production and overall plant size increase as the ploidy level increases (Hely, 1957; Kannenberg and Elliott, 1962; Baysal, 1974; Meares, 1975), but considerable variation exists for parent plant longevity and daughter plant frequency (Hill and Mulcahy, 1993). However, there is still considerable morphological variation within each ploidy level, both among and within populations (Kannenberg and Elliott, 1962; Stewart, 1979).

Seed size, seedling vigour, and adaptability increase with ploidy. Flowering tends to be earlier and more predictable in the diploids, but highly daylength dependent in the tetraploids, and variable in the hexaploids (Hill and Mulcahy, 1993).

Kannenberg and Elliott (1962) have shown that the average weights per 100 seeds for the 2n, 4n, and 6n ploidy levels in Caucasian clover are 0.1510, 0.1716, and 0.2986 grams, respectively. Screening indicated that separation of 2n and 4n seed mixtures would not be feasible, but that 6n seeds could be effectively screened from mixtures with 2n or 4n seeds. For example, a 1/14 inch mesh screen removed 40% of the 6n seed and none of the 2n and 4n seeds. A 1/15 inch mesh screen removed 74% of the 6n, 1% of the 4n, and none of the 2n seed. Seed weight may be used to identify ploidy level. When individual plants are grown under the same environment, 100-seed weight can be an effective means of identifying 2n, 4n, and 6n types. Most certainly it can be used to distinguish 2n or 4n plants from 6n plants, and it probably is adequate in separating 2n from 4n plants (Kannenberg and Elliott, 1962).

General agreement has been found between the effects of polyploidy in Caucasian clover and the effects of induced polyploidy in other species. However, some differences were noted. Induced polyploids are reported to have fewer flowers and rhizomes, but in Caucasian clover these characteristics increased directly with ploidy. Although induced polyploids generally have fewer florets per head, no decrease in floret number was found for polyploid Caucasian clover. These changes are probably a result of recombination and natural selection over an extended period (Kannenberg and Elliott, 1962).

Khabibov (1980) also reported that in Caucasian clover, marked variation was noted in quantitative characters of the flowers, fruit and seeds in the same populations in different years and at different altitudes in Dagestan. Populations from high-altitude meadows in the plateau-like territories of Dagestan were best for selecting forms with a high seed yield.

2.4 PLANT DENSITY

For any species it is advantageous to achieve the optimum sowing or planting distance, as plant density can markedly affect plant performance. Too dense a plant stand increases competition for certain essential growth factors (nutrients, sunlight and water) and increases the risk of disease, resulting in lower yield and quality because of thinner, weaker plants with lower tolerance to less favourable conditions. In contrast, with too low a population, weeds have the opportunity to enter the crop and can indeed overgrow it. Only a well developed plant with sufficient space at the fully grown stage, so that it can dry quickly after rain or dew, can produce high seed yield and quality (Janick, 1972; Vis, 1980; Phetpradap, 1992).

Competition occurs between plants when they are grown at a high density. Donald (1963) defined competition as "a phenomenon which occurs when two or more organisms seek the measure they require of any particular factor or thing, and when the immediate supply of the particular factor or thing is below the combined demand of the organisms". In general, competition occurs both within each individual plant (intra plant competition) or between individual plants (interplant competition). Intraplant competition is defined as the competition for limited growth factors between the organs within a plant. Every organ is in competition with every other organ. For example competition for light between leaves. In such cases it is common for leaves to be so heavily shaded by those above as to die because they are not supported by export of assimilates from other parts of the plant (Donald, 1963; Etherington, 1983). Each flower and fruit is also to some degree in competition with the other flowers and fruits on the plant (Addicott, 1982; Phetpradap, 1992). Interplant competition most commonly occurs as a result of limited supplies of light, nutrients, water, carbon dioxide and to a lesser extent, space (Donald, 1963; Rhodes, 1970; Etherington, 1983).

The two main factors involved in plant population are the number of plants per unit area (plant density) and spatial arrangement (plant rectangularity). Plant population density is a more important determinant of yield than planting arrangement (Field and Nkumbula, 1986). At a given density, the highest yield is normally obtained from an equidistant plant spacing since this offers a more desirable environment for the spread and development of plants than those with a spacing of unequal dimensions (Mack and Varseveld, 1982). Because there is little information available on the effect of plant density on seed production in Caucasian clover, this discussion, therefore, aims to review information supplemented by documented results of plant density effects in more common herbage legumes (e.g. white clover, red clover, lotus and lucerne). Further information on this aspect on Caucasian clover will be discussed in Chapter 5.

2.4.1 Effects of plant density during vegetative and reproductive growth

The sward microenvironment (i.g. light, temperature, moisture) has an important effect in the development of the plant (Zaleski, 1964). Light intensity affects vegetative growth and floral induction and, consequently inflorescence development. Low light intensity retards the vegetative growth of white clover, especially the elongation of stolons, even under a long day with optimum temperature. It appears that some plants can produce more inflorescences under a high light intensity, even for less than 15 h, than the same plants under a low intensity but long day (17 h). This suggests that the response of the clover plants to light intensity is great and varies between individual plants. Low intensity may lead to a complete failure of floral induction or to a complete or partial abortion of buds and inflorescences, even at suitable temperatures. The highly significant positive correlations between light intensity and length of stolons, and also number of inflorescences, emphasises the importance of high light intensity for both growth and inflorescence formation. The importance of light intensity in the growth of herbage plants and the significance of leaf area in growth are now widely recognised (Zaleski, 1964).

In lupin, a plant density study (ranging from 27 to 156 plants m^{-2}) showed that the canopy of cv. 'Wau11b' (*Lupinus angustifolius*) in low-density plots was more open, with plants having a more even leaf distribution from upper to lower levels. Thus light had greater penetration to basal branches. Herbage in this condition was of better nutrient quality, containing more leaf when dry weight was maximal. Low-density plants also had higher relative growth rates and net assimilation rates. In high-density plots a dense upper canopy was formed which possibly caused some self-shading (Herbert and Hill, 1978a). Reduction in seeds per pod at higher densities indicates increased competition, possibly as a result of earlier canopy closure. These reductions are similar to reductions that occur between inflorescence orders. This suggests that plants are quite sensitive to interplant competition, which also results in fewer branches at high densities. Thus factors influencing branch formation may be similar to those influencing seed set in pods on the subtending inflorescence (Herbert and Hill, 1978c).

Plant density influences the moisture level in the soil. *Lupinus albus* plants grown in medium- and high-density plots have been found to extract more water from the soil, which was below wilting point (11%) in the surface 20 cm at the beginning of flowering. Low-density plots have significantly more soil moisture at this growth stage (Herbert and Hill, 1978a).

2.4.2 Effects of plant density on seed yield

The fact that lowest density plants produce the greatest seed yield per plant has been reported in lucerne (Kowithayakorn, 1978), siratro (Juntakool, 1983), soybean (Chanprasert, 1988), *Salvia officinalis* L. (Macchia *et al.*, 1990), *Hyssopus officinalis* L. (Macchia *et al.*, 1990) and China aster (Phetpradap, 1992). This higher seed yield per plant at low plant density is in all cases due to a greater number of branches and seed heads. Seed numbers per seed head and seed weight were relatively less affected by plant density (Phetpradap, 1992). However, seed yield per plant was depressed but yield per unit area increased with increasing plant density (Kobza, 1987). So, maximum seed yield per unit area is only obtained from plants grown at an optimum sowing distance.

Wide rows and low population densities have resulted in high seed yields in red clover (Clifford, 1974) and lucerne (Kowithayakorn and Hill, 1982). Given the small seed size of both lucerne and red clover, seeding rates of less than one kilogram per hectare produce enough plants for maximum seed yields (Hare, 1984). The results of an experiment by Hare (1984) showed that *Lotus pedunculatus* (Cav.), syn. *L. uliginosus* (Schkuhr.) cv. Grasslands Maku seed yields of over 85 g m^{-2}, in the first year after transplanting, were produced in small plots established in 0.30 to 0.45 m spaced rows at population densities of 22 and 33 plants m^{-2}. Seed yields were significantly reduced in 0.15 m spaced rows at population densities of 66 and 133 plants m^{-2}. Wide row spacing and low population densities produced plants which had significantly more seeds per stem, by producing more umbels. However, pods per umbel, seeds per pod, and 1,000 seed weight were not affected by row spacing and population density.

In Caucasian clover, high seed yield per inflorescence and per plant was strongly associated with actively growing plants. Full development of each reproductive component depends on the partitioning of adequate resources. Seed yield from Caucasian clover appears to be maximised where plants have abundant space, nutrients and moisture to fulfil their reproductive potential (Widdup *et al.*, 1996). Recent studies have shown 45 cm as the optimal row spacing with a new selection cv. Endura (6n) (Guy, 1996). The hexaploid plants averaged 50 cm in width after 18 months, which would have resulted in full canopy cover. Diploid and tetraploid plants averaged 35 cm in width, suggesting a 30 cm row spacing as more appropriate to maximise seed yield with lower ploidy cultivars (Widdup *et al.*, 1996).

The fact that wide inter-row spacing gave seed yields as high as or higher than narrow inter-row spacing suggests that where a second seed crop is taken, cultural operations could be provided for without prejudicing seed yield (Clifford, 1974). On the other hand, wide row spacing have also been found to make management of herbage seed crops easier (Clifford, personal communication). Inter-row cultivation, spaying, and fertilisation can be carried out effectively without crop damage (Hare, 1984).

2.5 SEED YIELD AND QUALITY AS AFFECTED BY INSECT PESTS

2.5.1 Insect pests and their effects on seed quality

Insects compete with man for the produce of the world farming land, and, when they reduce the production below the artificial standards set by man, they are regarded as pests (Doull, 1949). In other words, those insects that are in some way harmful we refer to as pests (Fenemore, 1982). Of the one million or so different species of insects that exist, only a few thousand qualify for pest status. Although the number of species of insects that are regarded as pests is not large, their activities have major impact on human welfare (Fenemore, 1982). The Food and Agriculture Organisation of the United Nations has estimated that one third of all food grown is lost to pests and diseases, either from the growing crop in the field or in store after harvest. Although this figure includes losses from disease organisms as well as pests it may be safely claimed that at least half these losses are due to insects (Fenemore, 1982).

Insect pests can be organised into three categories: a). key pests which are the pests of major importance on specific crops; b). occasional pests which occur infrequently in damaging numbers (above the damage threshold); and c). pests which have the potential to reach pest status but are normally suppressed by natural regulating factors (Fenemore, 1982).

In terms of the types of injury they inflict on the plant, insect pests can be divided into those with biting/chewing mouthparts that remove solid portions of plant tissue and those with piercing/sucking mouthparts, which feed on plant sap. Feeding in the former case results in obvious holes in leaves, stems, fruits or other plant parts, or tunnels bored in stems, fruits or roots. The actual nature of the injury is often characteristic of a particular pest. In contrast, insects with piercing/sucking mouthparts, which comprise the insect order Hemiptera, feed only on plant sap and are quite incapable of removing solid tissue. In the act of feeding however, these insects inject saliva into the plant to assist with digestion and uptake of sap. In many cases this saliva is an irritant or toxic to the plant and results in stunting, distortion, or death of areas of plant tissue. The effects of such insect feeding may thus be much more serious than the mere drain on the plant from sap removal (Fenemore, 1982).

Chapman (1984) classified insect pests of agricultural seed crops into two groups: those which are generally polyphagous feeders and attack the roots or foliage of plants, e.g., grass grub, and those which cause direct injury to inflorescences, flowers, ovules, seed and flowering stems, e.g. thrips.

Basically, the degree of injury to a plant is obviously closely related to the density of the pest population, but the stage of development of the pest is also important. Large larvae eat more than smaller ones and adult insects may not feed at all. Generally, the greater the degree of injury to a plant the lower the yield will be, but the end result depends very much on whether yield forming or non-yield forming parts of the plant are attacked (Fenemore, 1982). For example White (1975) reported that larvae of *Megacraspedus calanogonus* (Lepidoptera: Gelechiidae) and *Diplotoxa neozelandica* (Diptera: Chloropidae) feed on the developing ovary of five *Chionochloa* species, and were shown to be capable of severely reducing seed production, at times causing 100% destruction in localised areas in New Zealand.

Pests, which cause injury directly to yield forming organs of plants, exhibit a very different relationship between pest density and yield. The damage threshold in such cases is extremely low (or in fact zero) and yield decreases linearly until, with high pest populations, it reaches zero (Fenemore, 1982). In seed production of red clover, seed losses of 43 per cent have been recorded and 30-40 thrips per flower head is considered a heavy infestation (Chapman, 1984).

Insect pests can greatly damage seed quality. Yates (1952) demonstrated that the adults of red clover thrips (*Haplothrios niger* Osb.) feed on the soft green ovary with its developing seed and produce a typical lesion in the form of a brown spot on the seed coat of red clover. The structure of the seed coat is modified in this area and allows access of water more readily than before. These seeds germinate very quickly in laboratory tests. Yates (1952) also reported that in red clover, thrips larvae hatch after the flowers have died back. They feed on the green seed pods, causing the developing seed to shrivel. Often there is also scarring of the seed coat itself. Hampton and Hill (1990) suggested that physical damage influences physiological damage. When the physical damage has been caused during seed development, seed vigour and storability could be affected.

2.5.2 Pests in pasture legumes

The major pests of legume seed crops in New Zealand have been identified as the Australian crop mirid (*Sidnia kinbergi*, Hemiptera: Miridae), the potato mirid (*Calocoris norvegicus,* Hemiptera: Miridae), the brown shield bug (*Dictyotus caenoses*, Hemiptera: Pentatomidae), the green vegetable bug (*Nezara viridula*, Hemiptera: Pentatomidae), the wheat bug (*Nysius huttoni*, Hemiptera: Lygaeidae) (Wightman *et al*., 1981); aphids (*Acyrthosiphon kindoi,* Hemiptera: Aphididae; *A. pisum,* Hemiptera: Aphididae; *Brevicoryne brassicae,* Hemiptera: Aphididae; *Therioaphis trifolii*, Hemiptera: Aphididae), Clover casebearers (*Coleophora spissicornis,* Lepidoptera: Coleophoridae; *C. frischella*, Lepidoptera: Coleophoridae), Clover mites (*Bryobia praetiosa,* Acari: Tetranychidae; *B. repensi,* Acari: Tetranychidae; *Tetranychus urticae*, Acari: Tetranychidae), and red clover thrips (*Haplothrips niger,* Thysanoptera: Phlaeothripidae) (Chapman, 1984).

The two particular insects, which are the target pests in this present study (Australian crop mirid and red clover thrips), are more fully described below.

Australian crop mirid

The Australian crop mirid is the most abundant and widely distributed mirid in legume seed crops. It occurs throughout New Zealand and was first recorded in 1922 causing damage to passionfruit vines (Chapman, 1984). Mirids have long been known to be pests of agricultural and horticultural crops (Johnson, 1962; Wightman, 1967; Erdelyi and Benedek, 1974) but were not considered to be of much significance in New Zealand until fairly recently (Baker, 1978; Watson and Townsend, 1981; Wightman and Whitford, 1982).

Australian crop mirid commonly occurs in pasture and forage legume crops while forage brassicas, asparagus and strawberries may also be infested (Chapman, 1984) It proliferates especially in warm, dry conditions (Wightman and Macfarlane, 1982) and peak numbers occur in late summer-autumn (Wightman and Whitford, 1982).

The life history of the Australian crop mirid is that adults are the overwintering stage. Egg laying commences in early spring when females insert eggs into plant tissue. Females may produce eggs until June and it is likely there are three to four overlapping generations per year (Chapman, 1984).

This mirid is a sap-sucking pest. While feeding it injects salivary enzymes into buds, flowers, seeds and growing points of legumes. This causes shrivelled (Macfarlane *et al.* 1981) and discoloured seed (Avery, 1963; Macfarlane and Pottinger, 1976;

Donovan, 1981; Macfarlane *et al.* 1981; Wightman *et al.* 1982; Chapman, 1984; Smallegange, 1992). Observations made by Pearson (1991) in white clover suggest that the effects of the feeding by Australian crop mirid may result from a reaction to toxic saliva injected by the feeding mirid rather than from simple mechanical feeding damage.

As has been found in white clover (Pearson, 1991), the younger the damaged buds, the greater the effect on seed yield. In lucerne, attack at an early stage causes flowers and small pods to fall off, while the seeds in pods attacked at a later stage fail to develop (Macfarlane *et al.* 1981). A high population (>100 mirids/20 sweeps of a sweep-net) prevents any seed forming in lucerne (Wightman *et al.* 1982). In white clover its feeding is more damaging than that of the other Hemiptera (Smallegange, 1992).

Thrips

Thrips are small insects (in New Zealand ranging from about 1-3.5 mm long), but if present in numbers and not controlled they can cause considerable damage. Thrips have rasping-sucking mouthparts and the resulting damage consists of flecks and markings, which often give a silvery appearance to the surface of flowers, leaves and fruit. Thrips, especially immature forms, often occur in greater abundance on the underside of leaves and leave small drops of excreta. Overseas, up to 12 generations per year have been recorded in heated greenhouses. In unheated houses thrips may hibernate in soil or rubbish (Chapman, 1984). In New Zealand, several thrips such as New Zealand flower thrips (*Thrips obscuratus* (Crawford)), onion thrips (*Thrips tabaci* Lindeman), and red clover thrips (*Haplothrips niger* (Osborn) are commonly found in pasture legumes such as white clover, red clover and Caucasian clover and of these the red clover thrips are considered the most important (Pearson, personal communication).

For many years prior to 1950 farmers reported the presence in red clover heads of a tiny red insect which they called "Red Mite". At that time no answer could be given to their enquiries as to whether the insect was responsible for any damage to the red clover seed crop (Doull, 1949). They were in fact, small insects from the order *Thysanoptera*, (common name Thrips) which are yellow, brown or black in colour, and are found among all kinds of vegetation, both on the flowers and the foliage (Bailey, 1936; Doull, 1949).

Over a number of years, periodic outbreaks of thrips have been reported in the red clover seed-growing areas of New Zealand. Thrips, which are normally present in the clover blossom in quite small numbers, become abundant in some seasons, and observers have associated outbreaks with poor seed yields in those years. A larger percentage of shrivelled and badly formed seed is harvested, and much of this is dressed out, representing a loss to the grower (Yates, 1952). One of these is the red clover thrips (*Haplothrips niger*) (Figure 2-2).

Red clover thrips had been reported as causing loss of seed in lucerne and red clover crops in Idaho in U.S.A. (Burrell, 1918), and in Finland (Linaniemi, 1935). Bailey (1948) stated that *H. niger* was abundant on red clover, particularly in the irrigated pastures of the high elevations and mountain meadows of California.

Doull (1949) observed that it is hard to find the tiny, almost colourless first stage larvae in the clover head. They are never found feeding in the open but always feed deep in the head. They are regularly found within the calyx of withered flowers. With age the larvae grow rapidly and assume a conspicuous bright red colour. Throughout the whole of the larval life the insect shuns the light, and remains in the clover head feeding on all parts of the head. The larval life is variable depending on temperature but it is of the order of two months. Following the drying of the main crop of clover heads the thrips population diminishes considerably, while the first generation is

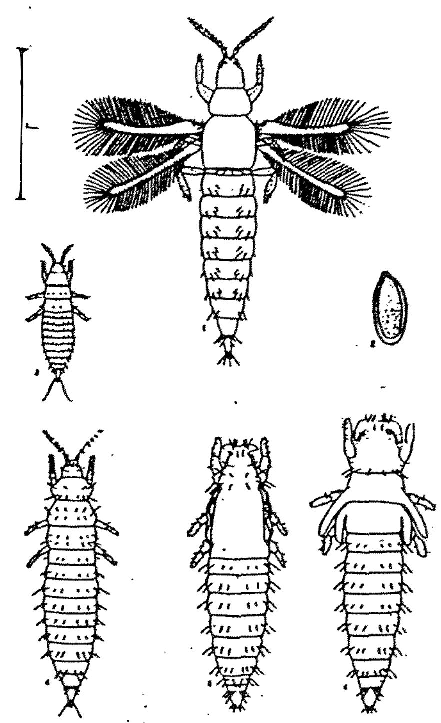


Figure 2-2. Red clover thrips (*Haplothrips niger* Osborn). Figure 1.--Adult, 2.--Egg, 3.--Larva I, 4.--Larva II., 5.--Prepupa., 6.--Pupa I (Yates, 1952).

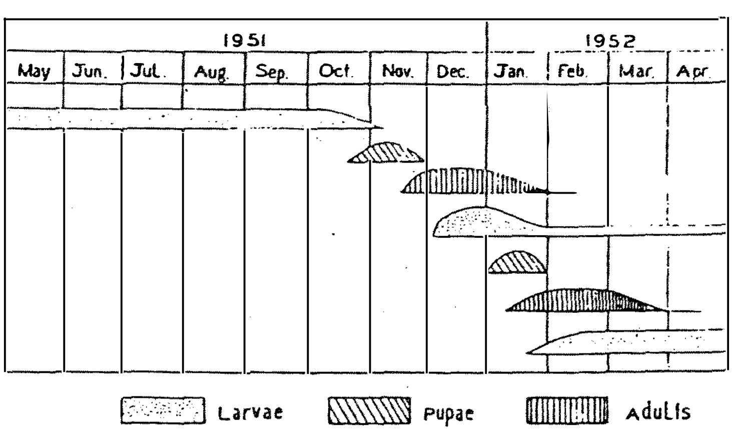


Figure 2-3. Diagram showing seasonal history of red clover thrips (*H. niger* Osborn) (Yates, 1952).

passing through the pupal stages. In January the clover seed crop commences flowering and at this time the second generation emerges. At the end of the flowering season, unopened heads are supporting a high population of second stage larvae. This is quite contrary to observations made earlier in the season.

Yates (1952) and Chapman (1984) have fully described a seasonal history of red clover thrips (Figure 2-3). There are two generations per year. Fully-grown larvae spend the winter in hollow clover stalks close to the ground. As the spring growth appears, they move up the stems and pupate under the stipules; adults become prevalent in early November.

Newly emerged adults are dark red, and the typical shiny black coloration develops within the first 24 hours. They are concentrated on the developing flower heads, but oviposition does not commence until the flowers are almost fully open. For the appearance of a new generation in the field, the eggs are attached singly by stalks to the hairs on the calyxs of individual florets. The incubation period is approximately 12 days, and the first larvae are found in early December.

The larvae avoid light, and feed in protected places in the flower head. They are commonly found either within the corolla tube or crowded between the green seed pods.

In mid-January, a second pupation occurs. The adults of the second generation continue to lay until mid-March, when the clover seed cut is taken, and they disappear with the first autumn frosts.

During February, the newly incubated larvae develop rapidly to maturity. There is, however, no further pupation. As the seed heads dry out, the larvae move down the stems in search of food, so that when the seed cut is taken most of the larvae population is concentrated close to the ground and remains undisturbed.

2.5.3 General pest control

It is not feasible to eliminate insects entirely, nor is it necessary. Control measures are aimed at reducing the incidence of insect attack. This can be done either by reducing the numbers of insects, or by giving the crop every opportunity to grow away from the insect attack (Doull, 1949). Fenemore (1982) stated that effective pest control might be described as a reduction or maintenance of a pest population below the damage threshold.

2.6 SEED PRODUCTION AND MANAGEMENT

Rhizomatous legumes are generally poor seed producers. Very little is known about the agronomy of their seed production. There is also limited agronomic information to assist with the establishment and management of farm-scale stands (Daly and Mason, 1987). So far, in New Zealand, Caucasian clover has been grown only for research purposes and there are few records of commercial areas outside their natural range in Europe and Western Asia (Gurung, 1991). Most of the papers available on clover seed production are concerned with traditional species such as white and red clovers and a limited attempt has been made to investigate Caucasian clover, particularly for seed production (Gurung, 1991). Therefore, this review of Caucasian clover seed production is when necessary, supplemented by information on seed production in more common legume species.

2.6.1 Seed yield and seed yield components

Well-grown legume seed crops produce large quantities of seed - much more than can be harvested. Even in a closely synchronised crop, there are considerable differences in the time at which individual seeds mature. New inflorescences develop progressively and each may then flower over a period of several days or even weeks. This pattern is further complicated by the continual turnover of seeds, which are shed progressively from the standing crop, as they ripen (Hill and Loch, 1993).

For general purposes, seed yield can be defined as a four-way product of inflorescence density, number of potential seed sites per inflorescence, percentage seed set and mean individual seed weight (Hill and Loch, 1993). Lorenzetti (1993) also suggested that seed yield can be considered as agricultural realised seed potential, depending on the number of seeds produced in the area; the average seed weight, and the percentage of seeds actually harvested.

Measurement of "seed yield" depends on the particular definition used. **Potential seed yield** comprises the total weight of seeds set per unit area, and includes all seeds produced by the crop over a period of several weeks or more. Where seed is allowed to accumulate on the ground before harvest, potential seed yield is related to the quantity produced over the entire growing season. Because of continual seed production and loss, only a proportion of the potential yield is present on the standing crop for harvest at any one time. This is the **presentation seed yield**, which is further reduced by harvest inefficiencies to give **harvested seed yield**, the usual criterion for assessing seed production (Hill and Loch, 1993).

The potential seed yield for clover species represents the cumulative expression of four principal components of yield: inflorescence numbers per unit area, floret numbers per inflorescence, seed numbers per floret and seed weight (Zaleski, 1970; Van Bogaert, 1977; Huxley *et al.*, 1979; Maldonado, 1985; Evens *et al.*, 1986; Clifford, 1987; Thomas, 1987). These components all differ in their relative contribution to total seed yield and change with genetic variability within species as well as with environmental conditions (Zaleski, 1961; Thomas, 1981).

As with herbage grasses (Hebblethwaite *et al.*, 1980) the development of legume seed crops can be divided into two stages: establishment of the yield potential and utilisation of the yield potential, where yield potential is defined as the number of florets (or potential seed sites) per unit ground area of the crop at anthesis. This yield potential is mainly dependent on the number of inflorescences and the number of florets per inflorescence. Utilisation of yield potential is determined by events at and after anthesis, through the developmental processes of pollination, fertilisation and seed growth. These processes determine the number of seeds per floret and mean seed weight (Hampton, 1990).

High seed yields are only achieved if the crop is harvested when the number of ripe inflorescences is at a maximum, as floret numbers per inflorescence, seed numbers per floret and seed weight remain constant over the harvest period and are not influenced by harvest date (Hollington *et al.*, 1989). It is generally agreed that the number of inflorescences is the most important seed yield determinant in various situations in white clover (Van Bockstaele and Rijckaert, 1988; Hollington *et al.*, 1989; Marshall *et al.*, 1989 Nordestgaard, 1989) and birdsfoot trefoil (Albrechtsen *et al.*, 1966; Mos, 1983; Stephenson, 1984; Mcgraw *et al.*, 1986; Li and Hill, 1988; 1989).

Seed yields are strongly affected by environmental factors as well as genotype (Thomas, 1987). Climatic considerations predominate in the success or failure of herbage legume seed production, as the weather the crop receives is a primary determinant of yield (Hampton, 1990). The first obvious requirement is for sites compatible with the day length, temperature and radiation requirements of the species or cultivar (Thomas, 1987). Genotypes originating from high altitudes tend to be long day plants and do not initiate inflorescences in warm short days; while genotypes originating from low altitudes also initiate inflorescences in cool short days, but tend to do so in the decreasing daylength of autumn, stopping as daylength and temperature increase (Hampton, 1990).

Temperature conditions may affect vegetative growth, floral induction, growth and differentiation of the inflorescence, flowering duration, pollen germination, seed setting and seed maturation. For example, Puri and Laidlaw (1984) showed that increasing temperature increased inflorescence number, florets per inflorescence, seeds per inflorescence and seed set in *T. prastense*. Decreasing temperature lowered pollen fertility in *T. repens*, but increasing temperature, particularly at a photoperiod of 14 h or less decreased florets per inflorescence (Thomas, 1987; Hampton, 1990). *Stylosanthes humilis* grown at 24° C, as compared to 17° C, had 3.6 times more inflorescences, 1.6 times more florets per inflorescence, 5.8 times greater seed setting and 5% lighter 1000 seed weight (Skerman and Humphreys, 1973). Optimum temperature and the shape of the temperature response will differ for each phase of vegetative and reproductive growth, and will also vary between and within species (Hampton, 1990).

The effect of light intensity on flower head development and ovule fertility of white clover cv. Grasslands Huia was tested under controlled environmental conditions by Pasumarty (1990) who found that in full light most florets developed fully, but about 20% of their ovules were sterile. In densely shaded conditions, however, not only did many flower heads abort completely, but also between 40 and 80% of ovules in flower heads that did not do so were sterile. These results clearly suggested that environment has a great influence on ovule fertility (Pasumarty *et al.*, 1995).

Within the legume species, types range from those which are mainly vegetative and produce few flowers, to those at the other extreme, which flower profusely. Thus in most species, there is considerable genetic variation available for components that together constitute a good seed yield and would be amenable to selection (Hampton, 1990). Regardless of the genetic variation however, seed yield might be considerably increased if the optimum conditions required for each component of seed yield were more fully understood. In optimum conditions suitable for initiation, growth and development, a large number of inflorescences each bearing a large number of florets which contain a high number of fertile ovules as well as fertile pollen would be formed. Subsequently a high percentage of these florets would undergo anthesis, be efficiently pollinated and fertilised and ultimately produce seed of maximum weight, vigour and germination capacity (Thomas, 1987; Budhianto, 1992).

Seed yield in many herbage legumes fluctuates widely within a cultivar, both within and between seasons, and yields of between 50-450 kg ha^{-1} are more common than those over 500 kg ha^{-1} (Hampton, 1990). In Caucasian clover, because of the slow seedling growth and a long establishment period (Speer and Allinson, 1985), seed yields the first year after planting can be as low as 70 kg ha^{-1}. Seed yields in later years are reported to range from 100 to 600 kg ha^{-1} (Bryant, 1974; Anon, 1977; Voloshenko *et al.*, 1977; Stewart and Daly, 1980; Henry and Taylor, 1989; Steiner, 1992), and cultivar Monaro is reported as averaging 300-400 kg ha^{-1} (Daly *et al.*, 1993; Coolbear *et al.*, 1994; Ehlke and Vellekson, 1997). The greatest recorded yield of cv. Monaro was 500 kg ha^{-1} (Widdup *et al.*, 1996) who also obtained 900 kg ha^{-1} for cv. Endura (6n) which is a selection from Monaro. Hexaploid Caucasian clover has the potential to produce high seed yields, which compare favourably with those of other legumes.

2.6.2 Obstacles to seed production

The constraints of Caucasian clover seed production in New Zealand were reviewed by Hampton *et al.* (1990), and included lack of seed supply and therefore little demand and very limited seed production information. "A constraint is the present lack of commercial seed supplies" for *T. ambiguum* and *T. medium* (Daly and Mason, 1987) due to poor seed production and poor establishment (Townsend, 1985; Sheaffer and Marten, 1991).

Problems typical of all herbage legumes have been encountered, i.e. indeterminacy, protracted flowering periods, pollination problems, poor seed set, abortion, uneven ripening, pod shatter, harvesting difficulties and low harvest indices (Hampton *et al.*, 1990). Indeterminate growth and responses to the environment result in plants flowering over an extended period during which inflorescence buds, blooming inflorescences, young pods and mature pods ready to dehisce can be present simultaneously on an individual plant (Efendi, 1993). This extended flowering period and the resultant range of flower ripeness categories in the crop makes it difficult to optimise harvest date. Early harvesting can result in unripe inflorescences being gathered but a late harvest can miss the older, earlier produced inflorescences which may have fallen below cutting height (Efendi, 1993). One of the reasons for the failure to commercialise this clover has been the poor or inconsistent flowering leading to low seed production (Hill and Mulcahy, 1993; Hill *et al.*, 1996).

Increased pollen abortion was noted in polyploid chromosome races as they have more erratic flowering, though variability between introductions has been reported to be as great as between chromosome races (Kannenberg and Elliott, 1962). The potential exists for the selection of dense flowering forms with a low degree of pollen abortion (Bryant, 1974).

Pod shattering in Caucasian clover may be a problem too; losses of up to 50% have been reported (Pellett, 1945; Khoroshailov and Fedorenko, 1973; Stewart and Daly, 1980).

2.6.3 Management for seed production

Management of legume seed crops is initially directed towards developing a strong vegetative framework to maximise the number of potential reproductive sites (Hill and Loch, 1993). Understanding crop growth is necessary before recommending suitable management practices which encourage both vegetative and reproductive growth and the attainment of maximum seed yields (Hill and Witchwoot, 1990).

A more exact understanding of the way climate influences the diverse processes leading to seed production is a necessary prelude to advances in efficient site selection, and in such agronomic fields as choice of sowing time and spatial arrangement, cultivar maturity type selections, risk assessment in fertiliser application, irrigation strategy, defoliation practice and harvest procedure (Humphreys and Riveros, 1986, Hampton, 1990). The ideal seed production climate has suitable radiation, temperature and rainfall for the vegetative growth of the crop, favourable photoperiods and temperature for floral induction, and even, dry relatively still weather during maturation and harvest (Hampton, 1990).

Crop establishment

Successful establishment of the desirable plant population is of prime importance in order to achieve high seed yields (Montgomery, 1983; McCartin, 1985). Preliminary studies for seed production from rhizomatous clovers (e.g. zigzag clover, red clover and Caucasian clover) were established at a 75 cm row spacing at Lincoln University, New Zealand (McCartin, 1985). Guy (1996) reported that in a trial with 30 cm or 45 cm row spacing with a sowing rate of 8 kg ha^{-1}, although there were no significant differences in the first year (101 kg ha^{-1} cf. 111 kg ha^{-1}), the 45 cm row spacing yielded significantly higher than the 30 cm row spacing in the second year (709 kg ha^{-1} cf. 371 kg ha^{-1}). Wider rows produced more flowers per unit area, 448 m^{-2} for the 45 cm spacing compared with 288 m^{-2} for the 30 cm spacing.

In Australia, excellent establishment of cv. Summit and Treeline was obtained from a seeding rate of 1.9 kg ha^{-1} in rows of 45-47 cm wide (Efendi, 1993). Daly *et al.* (1993) demonstrated that a relatively low population (20 000 plants ha^{-1}) of small plants was inadequate for seed production in the first year, but inflorescence numbers per square metre were sufficient to give high potential seed yields in the second year from the larger plants of Caucasian clover. This point was supported by Guy's report (1996). Using 2 kg or 6 kg ha^{-1} of coated and inoculated seed with a 45 cm row spacing, the lower sowing rate treatment of 2 kg ha^{-1} produced a significantly higher seed yield, of 208 kg ha^{-1}.

Inoculation with an effective strain of rhizobia is essential for nodulation (Pryor *et al.*, 1998). Although rhizobia strain ICC105 (ICMP4073b) was recommended as the New Zealand commercial inoculant, a new strain ICC148 was reported to be more effective than strain ICC105. Strain ICC148 increased the percentage of seedlings nodulated over that obtained with ICC105, from 23% to 49%. Dry matter weights of individual seedlings were increased, and dry matter of individual plants after 6 and 13 months increased 1.5 and .30 fold, respectively when inoculated with strain ICC148 (Pryor *et al.*, 1998).

Moorhead *et al.* (1994) compared three sowing techniques, i.e. broadcasting, sod seeding and strip seeding Caucasian clover in spring. By mid December, 48 and 38% establishment had occurred in the strip and sod seeding respectively, but only 9% from broadcasting. Strip seeding was the most successful technique, resulting in earlier rhizome and tap-root development and wider lateral spread of rhizomes. By using a strip technique Caucasian clover was established as rapidly as white clover, and plants of both species were similar in size after 5 months.

High rates (300 kg ha^{-1} compared with 150 kg ha^{-1}) of molybdic sulphur superphosphate applied at establishment accelerated Caucasian clover plant growth and rhizome development, particularly in the strip seeding treatment (Moorhead *et al.*, 1994; Strachan *et al.*, 1994). In an ungrazed field trial at Mt Possession only 3 t ha^{-1} of below-ground biomass was measured in plots which had received only 50 kg P 13 years previously, but 15 t were recorded with 200 kg of P and over 20 t where 800 kg of P was topdressed (Strachan *et al.*, 1994). This is partly the reason why Lucas *et al.* (1981) and Kee (1981) recorded lower top growth from Caucasian clover than from white clover and Maku lotus in the first four years of the trial; much of the biomass was accumulating underground (White, 1995). However the plots established at Mt. Possession were for grazing trials, not seed production.

For seed production, calcium, phosphorus, nitrogen, potassium, sulphur, molybdenum and boron are five major and two minor elements that tend to dominate the nutritional limitations (Clifford and Rolston, 1990; Marshall *et al.*, 1991). For example, phosphorus deficiency will result in dwarfed prostrate growth, nitrogen deficiency in general yellowing, leaf necrosis and death, calcium deficiency in collapse of the petiole, with the leaf initially remaining green and turgid, potassium deficiency in leaf necrosis and death, and sulphur deficiency in leaf necrosis and death (Dunlop and Hart, 1987).

For herbage legumes, nitrogen, phosphorus and potassium are used to promote vegetative growth and reproductive development, but high levels of these elements cause excessive vegetative growth and therefore, seed yield potential will be diminished (Clifford and Rolston, 1990). Calcium is used to balance soil acidity to a suitable level (pH 5.5-6.0) for seed production. Up to 6 ppm of exchangeable sulphur in the soil gives high seed yield (Clifford and Rolston, 1990). The major role of molybdenum in the level of 0.5-10 ppm in herbage is to ensure efficient nitrogen fixation by the root nodule rhizobia. Its main function in this symbiotic relationship is in the union with the root to ensure adequate plant carbohydrate transfer for bacterial use (Clifford and Rolston, 1990). Boron is important for reproductive growth and development. In pot trials, an available boron concentration of 1 mg l^{-1} is necessary to optimise reproductive growth and development (Marshall *et al.*, 1991). In the field, the requirements may be higher than this level as rainfall can cause boron leaching and the increase in soil acidity followed by lime application to correct such acidity is likely to reduce boron availability (Gupta, 1979; Budhianto, 1992).

In Caucasian clover, Spencer *et al.* (1980) reported that higher chromosome number was associated with a lower internal P requirement. Hexaploid cv. Monaro exhibited a high yield potential in both soil and solution culture, was ranked high with respect to efficiency of P utilisation, and had a relatively low external P requirement and a low critical P concentration in the tops. Spencer and Hely (1982) also reported that growth of Caucasian clover in the absence of applied P and responses to P were greater than for white clover. The hexaploid became more responsive to P than the diploid and tetraploid forms. This was interpreted as indicating that either the P requirement of Caucasian clover under field conditions was much less than that of white clover (at least in the establishment phase) or that another factor had limited the foliar response to P by the Caucasian clover. This factor may well have been the location of the fertiliser P, which had been applied to the soil surface after planting. The strong sorption characteristics of the soils concerned may have restricted P penetration and thereby rendered it less available to the deep-rooted Caucasian clover than to the shallower-rooted white clover.

Weed control

Seed yields can be reduced through competition in the field and the need to sacrifice some seeds during post-harvest cleaning to remove contaminant weed seeds. Problems are generally more acute with legumes than with grasses, especially in established perennial crops. For example, suitable broad spectrum herbicides have not yet been identified for broadleaf weed control in a number of legumes. The development of better herbicide recommendations is therefore a continuing process (Hawton *et al.*, 1990; Loch and Harvey, 1993; Hill and Loch, 1993).

Weed control, particularly of white clover, is essential if maximum seed yields of Caucasian clover are to be achieved (Guy, 1996), as in Caucasian clover seed production, the major problem can be rapid growth of white clover. Complete eradication is seldom possible. Since Caucasian clovers are very slow in growth at establishment, rapid growth of white clover is likely to threaten the newly-sown crop. Therefore, it is most important to choose a site which is free of white clover and if that is not possible, the growth of white clover must be checked as early as possible. If not controlled properly, white clover may cause difficulties during harvesting as flower-heads of white clover mature at the same time as those of Caucasian clover. This is particularly so with early flowering diploid plants (Gurung, 1991).

Pest control

Pests can affect seed yield, either reducing or destroying the seeds, and seed quality, resulting in reduced seed vigour (Lorenzetti, 1993). One of the most important aspects of pest control in clover seed crops, apart from yield, is quality - the appearance of the seed itself. Brightness and shine may be lost due to thrips attack, or honeydew deposited by aphids in the field may case dressing problems in stored seeds (Trought, 1980). In New Zealand the most important pests of clover crops are clover casebearer moths (*Coleophora* spp.), blue-green aphids (*Acyrthosiphon kondoi* Sinji), and red clover thrips (*Haplothrips niger* Osborn.) (Gurung, 1991).

Pests may be present in low numbers, but a set of favourable climatic and other conditions may arise which allows a minor pest suddenly to assume greater importance. Regular inspection and the judicious application of insecticides, if necessary, may prevent serious loss of crops. There are a number of insecticides available for pest control, but one with low toxicity to bees is essential (Gurung, 1991).

Pollination

Not all florets are potentially fertile, for morphological (Hill, 1980), genetic (Elgersma, 1985), or pest damage (Johnson, 1960) reasons, but it is the environment which strongly influences the success of pollination and fertilisation through its effects on anthesis patterns and duration, pollen production, availability and fertility, pollinator activity and effectiveness, and ovule fertility (Hill, 1980; Elgersma, 1985; Clifford and Scott, 1989; Hampton, 1990, 1991). In legumes fertilisation is promoted mainly by foraging bees; plant physiological processes which affect nectar secretion and thereby bee visitation must be carefully studied (Clifford, 1987). Improvements in pollination/fertilisation can be achieved by matching the crop to the appropriate environment (Hacquet, 1990, Hampton, 1991).

As a species with a high degree of self-incompatibility within all ploidy levels, cross pollination is essential to obtain high seed yields of Caucasian clover (Kannenberg and Elliott, 1962; Hely, 1963; Townsend, 1970). The flowers of Caucasian clover and their rich nectar strongly attract different moths, butterflies and honeybees. Since, Caucasian clover has a short corolla tube cross-pollination is not a problem if appropriate pollinators are present. Honeybees are efficient pollinators of Caucasian clover (Daly *et al.*, 1993). 10-12 honeybee hives per hectare is optimum for cv. Summit during the dense flowering period (Bryant, 1974).

Other practices

Closing date is taken to be the final time when the vegetative growth is controlled either by cutting, grazing or by the use of chemicals. Accordingly, the aim in deciding closing date is to ensure that as many inflorescences as possible are already initiated but not yet emerged at the time of closing. These inflorescences then emerge over a short period of time in the immediately following weeks to give the maximum inflorescence density and the minimum range of inflorescence ages (Thomas, 1987). Closing too early results in longer flowering spans, and hence fewer inflorescences mature at any one time. If it is too late, inflorescences are smaller and the density of inflorescences is reduced (Montgomery, 1983).

Defoliation is commonly used and considered to be a disturbance to normal plant development (Harris, 1978). It 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). The severity of defoliation and therefore the response of the plant may be considered in terms of *frequency*, *intensity* and *timing* in the year (Harris, 1978; Sheath, 1978; Maldonado, 1985).

Stewart and Daly (1980) reported that optimal management of Caucasian clover requites a long rotation -- around 2 months -- combined with heavy grazing or forage harvesting before closing. Gurung (1991) reported that seed production was increased by about 40% after defoliation at the vegetative stage in spring (27/09) in 1989. Daly *et al.* (1993) also indicated that highest yields were obtained from Caucasian clover cultivars after cutting at the vegetative stage (late September). In their study, closing treatments were aimed to concentrate the flowering period and change the times of peak flowering. Defoliation in spring before flower buds were visible enhanced flower numbers m^{-2} but later cutting decreased seed yields considerably. The defoliation treatments with cutting at 1 cm above ground in their study were probably excessive. Higher cutting may have resulted in more rapid recovery and increased flower numbers. Irrigation at the time of defoliation treatments may also have overcome the confounding effect of increasingly dry soil conditions with the later defoliations.

Removing a hay crop (haying) is a common practice used in some forage legume seed production systems to increase seed yields. As a result of hay removal, flowering is slightly delayed until ambient temperatures are more conducive to pollinator activity; the ratio of reproductive to vegetative growth is increased; new leaves are produced which are not infected with early-spring foliar leaf diseases; and the life-cycles of insect pests such as the clover seed weevil (*Tychius picirostis*) and ladino clover midge (*Dasineura gentreri*) are disrupted (Steiner, 1992). However, Steiner's report (1992) showed that Caucasian clover seed yield can be reduced by haying during late-spring, regardless of the time of haying. Similarly, seed yield can be reduced by haying, regardless of the time of stand establishment two seasons earlier. Average seed yields were reduced more than 90% in hayed treatments compared to the unhayed control. Seed yield reductions in hayed treatments were due to lower final flower counts than in the controls. According to his observation, hayed treatment plants are unable to regrow from the ground following haying. Thus to re-establish flowers for seed production, new reproductive stems must be produced after haying. Steiner (1992) noted that this followed Pellett's (1956) observation that Caucasian clover produces blooms only once per season and if cut will not bloom again until the next year. However, these findings seem contrary to Bryant's (1974) speculation that an early-season seed harvest may allow a second-season seed crop to be produced, but if sufficient quantities of new reproductive stems were produced, a second harvest may be possible.

Inter-row cultivation is an inappropriate management practice for Caucasian clover seed crops, as reproductive growth in this crop is dependent largely on prior crown development, and, in particular, the numbers of main crowns - the net effect of this management practice in Caucasian clover is likely to be deleterious, in that main crowns are inevitably going to be destroyed and this will negate any other potential benefits (Coolbear *et al.*, 1994).

The timely use of irrigation for seed production is one of the most effective methods of increasing seed yields, but if water is applied at the wrong time the result may be disastrous. Generally there should be adequate moisture up to closing, which normally comes from spring rainfall. Clifford (1986) suggested that, after closing, irrigation should not be applied until soil moisture in the top 20 cm is approaching wilting point. Application rate should not be high and total application just sufficient to restore the soil to about 50% of available soil water. Caucasian clover is tolerant to drought. However, after closing, irrigation may be needed to enhance its sometimes slow recovery from defoliation (Gurung, 1991).

Harvest

Not all the seed that is produced can be harvested because of the range in ripeness, lodging, shattering-pod dehiscence and harvest losses. Harvest losses ranging from 20 to 75% are often reported both in grasses and legume (Foster *et al.*, 1962; Andersen and Andersen, 1980; Clifford and McCartin, 1985; Meijer, 1985; Simon, 1987; Horeman, 1989; Elgersma, 1990; Hampton, 1991; Lorenzetti, 1993). The degree of loss is partially dependent upon species, cultivar, uneven crop development and inclement weather, but can also be affected by time of harvest, method of harvest and machinery setting. There is scope for significantly reducing harvest losses in both grasses and legumes (Hampton, 1991).

One of the biggest problems in seed production is the correct timing of the harvesting of the crop so that maximum seed yield is obtained but losses from seed shedding and in seed quality are minimal (Hill, 1987). The assessment of optimum harvest date is difficult. As the time for full seed development in individual florets from pollination to mature seed is 26 (± 5) days depending on weather conditions (Hyde, 1950; Hyde *et al.*, 1959), the most consistent method of determining an optimum harvest date may be associated with the time after peak flowering. At present, however, it has been suggested that the most accurate and consistent measurement of correct harvest timing is 'seed moisture content'. It is possible to predict harvesting time by monitoring changes in the seed moisture content of a crop, because there is a consistent relationship between seed moisture content and the dry weight of the crop. All crops have an identifiable seed moisture content at physiological maturity. This moisture content is relatively constant for each species and is independent of environmental factors (Hampton, 1994). For example, optimum time to commence harvesting in Maku lotus was 2-4 days after seed physiological maturity (maximum dry weight) when the seeds had reached 35% moisture content (Hare and Lucas, 1984).

In Caucasian clover in New Zealand, the peak flowering period is about mid December (Efendi, 1993; Widdup *et al.*, 1996). During seed development, the ripening stage took 8 to 12 days from mass maturity, and seed moisture content was about 30-40% during this period. This stage can be recognised in the field by the dark brown flower colour, yellowish brown pod and light yellow seed (Efendi, 1993). Also, it was suggested 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 the maturation stage, is influenced by the environmental conditions. Weather conditions may vary from year to year and the duration of each stage may slightly change. 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. Optimum harvest time for the clover could be around 34 days after peak flowering (Efendi, 1993).

Mowing or chemical desiccation may be employed as the first stage of harvesting Caucasian clover to reduce the risk of seed losses from pod shatter and enable the crop to dry more rapidly in the field before combine harvesting (Efendi, 1993). A rotary mower is preferred (Guy, 1996).

Climatic factors such as strong winds, heavy rain with large raindrops or hot, dry conditions can result in climatic losses. Inflorescences can fall below harvesting height in wet weather (Mohamed, 1981; Evans *et al.*, 1986). Hot, dry conditions lead to inflorescence shedding, hence a loss of seed pods and individual seeds (Holloway, 1987), and seed loss increases with time of exposure to unfavourable climatic conditions and plant breakdown. Therefore, to minimise seed loss, seed must be harvested as soon as it is ripe. Harvesting losses due to machinery inefficiencies and design can be as high as 40% (Clifford and McCartin, 1985). Crop condition at harvesting (green or desiccated) and time of harvesting (morning or afternoon) are also important factors that affect harvesting losses. In Canterbury, lowest losses (5%) were recorded from a desiccated white clover crop cut in the morning with a double reciprocating knife mower (Clifford and McCartin, 1985).

Chapter 3

ROOT SYSTEM DEVELOPMENT IN CAUCASIAN CLOVER CV. MONARO AND ITS CONTRIBUTION TO AERIAL COMPONENTS

3.1 INTRODUCTION

Caucasian clover is a strongly rhizomatous, low-crowned perennial legume (Khoroshailov and Fedorenko, 1973; Bryant, 1974; Stewart and Daly, 1980). The species has a deep semi-woody often-branching taproot from which many branched rhizomes grow. These eventually give rise to daughter plants, both terminally and from nodes (Bryant, 1974; Stewart, 1979). Because the rhizomes and growing points are below ground level (Allan & Keoghan 1994; Moorhead *et. al.* 1994) this gives the plant the ability to 'creep' through pasture, even under very hard grazing (Guy, 1996).

Compared with other traditional herbage legumes such as white clover and red clover, the Caucasian clover root system is complicated. It consists of several fractions described by Peterson *et al.* (1994a) as the primary crowns, primary taproots, rhizomes, secondary crowns, secondary taproots, fibrous roots, rhizome shoots and rhizome initials. This complex root system is associated with a large underground mass that can average 6600 kg ha^{-1} from a 5 year old stand (Peterson *et al.* 1994b). In general, one of the most common characteristics of this clover is its persistence, because of this root structure. The extensive root and rhizome system (Daly & Mason, 1987) also acts as an important store for nutrients (Strachan *et al.* 1994; Moorhead *et al.* 1994), that can be remobilised and utilised for growth.

The root system of Caucasian clover not only allows the plant to persist well under harsh conditions, but it may also play an important role in above ground development during seed production. Coolbear *et. al.* (1994) suggested that extensive root growth and the development of Caucasian clover crowns were an essential prerequisite for reproductive growth. This means that there should be a relationship between root development and reproductive development in Caucasian clover. The objectives of this experiment were therefore to investigate root system development during both the vegetative and reproductive growth stages and its contribution to aerial plant components; the relationship between root development and reproductive development; and the contribution both from primary and secondary crowns to seed yield in the first harvest season.

3.2 MATERIALS AND METHODS

3.2.1 Experimental site and management

This experiment was carried out at the Seed Technology Centre, Department of Plant Science, Massey University, Palmerston North beginning in May 1994 using Caucasian clover cultivar Monaro. Plants growing in a four-year-old stand established for seed production at the AgResearch Grasslands Research Unit, Aorangi, Palmerston North had been dug up in January 1992 and replanted in large containers at the Seed Technology Centre. They were observed for morphological characters in 1992-1993 and seeds were harvested in 1993-1994 (see Chapter 4).

One of these cultivated plants was randomly selected on 12 May 1994, and the soil on the roots was washed off. This plant was then separated by cutting 80 segments of secondary crowns that arose from rhizomes. These segments were placed immediately into small pots (8 cm diameter) containing potting medium (Yates Black Magic Seed Raising Mix) which were then placed in a glasshouse at 30/20

± 5° C at the Seed Technology Centre. They were watered twice a week. In early June (twenty days later) the young plants were moved outside to harden off for four weeks.

In order to observe root system development from the beginning of growth to seed harvest, a raised sand bed plot (6 x 4.5 x 0.3 m) was established adjacent to the Seed Technology Centre (Plate 3-1). A plastic sheet with multiple holes was placed on the bottom of the plot. Then, washed river sand was spread to a depth of 30 cm. The 56 clonal plants were randomly selected and transplanted into the sand bed on 1 July at a square spacing of 60 cm. Every two plant rows represented one replicate block, each containing fourteen plants. Four replicate blocks were used.

Mesurol Snail and Slug Bait (Methiocarb 20 g kg^{-1} bait) was spread at 10 kg a.i. ha^{-1} on the surface of the sand bed before transplanting on 15 June. Nitrophoska (N:P:K:S = 12:10:10:2; 1 kg (370 kg ha^{-1})) was applied immediately before the plants were transplanted, and again at the same rate before inflorescence buds were visible on 25 November. Weed control was carried out by hand hoeing when necessary. During the plants' development, irrigation was applied with a small spray irrigation system for four hours (600 litre hr^{-1}) at about 10 day intervals. During flowering, Mavrik insecticide (a.i. taufluvalinate 240 g $litre^{-1}$) was applied at 150 ml ha^{-1} on 9 and 28 February 1995 to control aphids, thrips and mites.

Destructive sampling involved removal of one plant in a row of two in each block at each sampling time. Ten samplings were made with a sampling interval of 15-30 days. In the early stages of plant development samples were taken every 30 days from 20 September to 20 December. The sampling interval was then reduced to every 15-20 days from first flowering until the final seed harvest. Seed was harvested twice during the season. The first seed harvest was carried out by hand on 10 February when some of the inflorescences from primary crowns had turned dark brown in colour and were regarded as mature. The second seed harvest (final

Plate 3-1. Layout of the experiment site for root system development of Caucasian clover cv. Monaro (7 plants/row; 8 rows).

harvest) was carried out by hand six weeks later on 25 March 1995. Inflorescences were harvested from both primary and secondary crowns.

3.2.2 Plant measurement and statistical analysis

During plant development, morphological characteristics and dry matter of each part of the plant were recorded. One plant was dug up from each of four plots in the sand bed, placed on a concrete surface separately, and the roots washed gently with running water. Plants were easily extracted from the sand bed, and it was assumed that the entire root system was obtained in this way. Each root system was then dissected, separating the primary crown from the secondary crowns. While a few tertiary crowns arising from secondary crowns were identified in late January when they began to develop, they were not recorded because they were very small and few in number. The separated crowns were divided further into the root (underground) and aerial components (above ground).

Measurements of the root system were made by counting the number of secondary crowns per plant, the number of rhizome tips on primary and secondary crowns per plant, dry weight of the primary crown root system including the taproot, rhizome initials (tender, white, new rhizome growth) and fibrous roots of primary crowns, and the dry weight of the secondary crown root system that consisted of taproots, rhizome shoots and fibrous roots of secondary crowns of each plant. Aerial components were measured by counting the number of leaves, shoots and inflorescences on primary and secondary crowns, respectively for each plant, floret number per inflorescence, seed number per inflorescence, thousand seed weight and seed yield on primary and secondary crowns, respectively. All dissected plant components were placed separately in different trays and dried in an air oven at 60° C for 48 hours. Dry weight of each item was then measured separately.

Flower number was recorded on two intact plants in each plot. Counts were made every five days, because it takes five days for flowers to fully open (Coolbear *et al.* 1994). This procedure began at first inflorescence appearance (November 15, 1994) and continued through to final seed harvest (March 25, 1995). Open inflorescences were regarded as those in which 70% of florets were fully open.

Seed was harvested by hand on February 10 and again on March 25. At each harvest, inflorescences removed from each plant in each block were kept in individual paper bags in the laboratory and air dried at room temperature (about 24° C) for four months. After this, florets per inflorescence and seed number per inflorescence were determined for both first and second harvested inflorescences. Four inflorescences from each bag were used. Florets per inflorescence were counted by taking all the florets from each single inflorescence, and seed number per inflorescence was counted by rubbing those florets on a stippled rubber pad, and removing light chaffy material with air blowing at 345 rpm for 30 seconds.

The percentage of pods with zero, one or two seeds was also investigated with X-ray radiography, using inflorescences harvested on February 10 and March 25. This method is based on the principle that different parts of a seed absorb soft X-rays to a different extent, and can consequently be differentiated in an X-ray photograph (Hill and Hill, 1995). Four inflorescences were taken randomly from the samples harvested at each of the two different dates. Fifty pods from each inflorescence were used to determine seed numbers per pod. Results were taken from Polaroid X-ray negatives obtained using the Hewlett Packard X-ray System (Model 43804 N) after exposure at 15 KVP tube voltage for 3 minutes.

Before the final harvest, the frequency of pods with zero, and one or two seeds was also investigated by dissecting 360 fresh pods from 12 inflorescences collected in late February. An attempt was also made to determine the percentage of pods with different numbers of seeds appearing on the top, middle and bottom positions of an inflorescence. Thirty fresh pods of an inflorescence were taken from three different positions (ten fresh pods from each position) and counted in 4 replicates (10 x 3 x 4) per position. After dissection, the percentage of 0-seeded, one-seeded and two-seeded pods was determined for each position of the inflorescence.

The seed cleaning procedure involved rubbing air dried inflorescences on a stippled rubber pad. Straw and light material was removed by sieving through 1 mm and 2 mm sieves. The remaining material was purified with an air blower at 345 rpm for 30 seconds (Burrows. Model 1836-4). Seed yields for primary and secondary crowns per plant were calculated (from four plants). Also, thousand seed weight and germination of early and final harvested seeds was measured under the prescribed rules (ISTA, 1996).

The experimental data were processed using the analysis of variance and Fisher's LSD test at P = 0.05 of the SAS package (SAS Institute Inc., 1985-1990).

3.3 RESULTS

3.3.1 Structure of Monaro plants

Caucasian clover plants eventually developed well in this trial, although both aerial and root development was slow in the first four months after transplanting. Typical plants (Plate 3-2) had developed primary, secondary and tertiary crowns before reaching peak flowering on 5 February (Plate 3-3). From the primary crown in the centre of the plant, about ten secondary crowns developed radially from early January 1995. Only a few small tertiary crowns had formed from secondary crowns per plant by late January (7 months after transplanting). Both primary and secondary crowns were capable of producing reproductive shoots in the first growing season but not tertiary crowns (Plate 3-3). In root system development,

Plate 3-2. Typical plant structure (primary crown (A) and secondary crowns (B)) and root system development of Caucasian clover cv. Monaro 7 months after transplanting.

Plate 3-3. Development of primary crown (A), secondary crown (B) and tertiary crown (C) of Caucasian clover cv. Monaro 7 months after transplanting.

the number of rhizome initials (the sites of rhizome extension, and rhizome shoot and secondary crown development (Peterson *et al.* 1994)) did not change before December. Rhizome initials mainly arose from the primary taproot. By early February over 100 rhizome initials per plant were present on both primary and secondary taproots and corresponding numbers rose dramatically for secondary taproots after early March (Figure 3-1).

3.3.2 Development of aerial components and dry matter accumulation

There was no significant change in the number of leaves per plant before the middle of December, but after that, leaf number dramatically increased and reached a maximum of over 1100 leaves plant^{-1} in late March (Table 3-1). Reproductive development began from early November and reached a peak of reproductive shoots and inflorescences around the middle of February. More than 100 reproductive shoots and about 140 inflorescences were generated from a single plant. Secondary crowns began to develop before the middle of December.

These secondary crowns increased to 26 per plant during peak flowering and then continued to increase after peak flowering with continuous accumulation of root dry matter (Table 3-1). Whole plant dry matter did not increase significantly before early January. Root to shoot ratio was 1.82 at the beginning of plant development because dry matter accumulation was slow above ground. Then, it increased to 2.45 before reproductive development. Dry matter of the whole plant accumulated quickly in the one month before plants reached peak flowering. During peak flowering, however, the total weight of above ground dry matter quickly increased, and exceeded the total weight of underground dry matter. Root to shoot ratio decreased to 0.65. Then, root dry matter increased again and the ratio went back to 1.34 when seed was harvested in late March. More dry matter was quickly accumulated in the root system and the root shoot ratio reached 2.89 nine months after transplanting (Table 3-1).

Table 3-1. Development of the root system and aerial components in Caucasian clover cv. Monaro in the 1994-1995 season. Data are per plant.

Date	Leaf No.	Reprod. shoot No.	Infl. No.*	Sec. crowns	Root DM (g)	Aerial DM (g)	Root/Shoot ratio
20/09	4.3 ±0.25	0	0	0	0.71 ±0.18	0.39 ±0.08	1.82
15/10	9.8 ±2.51	0	0	0	2.59 ±0.5	1.0 ±0.02	2.59
15/11	6.3 ±1.09	3.0 ±0.94	1.8 ±0.55	0	4.14 ±0.64	1.69 ±0.22	2.45
15/12	50.0 ±14.43	4.3 ±1.19	11.8 ±8.41	1.8 ±0.29	9.39 ±1.62	8.64 ±3.36	1.08
10/01	130.0 ±12.37	29.8 ±2.64	11.0 ±2.63	9.3 ±0.55	16.06 ±3.01	19.49 ±2.03	0.82
30/01	366.0 ±105.25	82.0 ±26.38	79.3 ±36.78	13.8 ±6.55	51.57 ±17.74	72.78 ±25.27	0.71
10/02	482.5 ±80.46	113.8 ±29.07	139.0 ±55.43	26.0 ±5.06	63.66 ±7.99	98.11 ±24.09	0.65
25/02	653.8 ±77.96	105.5 ±12.35	196.0 ±31.80	32.8 ±6.25	89.92 ±10.72	152.0 ±43.79	0.59
25/03	1154.0 ±78.55	120.0 ±7.50	44.8 ±9.69	67.5 ±11.35	303.57 ±84.15	227.17 ±6.97	1.34
10/05	880.5 ±92.53	62.0 ±6.89	0	118.0 ±12.86	405.24 ±51.22	140.21 ±23.07	2.89

* Number of inflorescences with 70% or more of florets open.

Plant development emphasised the development of primary and secondary crowns in this clover in the first season. Aerial components dramatically changed when secondary crowns developed. Leaf number was not high before the middle of December when secondary crowns had not appeared. After that by mid March there were over 1000 leaves per plant, most from secondary crowns (Figure 3-2). The development of reproductive shoots started on primary crowns from early November, reaching a peak around the middle of February. Over thirty reproductive shoots (about 40% of the total number) emerged from the primary crown. (Figure 3-3). During this period, secondary crowns also developed well, and about eighty reproductive shoots (about 60% of the total number) were produced from secondary crowns. The inflorescences from both primary and secondary crowns contributed to a peak of over 200 per plant (Figure 3-4) at this time, but secondary crowns made the major contribution, producing about 140 inflorescences per plant.

Dry matter accumulation quickly increased from the middle of January, particularly the dry matter of secondary crowns (Figure 3-5). During peak flowering, dry matter accumulation was over two times more in secondary crowns compared with that in primary crowns. Secondary crowns produced over six times more dry matter than primary crowns by early May. In contrast, primary crowns accumulated less than 100g DM plant^{-1} by 10 May, usually from the roots.

3.3.3 Flowering pattern

Caucasian clover cv. Monaro showed a characteristic protracted flowering period from the middle of December to late March (Figure 3-6). The peak of flowering was around the middle of February in 1995 with inflorescences being produced from both primary and secondary crowns. However, most inflorescences arose from reproductive shoots arising from secondary crowns during this period (Figure 3-4). Before early January, all inflorescences were predominantly produced from

Figure 3-1. Production of rhizome initials from primary (RHIPT) and secondary taproot (RHIST) systems of Caucasian clover cv. Monaro in the first growing season (1994-1995). Vertical bars represent individual standard errors.

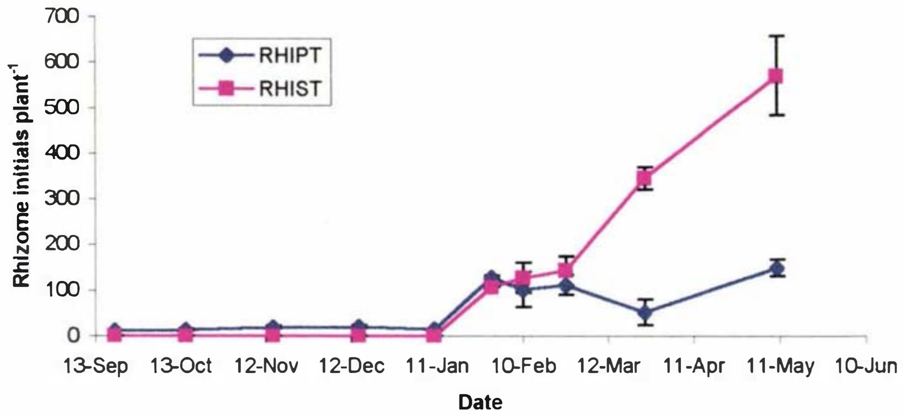


Figure 3-2. Leaf production on primary (PC) and secondary crowns (SC) of single plants of Caucasian clover cv. Monaro in the first season (1994-1995). Vertical bars represent individual standard errors.

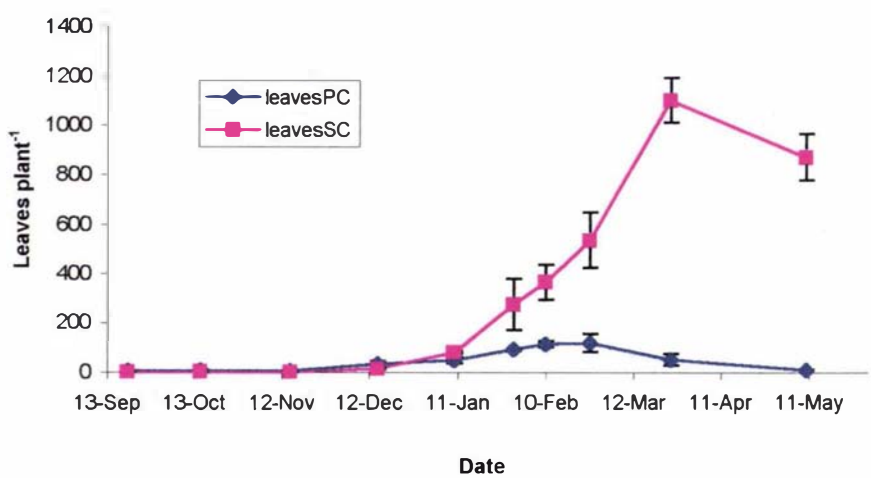


Figure 3-3. Development of reproductive shoots on primary (PC) and secondary crowns (SC) and number of secondary crowns in Caucasian clover cv. Monaro plants in the first season (1994-1995). Vertical bars represent individual standard errors.

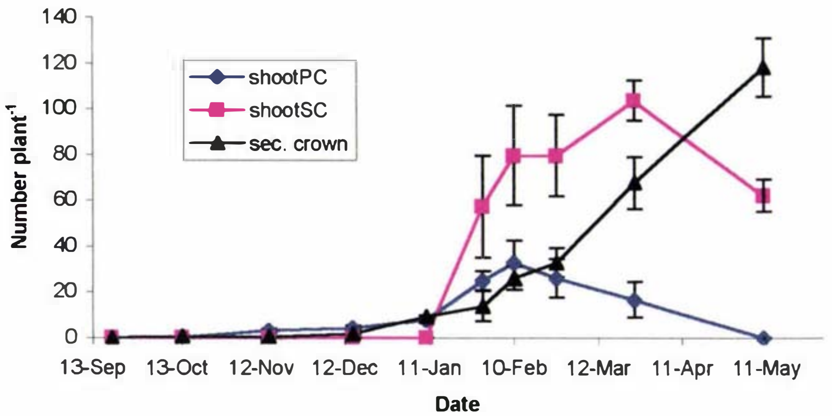


Figure 3-4. Production of inflorescences from primary (PC) and secondary crowns (SC) of Caucasian clover cv. Monaro in the first growing season (1994-1995). Vertical bars represent individual standard errors.

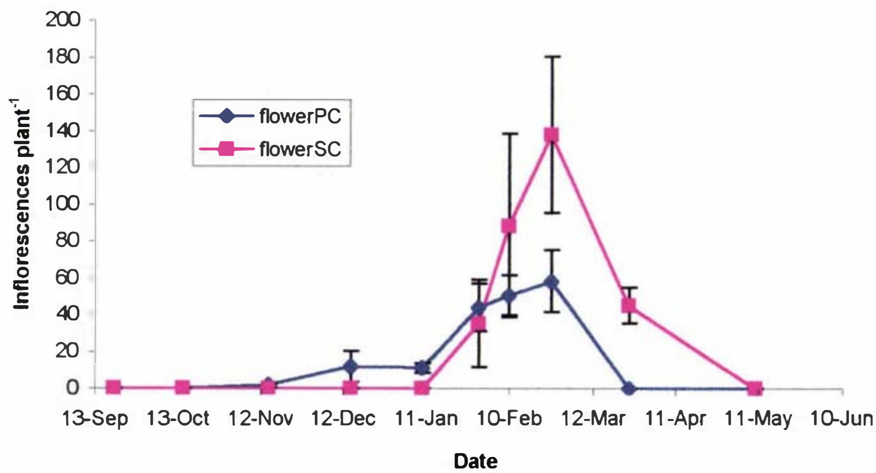


Figure 3-5. Status of aerial and underground dry matter of primary crown (ADMPC, UDMPC) and secondary crowns (ADMSC, UDMSC) of Caucasian clover cv. Monaro in the first growing season in 1994-1995

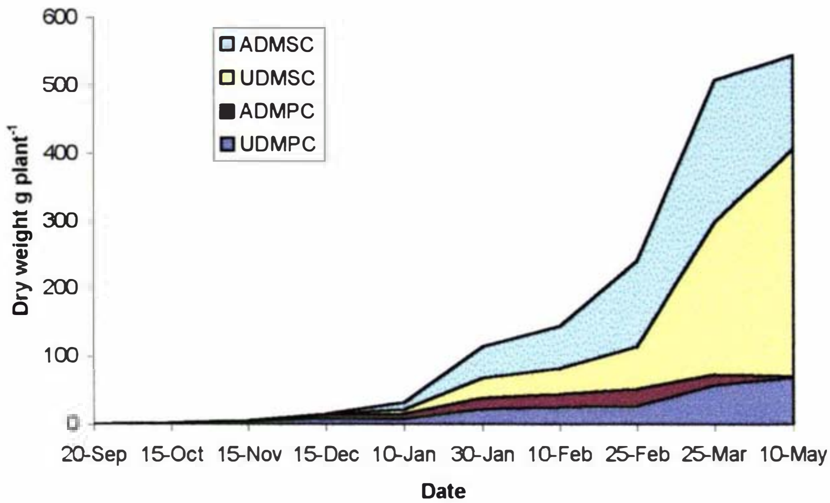
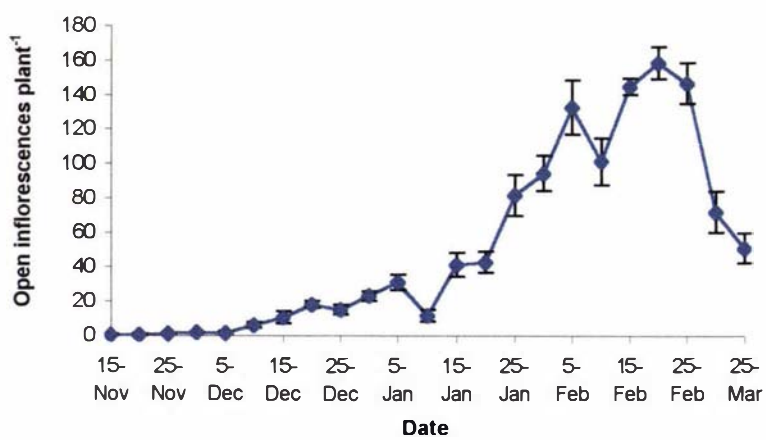


Figure 3-6. Flowering pattern of Caucasian clover cv. Monaro in the 1994-1995 season. Vertical bars represent standard errors



primary crowns (Figure 3-4). Tertiary crowns remained vegetative and no inflorescences were produced in the first season. Although there is a difference in the number of inflorescences between Figure 3-4 and Figure 3-6, this is because data in Figure 3-4 were collected from the plants at the time of destructive sampling.

3.3.4 Relationship between underground dry matter and other plant components

Regression analysis showed that the number of leaves (Figure 3-7), reproductive shoots (Figure 3-8), inflorescences (Figure 3-9) and secondary crowns (Figure 3-10) increased significantly as root dry matter increased during the period from the beginning of plant development to peak flowering. Rhizome initials on the root system also increased as root dry matter increased (Figure 3-11).

3.3.5 Characteristics of the primary and secondary crowns

Development of primary and secondary crowns was different (Table 3-2). During peak flowering significant differences between primary and secondary crowns occurred in leaf, reproductive shoot and inflorescence number, and above ground and underground dry matter per plant, and only primary crowns produced seed. At the harvest on 25 March, primary and secondary crowns produced 3.79 and 21.07 g seed, respectively.

3.3.6 Characteristics of the inflorescences harvested on different dates

Inflorescences harvested on 10 February (early stage of flowering) had fewer seeds per inflorescence, but higher thousand seed weight than inflorescences harvested on 25 March. However, there was no significant difference in floret number per inflorescence or germination percentage for inflorescences harvested on 10 February and 25 March, respectively (Table 3-3). There were differences in

Figure 3-7. Relationship between underground dry matter and leaf number in cv. Monaro

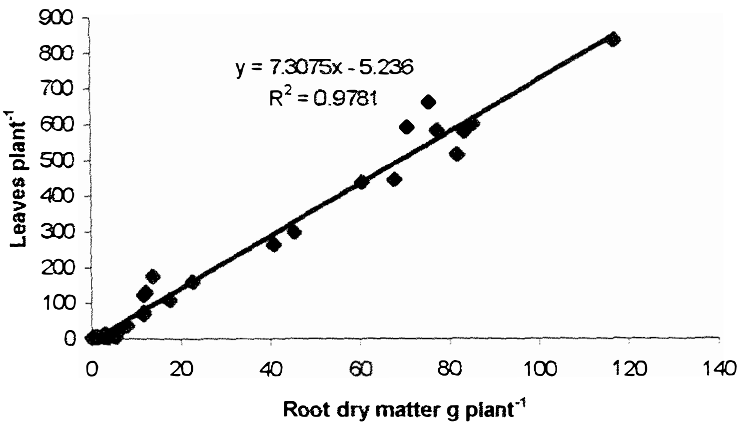


Figure 3-8. Relationship between underground dry matter and reproductive shoot number in cv. Monaro

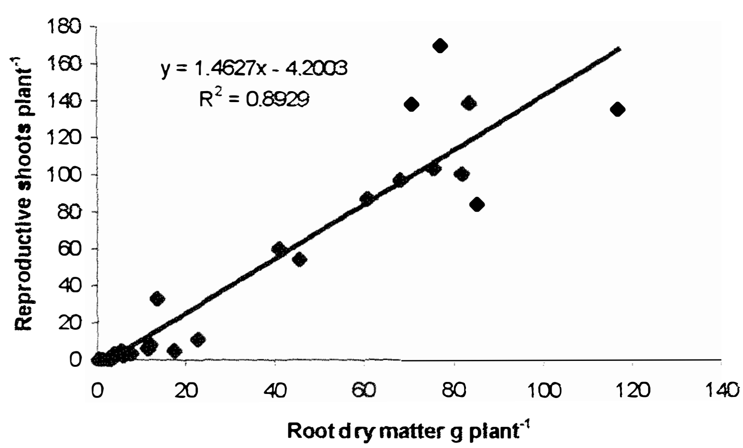


Figure 3-9. Relationship between underground dry matter and inflorescence number in cv. Monaro

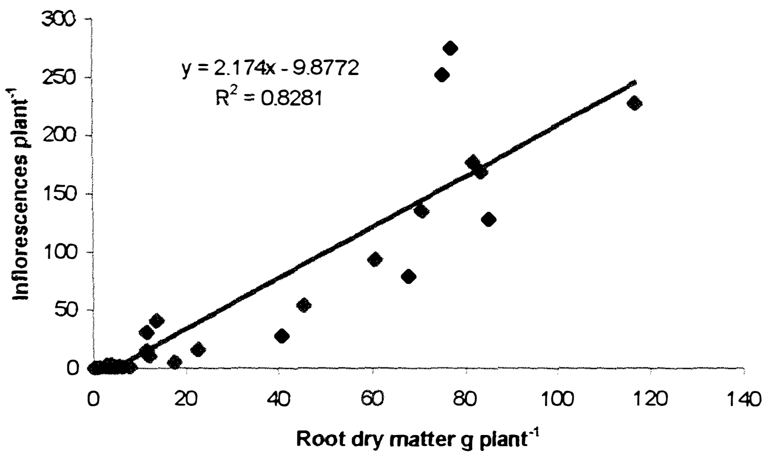


Figure 3-10. Relationship between underground dry matter and secondary crown number in cv. Monaro

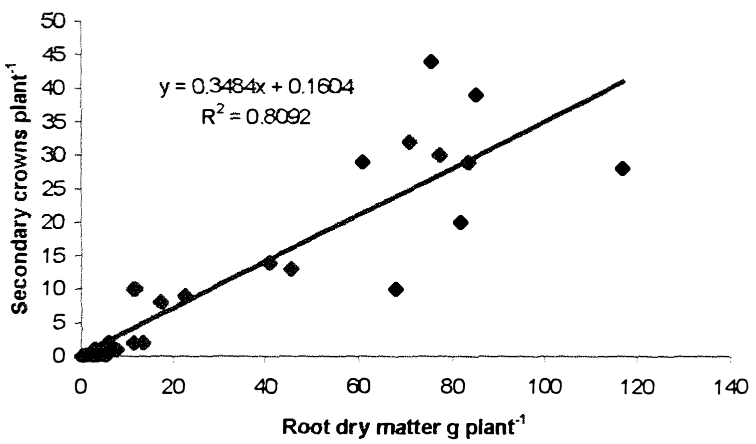


Figure 3-11. Relationship between underground dry matter and number of rhizome initials in cv. Monaro

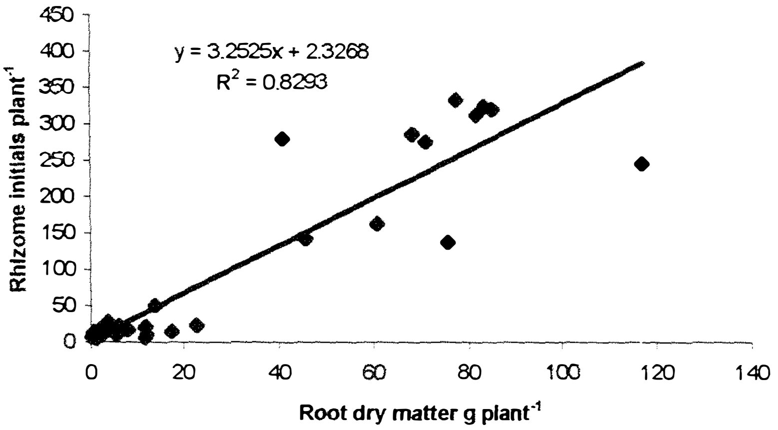


Table 3-2. Characteristics of primary and secondary crowns in terms of leaf number, reproductive shoot number, inflorescence number, seed yield and aerial and root dry matter per plant during peak flowering[1].

Crown type	Crown No.	Leaf No	Shoot No.	Infl. No.	Seed yield (g)	Seed yield[2] (g)	Aerial DM (g)	Root DM (g)
Primary	1	113	33	51	1.78	3.79	26.54	25.17
s.e.	±0	±11.6	±8.45	±9.45	±0.4	±1.15	±5.73	±3.97
Secondary	26	369	80	89	0	21.07	71.62	38.49
s.e.	±4.38	±61.71	±18.75	±43.27	±0	±7.44	±19.26	±6.36

[1] assessed 10 February

[2] seed harvested on 25 March.

Table 3-3. Characteristics of inflorescences harvested in early February or in late March in terms of floret number, seed number per inflorescence, thousand seed weight (TSW), and germination percentage.

Date of harvest	Florets Inflorescence^{-1}	Seeds Inflorescence^{-1}	TSW (g)	Germination (%)
10/2	79.1	35.9	2.834	96.0
25/3	82.9	61.5	2.596	95.5
LSD (0.05)	NS	23.9	0.169	NS

the percentage of pods with zero and one seed for those inflorescences harvested on 10 February and those harvested on 25 March. Pods harvested on 10 February had a significantly higher percentage of empty pods and a lower percentage of pods with one seed (Plate 3-4) compared with pods harvested on 25 March (Table 3-4). The percentage of pods with two seeds was similar whether pods were harvested on 10 February or 25 March (Table 3-4), although the figures obtained and the comparison in Plate 3-4 suggest the possibility of higher numbers of two seeded pods in later harvested inflorescences.

3.3.7 Distribution of pods with zero, one or two seeds

Two-seeded pods occurred more frequently at the top of the inflorescence, and their frequency fell from the top to the bottom of the inflorescence. The florets in the top position also contained the lowest percentage of empty pods. The pods in the middle of the inflorescence had an intermediate number of one and two seeded pods. However, pods produced at the bottom of the inflorescence showed a high percentage (30%) of empty pods, more one seeded pods (52.5%) and less two seeded pods (17.5%) (Table 3-5).

3.4 DISCUSSION

3.4.1 The development of aerial components and the root system

Caucasian clover developed slowly in the first six months (early July to early January) after transplanting in terms of its production of both leaf and stem tissue (Table 3-1). Plant development subsequently accelerated with a dramatic increase in leaf number (Table 3-1, Figure 3-2) and stem number (Figure 3-3) as temperature increased (17.3° C in November to 20.5° C in December) and sunshine hours increased (168 in November to 200 in December) (Appendix 1). This increase in plant size was mainly due to the development of secondary

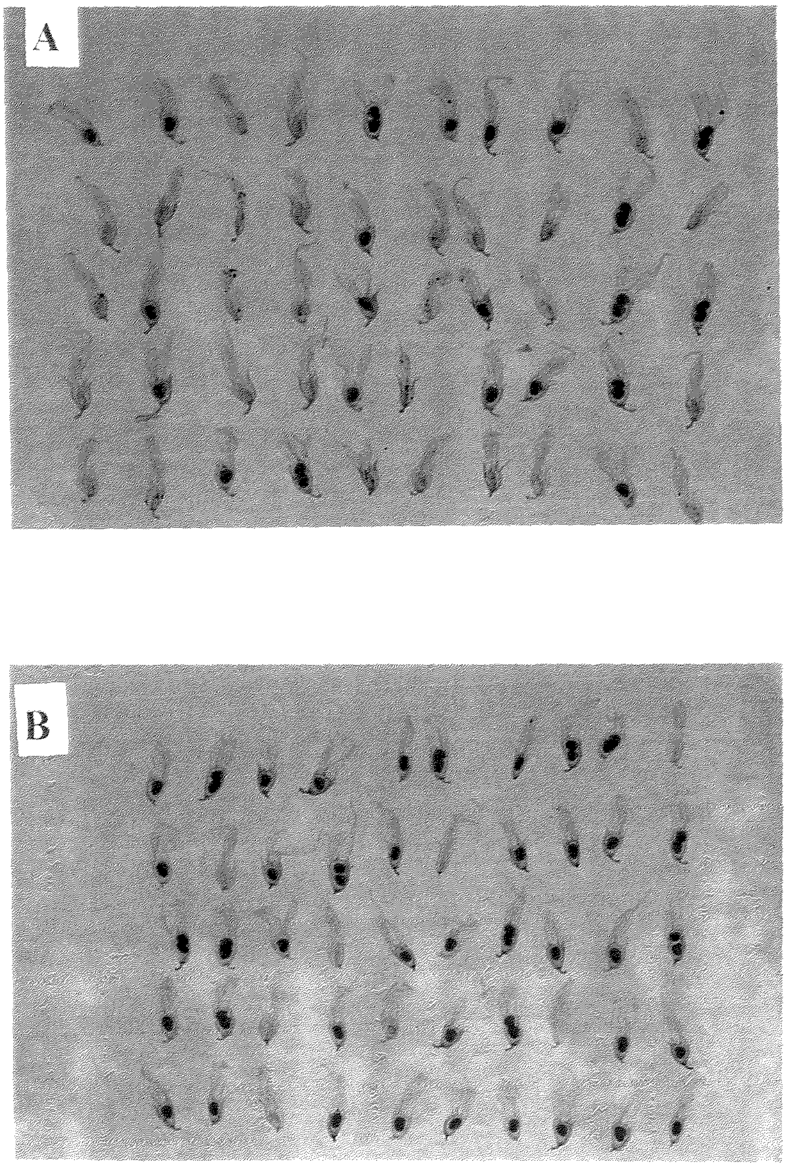


Plate 3-4. X-ray photograph (one replicate only) of differences in seed setting percentage of the pods from an inflorescence harvested on 10 February (A) and 10 March (B) 1995.

Table 3-4. The percentage of pods with zero, one or two seeds harvested in early February and in late March.

Date of harvest	No seeds (%)	One seed (%)	Two seeds (%)
10/2	56.8	30.5	12.8
25/3	18.2	64.5	17.2
LSD (0.05)	24.25	24.37	NS

Table 3-5. Position of florets within an individual inflorescence[1] bearing zero, one or two seeds (%).

Pod position	No seeds	One seed	Two seeds
Top	3.6	10.9	85.5
Mid	7.5	29.2	63.3
Bottom	30.0	52.5	17.5
LSD (0.05)	12.73	11.76	11.91

[1] harvested 25 March)

crowns (Plate 3-2). The numbers of leaves and reproductive shoots rapidly increased from the middle of January, with both leaf and reproductive shoot numbers reaching a peak at 1100 plant^{-1} and 130 plant^{-1}, respectively (Figures 3-2, 3-3) during reproductive growth. Warm temperature promoted plant development. Typically, plants developed primary, secondary and tertiary crowns in the first growing season (Plate 3-2), with primary and secondary crowns being also capable of producing reproductive shoots and inflorescences in the first year (Plate 3-3). Tertiary crowns remained vegetative and in low number in the year of appearance. In the absence of competition, secondary crowns developed reproductive shoots and inflorescences in the first season and dominated the number of reproductive shoots and inflorescences per plant because of the formation and development of many secondary crowns before peak flowering.

Accumulation of whole plant dry matter was slow at the beginning, when plants were in the vegetative stage. However, root dry matter was increasing while leaf production was increasing, and there was a difference in dry matter accumulation between under ground and above ground parts. Root to shoot ratio reached a maximum of 2.59 in October, slightly decreased to 2.45 in November and fell to 1.08 in December (Table 3-1). This demonstrates that dry matter accumulation is faster in underground parts than above ground parts during the vegetative stage. Rapid early root development is a characteristic of Caucasian clover (Moorhead *et al.*, 1994). It is believed that unlike many other clovers, Caucasian clover appears capable of allocating a much higher proportion of photosynthate into root and rhizome development than into the growth of leaves (Spencer *et al.* 1975; Moorhead *et al.* 1994). The results obtained in this trial confirmed this. The accumulation of under-ground dry matter was faster than the accumulation for aerial components in early plant development because a higher proportion of photosynthate could be transported and stored in the root system. This also suggests that the reason for the slow development of aerial components in early vegetative growth is a shortage of nutrients available for their growth, because the root system enjoyed accumulative priority. This could be confirmed using C^{14} tracing. Again, the results in this experiment suggested that Caucasian clover root system is a place for reserves of nutrients. These reserves can be an important guarantee for the following production of reproductive shoots and inflorescences, seed formation and development.

From the middle of December the accumulation of plant dry matter was accelerated under warm summer condition (20.5° C) after an initial slow beginning, particularly from the middle of January to late February. During this period the plants were in reproductive mode under warmer temperature (around 23° C) and high sunshine (186-258 h). Strong reproductive growth resulted in fast dry matter accumulation in the aerial components. In contrast, dry matter accumulation was slowed in the root system. Root to shoot ratio decreased from 2.45 in the middle of November (beginning of reproductive growth) to 0.59 by late February (at the end of peak flowering) and remained at this low level (0.59-0.82) throughout subsequent reproductive development (Table 3-1). It was clear that competition for nutrients between underground and above ground plant components was most intense during reproductive growth. This competitive requirement for nutrition was also evident in the change of root to shoot ratio; accumulation of root dry matter being almost inhibited by the strong reproductive growth. However, the accumulation of dry matter in the root system did not totally stop. This implies that Caucasian clover has the capability of allocating part of its photosynthate to the root system, even if a high proportion of photosynthate was still strongly required for reproductive growth above ground.

At harvest on 25 March, root to shoot ratio had increased from 0.59 at the end of peak flowering to 1.34 at the end of flowering and up to 2.89 by early May (9 months after transplanting). This quick recovery of root to shoot ratio after reproductive growth suggested that a large amount of nutrients were again being allocated preferentially to the root system. Dry matter of the root system increased quickly, from 89 g to 303 g plant^{-1} in one month. This development was reflected by the continuous increase in root dry matter (Figure 3-5), secondary crowns (Figure 3-3) and rhizome initials from both primary and secondary crowns (Figure 3-1) in the first season. This high root to shoot ratio (2.89 9 months after transplanting) in the present study was similar to the 2.36 (diploid) and 3.13 (hexaploid) of Caucasian clover plants reported by Spencer *et al.* (1975), 2.73 by Daly and Mason (1987), 2.3-4.0 (for cv. Monaro) by Coolbear *et al.* (1994) and 2.49, 3.15 and 3.36 for three sowing methods by Moorhead *et al.* (1994).

3.4.2 Interrelationship between root growth and reproductive growth

Priority for root development at an early stage appears to be a characteristic in Caucasian clover. This priority was significant in affecting the slow development of aerial components. Regression analysis of the data in this trial demonstrated that there was a significant relationship between root system dry matter and aerial components, and root system dry matter and the number of secondary crown and rhizome initials. Root dry matter was positively correlated with the number of leaves (Figure 3-7), the number of reproductive shoots (Figure 3-8), and the number of inflorescences (Figure 3-9), as well as the number of secondary crowns (Figure 3-10), and rhizome initials (Figure 3-11) when plants had reached peak flowering. All of these parameters increased when root dry matter increased. The high correlation coefficients (R^2 = 0.80-0.97) clearly showed that the development of aerial components greatly depended on root dry matter accumulation. Root dry matter accumulation was 'driving' both leaf production and the extent of reproductive development above ground. The increase of root dry matter promoted primary and secondary crown development, although this increase was more significant in terms of secondary crowns. Not only were more secondary crowns produced, but also some of these developed sufficiently to produce reproductive shoots and inflorescences. These made a major contribution to reproductive growth.

Flowering in Caucasian clover has been reported to occur in late spring to early summer (Kannenberg and Elliot, 1962; Townsend, 1970; Baysal, 1974). However, the flowering pattern in this study showed that peak flowering occurred in the period between 5 and 25 February when a large number of inflorescences were produced from both primary and secondary crowns as root dry matter trebled (Figure 3-6). A smaller number of inflorescences (about 20-30 inflorescences per plant) were produced from primary crowns before the middle of January (Figure 3-4). This delayed peak flowering period suggested that flowering pattern in cv. Monaro is likely to be related to plant age and root dry matter accumulation in the first growth season, if climate conditions are not limiting. Gurung (1991) noted that young plants have less root reserves for flower production early in their first spring. In this study, while some inflorescences appeared in the middle of November, all inflorescences produced before late January were from primary crowns, and secondary crowns did not produce any inflorescences until early February. In this case, reproductive development was associated with crown age in Caucasian clover. This suggests that reproductive development occurs only from the crowns that mature early enough and have sufficient root reserves available to make reproductive growth possible. Young secondary crowns do not produce reproductive shoots until they are well developed. This development is associated with a dramatic increase of root dry matter accumulation before peak flowering. Reproductive development greatly depends on crown age and root reserves. Coolbear *et. al.* (1994) also suggested that it is likely that much reproductive development is driven by root reserves rather than local photosynthesis.

Caucasian clover had a protracted flowering period (four months) in this study (Figure 3-6). In fact, new inflorescences were still being produced at the time of harvest on 25 March (Figure 3-4). This protracted flowering was caused by the continued production of reproductive shoots from primary crowns, and an extended production of reproductive shoots from secondary crowns when primary crown shoot production had ceased (Figure 3-3). This was also noted by Coolbear *et al.* (1994).

3.4.4 Seed yield and yield components

The total of seed yield obtained was 24.8g $plant^{-1}$. Seed yield in this study largely depended on the numbers of reproductive shoots and inflorescences, and the number of secondary crowns. At peak flowering, inflorescence number was nearly five times greater than the inflorescences produced before the middle of January, reaching an average of about 150 inflorescences per plant (Figure 3-6). Reproductive shoot number per plant and inflorescence number per plant were higher on secondary crowns than on primary crowns. Secondary crowns dominated both numbers of reproductive shoots and inflorescences per plant and made the greatest contribution to inflorescence production.

During the first season, individual plants developed on average one primary crown bearing 33 shoots and 51 inflorescences and 26 secondary crowns each bearing 3 shoots and 3.4 inflorescences (Table 3-2). Although a single primary crown produced an average seed yield of 3.8 g compared with secondary crowns each producing an average seed yield of 0.81 g, total seed yield produced by 26 secondary crowns greatly exceeded the seed yield from one primary crown, reaching 21.1 g per plant. That was five times more than the seed yield from a single primary crown. These results demonstrate that because of high reproductive shoot and inflorescences numbers from secondary crowns per plant, seed yield per plant therefore is almost entirely dominated by the number and size of the secondary crown population.

Because both primary and secondary crown vegetative and reproductive development overlapped in time, a protracted flowering period happened. The characteristics of inflorescences from early (primary crown) and from later harvested inflorescences (primary and secondary crowns) were also studied. It was found that later harvested inflorescences had a higher number of seeds per inflorescence but lower thousand seed weight than those harvested early (Table 3-3). Each floret of Caucasian clover contains an ovary bearing two ovules. However not every ovule was capable of developing a seed. The results of samples from inflorescences harvested on 25 March indicate that about 80% of florets can survive to the pod stage, and that pods containing one seed were more common than pods with two seeds. However, the percentage of one seeded pods per inflorescence was twice that of the early harvested inflorescences, and the percentage of empty pods was lower (18% versus 57%) (Table 3-4). This difference in seed number per inflorescence between early and late harvested inflorescences implicates competition for root reserves during reproductive development. Early inflorescences developed at a stage when accumulation of root dry matter was not high (16.1 g plant^{-1}), and therefore only limited root reserves would be available for reproductive development above ground and for root system development. With such a shortage of assimilates, many seeds in a single inflorescence may abort or fail to mature. Of course, another possibility may be the presence of pre- or post-fertilisation problems. When the plant was at peak flowering, accumulation of root dry matter had increased five times (89.9 g plant^{-1}), and as more root reserves were available for seed development, higher seed numbers per inflorescence would be possible.

Further competition for nutrients could occur between primary and secondary crowns during peak flowering as 21.1 g seed was produced from 80 reproductive shoots on 26 secondary crowns on an individual plant, while only 3.79 g seed was produced from 33 shoots on a primary crown (Table 3-2). This disproportionate seed yield could indicate nutrient competition. Secondary crowns were smaller in size compared with primary crowns, each produced only 3 reproductive shoots, and each shoot produced 1.1 inflorescences or 0.26 g seed. A single primary crown can produce 33 reproductive shoots, each shoot generated 1.6 inflorescences, however, and only 0.11 g seed was produced from each shoot. Although a single secondary crown produced fewer reproductive shoots and inflorescences, seed set was higher than that from the early harvest. The difference in seed number per inflorescence could be a result of competition for nutrients. Low numbers of seeds per inflorescence from primary crowns may result from fewer nutrients available for seed development as more nutrients were consumed by a greater number of reproductive shoots. If a single primary crown supplied the nutrients for the development of 33 reproductive shoots, the secondary crowns would have less nutrient supply stress, as only three reproductive shoots were supported by a single secondary.

It is possible that differences in pod set and seed set in early and late harvested inflorescences could be associated with differential pollinator activity as influenced by weather conditions during flowering. Warm, dry and sunny days are favourable for bee foraging on inflorescences (Bryant, 1974; Stewart, 1979; Gurung, 1991). Warm temperature (14.5-23.3° C) in February would be likely to have increased the chance for bee visits, and therefore a higher pod or seed set could be possible for inflorescences harvested later. Although low seed numbers were found in early harvested inflorescences, this does not necessarily mean pollination is a problem in Caucasian clover, since over 80% of florets from late harvested inflorescences survived to the pod stage (Table 3-4). This frequency compares well with results reported by Coolbear *et al.* (1994) in the same cultivar.

This study revealed that pods with different numbers of seeds were distributed differently in the top, middle or bottom positions of the inflorescence. Two-seeded pods appeared more likely in the middle or top positions, while florets at the bottom of the inflorescence produced more one-seeded pods. This result is opposite to results from white clover (S. Pasumarty, personal communication). At present, it is not known why this occurred. Perhaps it was associated with pollinator activity. The Caucasian clover inflorescence is globose at first, but oblong or ovoid finally, and florets open from bottom to top. At first, a small number of open florets on the inflorescence may have less attraction to pollinators, so that lack of pollination and fertilisation could occur (30% of florets may be sterile) and this led to less two seeded pods being produced. When most florets are open in the later development of the inflorescences, they are likely to be more attractive to pollinators, allowing more two seeded pods to be formed.

Nearly 85% of the total seed yield was generated from secondary crowns in the first season. If the root system of a plant had developed well, that would guarantee the production of more secondary crowns, allowing increased seed yield potential in the first season. Improved seed yield in Caucasian clover is likely to occur under suitable management conditions, with the application of suitable rhizobium, correct sowing time and higher fertiliser rates i.e. any management system likely to increase the rate and extent of the root system. Rhizobia strain ICMP4073b suggested by Lowther *et al.* (1992) can result in a 64% increase in seedling nodulation (Patrick *et al.*, 1994). Similarly, early sowing (in January) has been reported to be critical as time is needed to allow plants to become established and well developed before the onset of winter (Guy, 1996). High fertiliser rates (300 kg ha^{-1} of molybdic sulphur superphosphate) can also improve establishment and plant growth (Moorhead *et al.*, 1994). A well-developed root system would promote the development of secondary crowns and seed yield could be increased, as it is likely that in the next growing season these secondary crowns would become major sites for inflorescence production. However, this is yet to be confirmed.

Chapter 4

DIFFERENCES IN MORPHOLOGICAL AND REPRODUCTIVE CHARACTERISTICS AMONG INDIVIDUAL PLANTS OF CAUCASIAN CLOVER CV. MONARO

4.1 INTRODUCTION

Caucasian clover has been evaluated agronomically for more than 20 years in New Zealand. Work with cv. Summit (2n) (Paljor, 1973), cv. Treeline (4n) (Stewart, 1979) and cv. Monaro (6n) (Gurung, 1991) has shown that these cultivars exhibit several characteristics which identify Caucasian clover as a promising legume for pastures, as well as for eroded slopes for high country environments (Efendi, 1993). However, reliable seed supply has been one of the main factors limiting further evaluation work and commercialisation (Guy, 1996). Current cultivars from Australia, such as Prairie and Monaro, have shown variable and poor seed yields (Widdup *et al.* 1996). To date actual commercial seed yields have ranged from 0-200 kg ha^{-1}. Experimental seed yields from plot trials have also been variable, ranging from 40-900 kg ha^{-1} (Bryant, 1974; Voloshenko *et al.* 1979; Stewart and Daly, 1980; Efendi, 1993; Guy, 1996).

Morphologically, Caucasian clover is a very diverse species (Townsend, 1970) with a considerable genetic mixture. Plant height, leaf size, petiole length, rhizome production and overall plant size not only increase as the ploidy level increases (Hely, 1957; Kannenberg and Elliott, 1962; Baysal, 1974; Meares, 1975), but also similar variation exists among individual plants within the same cultivar. From an observation of thirteen individual plants of cv. Monaro in 1993-1994, it was noted that not every individual produced inflorescences and yielded seed under the same conditions (Fu, unpublished data). Even for those plants which generated inflorescences, seed yield differed from plant to plant. This variation could be associated with unreliable seed yield in the cultivar.

The purpose of this study was to investigate the morphological variation of three different genotypes selected from within a population of cv. Monaro, their flowering behaviour, the variation linked with seed yield and seed yield components of the three genotypes, and suggest possible factors affecting seed yield in cv. Monaro.

4.2 MATERIALS AND METHODS

4.2.1 Experimental site, management and treatments

This trial was carried out at the campus plot no. 21 of the Pasture Research Unit, Department of Plant Science, Massey University, Palmerston North beginning in September 1994. The experimental site was maintained in an area of 9 x 4.8 m, which held twelve plots. Each plot was 3 x 1.2 m (Appendix 2). The soil type was an Ohakea silt loam, which is a flat soil occurring on low terraces from old colluvium overlying stony alluvium. It is considered to have low internal drainage and is overall imperfectly to poorly drained. The natural nutrient status is low phosphorous, medium calcium, and low potassium, and in general it is used for intensive sheep and cattle farming and some horticulture (Cowie, 1974; Southward, 1997). A soil analysis (November 1994) is presented in Appendix 3.

Thirteen plants were originally dug up from a four year old established stand of Caucasian clover cv. Monaro (Rolston, personal communication) at the AgResearch Grasslands Research Unit, Aorangi, Palmerston North, New Zealand in January 1992. These were cultivated in buckets to observe plant development. Seed was harvested from these plants in the 1993-1994 season (Appendix 4). Three (No. 2, 9, 12) of the thirteen plants cultivated in the buckets were selected and used as the genotypes in this study. These three plants were chosen because of their apparent differences in inflorescence production i.e. high (No. 2), medium (No. 12) and zero (No. 9).

Fifty young crown segments were cut from rhizomes of each genotype on 2 September 1994. The total of 150 crown segments from the three genotypes were planted immediately in rootrainers (5 x 6 x 25 cm) containing seed raising mix (Yates Black Magic) and were kept in a glasshouse (30/20 ± 5° C) at the Seed Technology Centre. Forty plants of each genotype were then randomly selected for use in this experiment. They were watered once a week. In the middle of October, when the young plants were 10-20 cm high, they were placed outside to harden off. The field experiment was conducted as a randomised block design (RBD). Four replicates, each containing ten plants (4 x 10) were set up for each genotype. The young plants were transplanted into the field on 5 November 1994. In each plot, plants were placed at a distance of 60 cm between plants and between rows.

After transplanting, the plants were irrigated immediately with a garden sprinkler for four hours (600 litre hr^{-1}). From then, they were watered for four hours every week (except during wet weather) to ensure they established well in the first month after transplanting. Subsequent irrigation was applied with the same sprinkler for six hours (because of low soil moisture during a low rainfall period from December to February (Appendix 3-1) at ten day intervals until the middle of February 1995. Before transplanting the young plants, the herbicide Trifluralin was applied at 800 g a.i. ha^{-1} to control weeds. After transplanting, slug and snail bait (Mesurol (Methiocarb, 20 g kg^{-1} bait)) was broadcast at 50 g m^{-2} on the soil surface on 30 November. Attack (pirimiphos-methyl, 475 g $litre^{-1}$) was sprayed at 0.5 kg a.i. ha^{-1} to control insect pests on 1 December 1994. Hand weeding was carried out when it was necessary during plant development. Nitrophoska (N:P:K:S = 12:10:10:2) was applied in the rate of 0.9 kg (200 kg ha^{-1}) before inflorescence buds were visible on 5 December.

Seed production potential was assessed on individual plants during the 1994-1995 growing season. At the beginning of plant development, four plants from each plot (a total of 16 plants from each genotype) were randomly selected and identified by a coloured label for later data collection at seed harvest. During reproductive development, leaf size was visually assessed in five grades from large to small (5-1) (Plate 4-1). Flowering date was recorded as when the first inflorescence appeared in each plot. An inflorescence was regarded as flowering when over 50% of the florets were open. Also, the number of plants with inflorescences was counted in each plot of the three genotypes on 1 March 1995.

At harvest, all labelled plants in each plot were measured for diameter (the mean of two measurements taken on a plant's north/south and east/west axis). Then, they were cut at the base and leaf number, stem number and inflorescences per plant were counted for each labelled plant. Mature inflorescences were harvested by hand from each plant and sealed separately in a paper bag for air drying. Aerial components of each plant were kept in a paper bag and dried in an oven at 60° C for 48 hours to obtain aerial dry matter values.

All harvested inflorescences were kept in the laboratory at the Seed Technology Centre and naturally air dried at room temperature (about 24° C) for two months. After drying, four inflorescences were randomly sampled from each bag and the number of florets per inflorescence was counted. The florets from each inflorescence were threshed by rubbing between stippled rubber. Seed number per inflorescence was recorded. The remainder of the inflorescences from each bag were threshed in the same way. Then, straw and light material was removed by sieving through 1 mm and 2 mm sieves. The remainder was purified in an air blower at 345 rpm for 30 seconds (Burrows. Model 1836-4). Seed yield per plant was measured by processing all inflorescences harvested from a single plant.

Plate 4-1. Leaf size scale from large to small (5 - 1) in Caucasian clover cv. Monaro.

Germination percentage and thousand seed weight were tested under the rules of the International Seed Testing Association (ISTA, 1996).

All data were processed using a SAS package (SAS Institute Inc., 1985-1990) for analysis of variance and Fisher's LSD test at P = 0.05.

4.3 RESULTS

4.3.1 Morphological characters

Plants of the three genotypes differed significantly for leaf size, stem number per plant, plant diameter and plant dry matter, but not leaf number per plant (Table 4-1). Genotype 9 had fewer stems, smaller sized plants and less dry weight than genotypes 2 and 12, but did not differ for leaf size from genotype 2. Its darker green leaf colour was obvious (Plate 4-2). There was no difference in stem number per plant, plant diameter and plant dry matter between genotypes 12 and 2. However, genotype 12 had much larger leaves than genotypes 2 and 9. This is also demonstrated clearly in Plate 4-2.

4.3.2 Reproductive characteristics

Genotypes 2 and 12 produced their first inflorescences on 15 and 17 January 1995, respectively and by early March had 13 (genotype 2) and 20 (genotype 12) inflorescences per plant. The first inflorescences did not appear in genotype 9 until 20 February, one month later than genotypes 2 and 12 (Table 4-2). This genotype produced on average less than one inflorescence per plant. While an average of three plants in each plot (10 plants) of genotype 9 produced several inflorescences, most plants of genotype 9 produced no inflorescences during the reproductive development (Table 4-2).

Plate 4-2. Plants of genotypes 2, 9 and 12 selected from Caucasian clover cv. Monaro.

Table 4-1. Comparison of above ground characters (leaf number plant^{-1}, leaf size, stem number plant^{-1}, plant diameter and dry weight) in cv. Monaro genotypes 2, 9 and 12 at seed harvest (7 April 1995).

Genotype no.	Leaves plant^{-1}	Leaf size	Stems plant^{-1}	Plant diameter (cm)	Plant DM (g)
2	160.1 a	1.5 b	12.0 a	36.3 a	19.1 a
9	132.4 a	1.1 b	1.0 b	25.9 b	9.9 b
12	158.9 a	2.8 a	15.6 a	35.6 a	22.9 a
LSD (P=0.05)	NS	0.5	4.7	2.1	7.3

Means with the same letter do not differ at $P \leq 0.05$

Table 4-2. Comparison of flowering behaviour in genotypes 2, 9 and 12 of cv. Monaro.

Genotype No.	First flowering date*	Flowering plants plot^{-1}	Inflorescences# plant^{-1}
2	15/01	8.5 ± 0.6	13.3 ± 2.7
9	20/02	3.3 ± 0.7	0.9 ± 0.5
12	17/01	8.0 ± 0.5	20.5 ± 2.9

* The date the first inflorescence bloomed.

on 1 March 1995

Seed yield per plant and inflorescence numbers per plant differed significantly between genotype 9 and genotypes 2 and 12, although there was no difference between genotypes 2 and 12. Significant differences also occurred in thousand seed weight, but not in floret number and seed number per inflorescence (Table 4-3). Genotype 9 had a lower inflorescence number per plant and lower seed yield per plant compared with genotypes 2 and 12. Thousand seed weight was highest in genotype 2, followed by 9 and 12 (Table 4-3).

There were small but significant differences in seed germination among the three genotypes (Table 4-4). Genotype 12 had the best germination percentage, followed by genotype 9 and 2. There were no differences in abnormal seedlings or hard seed percentage although more dead seeds were found in genotype 2 than in the other two.

4.4 DISCUSSION

These three genotypes of Caucasian clover cv. Monaro differed significantly in their morphological and reproductive characteristics. This suggests that this cultivar of Caucasian clover has a considerable genetic mixture, as indicated by the variation occurring among the individual plants within the cultivar. Morphological differences among individual plants can occur not only at species level (Townsend 1970) but also within ploidy level and in different populations (Kannenberg and Elliott, 1962; Stewart, 1979). Genotype 9 in the current study was an example of this, as it had a smaller leaf size, darker green leaf colour, smaller plants and less dry matter than the plants from genotypes 2 and 12. However, genotype 12 had larger leaves than genotypes 2 and 9. These morphological variations were significant even though all three genotypes came from the same original seed lot sown as cv. Monaro. This suggests that genetic

Table 4-3. Comparison of seed yield and seed yield components (inflorescences plant^{-1}, floret and seed number inflorescence^{-1}, seed yield plant^{-1} and thousand seed weight) in genotypes 2, 9 and 12 of cv. Monaro.

Genotype no.	Infl*. plant^{-1}	Florets infl^{-1}.	Seeds infl^{-1}.	TSW (g)	Seed yield (g) plant^{-1}
2	34.7 a	80.8 a	49.4 a	2.575 a	3.65 a
9	1.1 b	86.5 a	60.0 a	2.125 b	0.18 b
12	41.6 a	87.6 a	56.7 a	1.900 c	2.99 a
LSD (P=0.05)	13.8	NS	NS	0.065	1.48

Means with the same letter do not differ at $P \leq 0.05$

* Inflorescences harvested on 7 April 1995

Table 4-4. Comparison of germination characters among seed from genotypes of cv. Monaro.

Genotype no.	Normal seedlings* (%)	Abnormal seedlings (%)	Fresh ung. (%)	Dead (%)	Hard seed (%)
2	91 c	2 ab	4 a	3 a	92 a
9	97 b	3 a	0 b	0 b	95 a
12	99 a	1 b	0 b	0 b	90 a
LSD (P=0.05)	1.63	1.63	2.83	0.99	NS

Means with the same letter do not differ at $P \leq 0.05$

* These data were obtained after hard seededness was removed by cutting the seed coat with a scalpel (ISTA, 1996).

differences exist among the individual plants within the cultivar, a conclusion also reached by Widdup *et al.* (1996).

This variation also occurred for reproductive growth among the three genotypes, which had different flowering behavior. Both genotype 2 and genotype 12 began to flower in the middle of January and reached peak flowering in early March in the first season. However, genotype 9 was later flowering, blooming one month later than the other two, and failed to reach a peak flowering, since few inflorescences appeared. Although cultivar Monaro does contain plants that flower at different times during a growing season (Efendi, 1992; Hill *et al.* 1993), different flowering times among individual plants lead to a longer flowering period. This may also indicate greater variability among individuals within the cultivar (Gurung, 1991). This protracted flowering among individual plants made it difficult to produce high numbers of inflorescences over a short period. Although this was a single season study and only three genotypes were used, it was also obvious that not all plants bloomed at a similar time. Such flowering behaviour could be one of the reasons for the protracted flowering period of the cultivar (Widdup *et al.* 1996), although it is believed that protracted flowering also results from the continuous production of reproductive shoots from crowns. Coolbear *et al.* (1994) have shown that as stems appear from the crowns and continue to develop flower-bearing branches from each leaf axil, the youngest flowers are at the tip and older flowers are located further down the stem.

Only 30% of the plants of genotype 9 produced inflorescences, the rest staying vegetative during the entire growing season. This suggests that in the at least some genotypes of Monaro there is an obligate need for a flowering induction effect. Kannenberg and Elliott (1962) have earlier reported that all ploidy levels of Caucasian clover flower under a 17-hour day and temperature ranging from 4.5° C at night to a daytime minimum of 21.5° C. At present, however there is a general lack of knowledge on the photoperiod and temperature effects on flower induction in Caucasian clover, although low temperature appears to be required for flower induction (Taylor, 1994; Widdup *et al.*, 1996).

The results in this study have clearly demonstrated that differences in the capability for reproductive development exist among individual plants of cv. Monaro and that there are significant differences in seed yield per plant and seed yield components between different genotypes. The main components affected are reproductive stems and inflorescence numbers per plant. Significant differences between genotype 9 and genotypes 2 and 12 showed that genotype 9 had poor reproductive capability and not every individual plant in the genotype produced inflorescences and seed. This could be an inherent genetic character. Nevertheless, some plants did flower. A similar situation was seen in a cv. Monaro population in an earlier study, where four (No. 3, 8, 9 and 13) of thirteen individual plants did not flower in the 1993-1994 season (Appendix 4-3). This poor reproductive ability in some individuals could be related to the inherently poor seeding ability of Monaro (Hampton *et al.* 1990; Hill *et al.* 1993).

For seed production it is essential to produce a high number of reproductive stems and inflorescences per unit area. In legumes in general, the number of inflorescences is the most important seed yield determinant e.g. for white clover (Van Bockstaele and Rijckaert, 1988; Hollington *et al.*, 1989; Marshall *et al.*, 1989; Nordestgaard, 1989) and birdsfoot trefoil (Albrechtsen *et al.*, 1966; Mos, 1983; Stephenson, 1984; McGraw *et al.*, 1986; Li and Hill, 1988; 1989). Caucasian clover seems to be similar. In the present study, because of significant differences in the number of stems and inflorescences produced per plant, the three genotypes showed large differences in seed yield. Genotypes 2 and 12 had seed yields of 3.65 g and 2.99g per plant, respectively, while genotype 9 had a seed yield of only 0.18 g per plant. Considerable variation for seed yield per plant within a hexaploid population has also been reported by Widdup *et al.* (1996). The variation in seed yield among individual plants in the same population can affect seed yield per unit area. Although it is not known what percentage of plants without inflorescences were in the original population, the phenomenon that plants fail to produce inflorescences, or produce only a few inflorescences, can be considered as a factor that limits or affects seed yield of the cultivar, particularly in the first few years, and would also lead to genetic shift if a proportion of the population failed to produce seed.

Plant variation in the cultivar not only occurred in morphological and reproductive characteristics but also in some aspects of seed quality. Thousand seed weight differed significantly between the three genotypes. Genotype 2 had a thousand seed weight of 2.575 g, compared with 2.125 g for genotype 9 and 1.900 g for genotype 12. This compares with a weight of 2.986 g (for 6n) reported by Kannenberg and Elliott (1962). This suggested that thousand seed weight of cv. Monaro was variable as the differences occurred among genotypes. Hard seed percentage was similarly high (over 90%) in all three genotypes.

The differences in morphological and genetic characteristics among individual plants in cv. Monaro suggest there is considerable genetic variation available for components that together constitute a good seed yield and would be amenable to selection (Hampton, 1990). Because of these intra cultivar variations, selection for plants that have uniform flowering and produce more inflorescences would be a significant and important way of improving seed yield. The newly released cultivar Endura is such an example (Norriss, 1995). This cultivar has more uniform flowering, higher seed yield potential and good agronomic performance (Guy, 1996) although there is still sufficient variation to enable further selection gains (Widdup *et al.*, 1996).

Chapter 5

EFFECTS OF PLANT DENSITY ON VEGETATIVE AND REPRODUCTIVE DEVELOPMENT AND SEED YIELD OF CAUCASIAN CLOVER CV. MONARO

5.1 INTRODUCTION

Plant density is a primary factor to be considered in studies on plant production. The number of plants per unit area can have a major effect in altering the micro-environment (i.e. light, water, nutrients etc.) which can result in changes in both vegetative and reproductive crop yields (Phetpradap, 1992). Donald (1963) and Etherington (1983) have defined two kinds of competition in plant populations, i.e. intraplant competition and interplant competition. Different plant densities are investigated usually with the intention of determining an optimal density for maximum yield per unit area (Holliday, 1960; Willey and Heath, 1969). At a given density, the highest yield is normally obtained from an equidistant plant spacing, since this offers a more desirable environment for the spread and development of plants than those with a spacing of unequal dimensions (Mack and Varseveld, 1982).

There is a relationship between seed yield and plant density, but this relationship is complicated because of the plasticity of yield components and the interaction between the environment and genotype (Phetpradap, 1992). In general, the relationship between seed yield and plant density is expressed with parabolic or asymptotic curves (Willey and Heath, 1969; Chapman, 1981) although Holliday (1960) considered the parabolic relationship was more suitable for reproductive forms of yield (i.e. grains and seeds).

As one example, seed yield of white clover (*Trifolium repens* L.) comprises the number of inflorescences per unit area, number of florets per inflorescence, number of seeds per floret and seed weight (Thomas, 1981; Maldonado, 1985; Pasumarty, 1990). These yield components relate to plant structure and in turn are determined by a combination of plant and environmental factors (Adams, 1975). In white clover, the number of inflorescences is the most important single seed yield determinant in various situations (Van Bockstaele and Rijckaert, 1988; Hollington *et al.*, 1989; Marshall *et al.*, 1989; Nordestgaard, 1989), and the development of inflorescences can be considerably influenced by plant density (Zaleski, 1961). This could also be the situation for Caucasian clover, since seed yield per plant is almost entirely dominated by the number of inflorescences which in turn is dominated by the number and size of the secondary crown population (Chapter 3). A previous study had also shown that seed yield increases when more inflorescences per unit area are produced from a wider row spacing in hexaploid Caucasian clover (Guy, 1996).

There is only limited information available on Caucasian clover cultivation under New Zealand conditions, e.g. the effect of sowing method (Moorhead *et al.* 1994), and row spacing (Guy, 1996), but little on plant density, and more information is required to determine how plant density affects seed yield in this clover. A study of the effect of plant density on plant vegetative growth and reproductive development using a wide range of plant populations would be useful to provide a basic understanding of the factors affecting seed yield. The objectives of this experiment were therefore to determine the effects of plant density on vegetative and reproductive growth, seed yield, and yield components of Caucasian clover cv. Monaro, and to determine differences in seed yield among twelve genotypes of cv. Monaro under different plant densities.

5.2 MATERIALS AND METHODS

5.2.1 Experimental site and soil conditions

The field experiment was conducted in the campus plots (paddock number 21) of the Pasture Research Unit, Department of Plant Science, Massey University, Palmerston North in two growing seasons from 1994-1996. Prior to 1994, the area was a demonstration field for agriculture engineering. The land was ploughed in the middle of March 1994 and power harrowed three weeks later in early April and again on 22 April before planting. The soil type was an Ohakea silt loam (see Chapter 4). Soil samples from depths of 0-15 and 15-30 cm were taken from the area where Caucasian clover plants were to be established and sent to the Fertiliser & Lime Research Centre, Massey University, Palmerston North for analysis. The analysis of the soil samples was completed for seed sown and seedling transplant radial trials on 10 June 1994. The results of the analysis are given in Appendix 5. Climate data are shown in Appendix 1 for 1994-1995.

5.2.2. Experimental design and material used

For plant spacing studies Nelder (1962) suggested the use of a systematic radial design which was later modified by Bleasdale (1967). For these two experiments plant density responses were studied using the 4.5° radial design (Figure 5-1). With this design a large range of plant densities are expressed in a small area. All radii radiate in rows out from a central point. The radial trial consisted of 25 concentric circles representing 25 plant densities with 80 plants in each circle. The two outer-most and four innermost circles were used as border rows. Therefore 19 plant densities ranging from 2.6 to 44.7 plant m^{-2} were set up. Five densities were selected as low (3.1, 5.8 plants m^{-2}), middle (10.8 plants m^{-2}) and high densities (20.3, 38.2 plants m^{-2}) for studying the effect of plant population on vegetative and reproductive development and seed yield (Table 5-1). In this design the distance

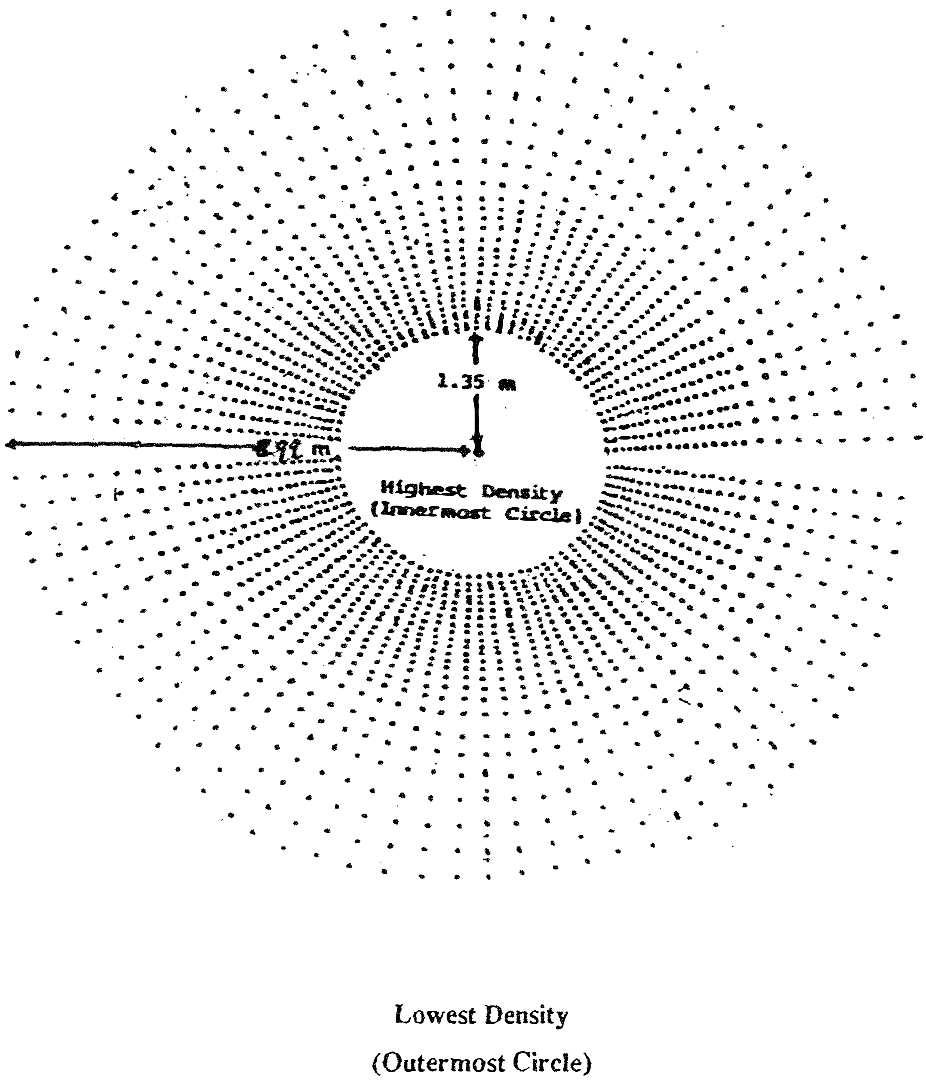


Figure 5-1. The layout of the Nelder radial spacing design (Type Ia).

Table 5-1. Numerical data for the layout of the radial spacing trials.

Arc number (from innermost)	Distance along radii (m)	Area plant^{-1} (cm^2)	Plant density (plant m^{-2})
1#	1.35	119	83.9
2#	1.47	140	71.6
3#	1.59	163	61.2
4#	1.71	191	52.3
5	1.86	224	44.7
6*	2.01	262	38.2
7	2.18	307	32.6
8	2.35	359	27.8
9	2.55	421	23.8
10*	2.76	493	20.3
11	2.89	578	17.4
12	3.23	675	14.8
13	3.49	790	12.7
14*	3.78	925	10.8
15	4.09	1083	9.2
16	4.42	1268	7.9
17	4.79	1484	6.7
18*	5.18	1738	5.8
19	5.60	2034	4.9
20	6.06	2382	4.2
21	6.65	2788	3.6
22*	7.10	3264	3.1
23	7.68	3821	2.6
24#	8.31	4474	2.2
25#	8.99	5238	1.9

Border rows.

* Selected plant densities used for reproductive studies.

between each pair of plants along a radius was approximately equal to the distance between the radii at that point. An almost rectangular (square) arrangement was set up (Appendix 7). The calculations of distances between each radius and between plants along the radii were based on Bleasdale's method (1967).

This experiment consisted of two radial trials, one involving sown seed and the other involving seedling transplants (twelve genotypes) in a semi-radial trial (Plate 5-1). Each of the radial trial plots was 19 m in diameter. Seeds or seedlings of cv. Monaro were used in these two radial trials.

5.2.3 Planting, measurement and statistical analysis

Seed sown radial trial:

In the seed sown radial trial, seeds of cv. Monaro were autumn sown on 26 April 1994, following inoculation with Nitrobug Legume Inoculant (Coated Seed Ltd, Christchurch N.Z.) following the packet directions for use. Trifluralin (800g a.i. ha^{-1}) was sprayed and incorporated by rotary hoe before sowing the seed. Seeds were sown within two hours of inoculation using three seeds per hole along each radius. A metal rod (9 m steel pipe) marked at the specified distances was laid on the soil surface and the inner end was fixed at the center of the circle to set up the first radius. The inoculated seeds were sown at the marked positions. When the first line was sown, the outer end of the rod was moved 70.5 cm, while the inner end was kept fixed at the center until the whole circle was completed. A small area (about 2 m^2) was also sown near the experimental plot for later replacement of seedlings that failed to establish. At the 3 leaf stage, emerged plants were thinned to one plant per position (Nelder, 1962).

Plate 5-1. Layout of seedling transplant (genotype) radial trial (back).

Irrigation was applied immediately after sowing using a garden sprinkler for two hours (600 litres hr^{-1}) and the same amount of irrigation was applied every 7-10 days until the end of June and as required from early September to the end of December, except for wet period during vegetative growth. Mesurol Slug and Snail Bait (Methiocarb, 20 g kg^{-1}) was broadcast at 5 g m^2 two weeks after seed sowing and again three weeks later. A shieded spray of Buster (Glufosinate-ammonium 200 g l^{-1}, 5 l ha^{-1}) was carried out in August and September, respectively. Fusilade (Fluazifop-P-butyl 250 g kg^{-1}, 2 l ha^{-1}) was also applied to control grass weeds in the experimental site on 30 November 1994. Nitrophoska (N:P:K:S = 12:10:10:2) was applied at 200 kg ha^{-1} before inflorescence buds were visible on 5 December.

Seedling transplant (genotype) radial trial:

Preparation of seedlings for the transplant radial trial began on 2 September 1994. Twelve randomly selected plants of cv. Monaro, that had been cultivated in large buckets (30-cm diameter, 440 cm deep) since January 1992 (see Chapter 4), were soaked in water in the buckets for two hours, removed from the buckets, and the soil on the roots was washed away gently with running water from a tap. These plants had shown great differences in their reproductive capability in the 1993-1994 season (Appendix 4) and were regarded as different genotypes of the cultivar.

In each of the twelve genotypes, 120 secondary crown segments were cut from rhizomes arising from the primary crown (80 were used in this study). These crown segments were transplanted immediately into root trainers (45 mm x 30 mm x 80 mm) containing potting medium (Yates Black Magic Seed Raising Mix) which were then placed in a glasshouse (20 ± 5° C) at the Seed Technology Centre, Massey University. They were watered once a week. One month later, the seedlings were moved outside to harden off for six weeks. These seedlings from the twelve genotypes were hand-transplanted into the field on 15 November 1994. The seedlings of each genotype were planted in three rows along the radius in a half radial trial plot (Figure 5-2). Three replicates were planted for all densities. One plant represented one replicate. The same planting method described for the seed sown radial trial was applied. In the site for the seedling transplant radial trial, no extra cultivation was applied before transplanting.

Irrigation was applied immediately with a garden sprinkler for one hour (600 litres h^{-1}) and the same amount of irrigation was applied once a week until the end of December, except for wet period during vegetative growth. Trifluralin (800g a.i. ha^{-1}) was sprayed and incorporated by rotary hoe before transplanting. Mesurol Slug and Snail Bait (Methiocarb, 20 g kg^{-1}) was broadcast at 5 g m^{-2} after transplanting and again three weeks after the first application. Shieded sprays of Buster (Glufosinate-ammonium 200 g l^{-1}, 5 l ha^{-1}) were applied in August and September, respectively. Fusilade (Fluazifop-P-butyl 250 g kg^{-1}, 2 l ha^{-1}) was used to control grass weeds on 30 November 1994. Nitrophoska (N:P:K:S = 12:10:10:2) was applied at 200 kg ha^{-1} before inflorescence buds were visible on 5 December.

During reproductive development (January 1995) sheep accidentally entered the trial area. They grazed many of the inflorescences and newly released leaves and treading damage also occurred. Data were therefore lost in the first growing season (1994-1995).

Both the seed sown and seedling transplanted radial trials were mown with a rotary lawn mower to 2-cm height above the ground and cut material removed on 3 October 1995. Buster (Glufosinate-ammonium 200 g l^{-1}, 5 l ha^{-1}) was sprayed on the trials two weeks after mowing, but before spraying the herbicide, plants of the clover were covered with vertically cut pipes (Plate 5-2) to protect them from chemical contact. Hand weeding was also carried out twice (early November and

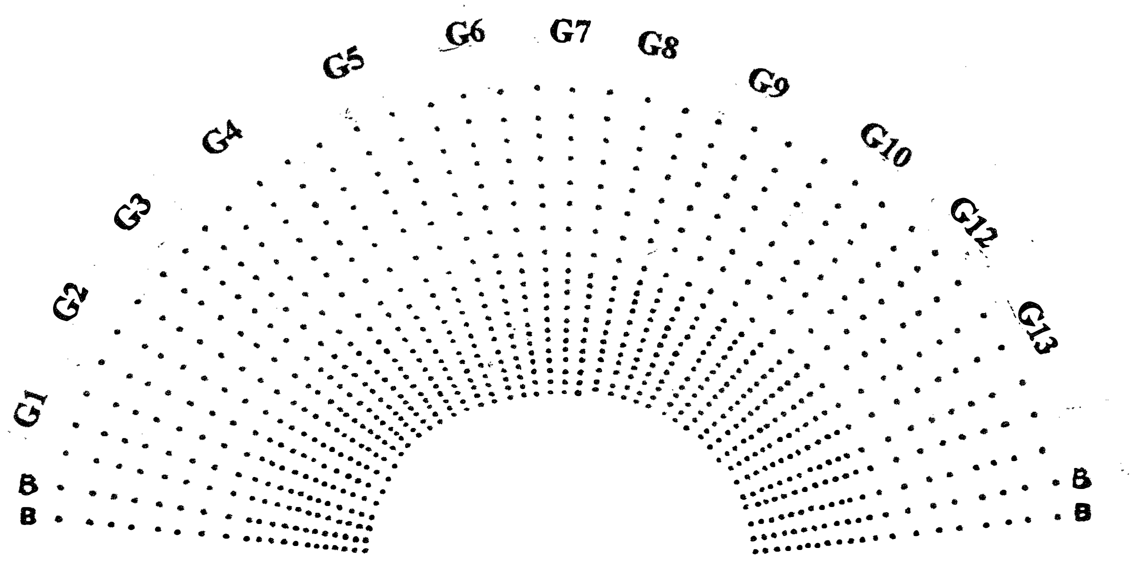


Figure 5-2. Arrangement of twelve genotypes (G1-G13) in a semi-circle radial trial.

B = border radii.

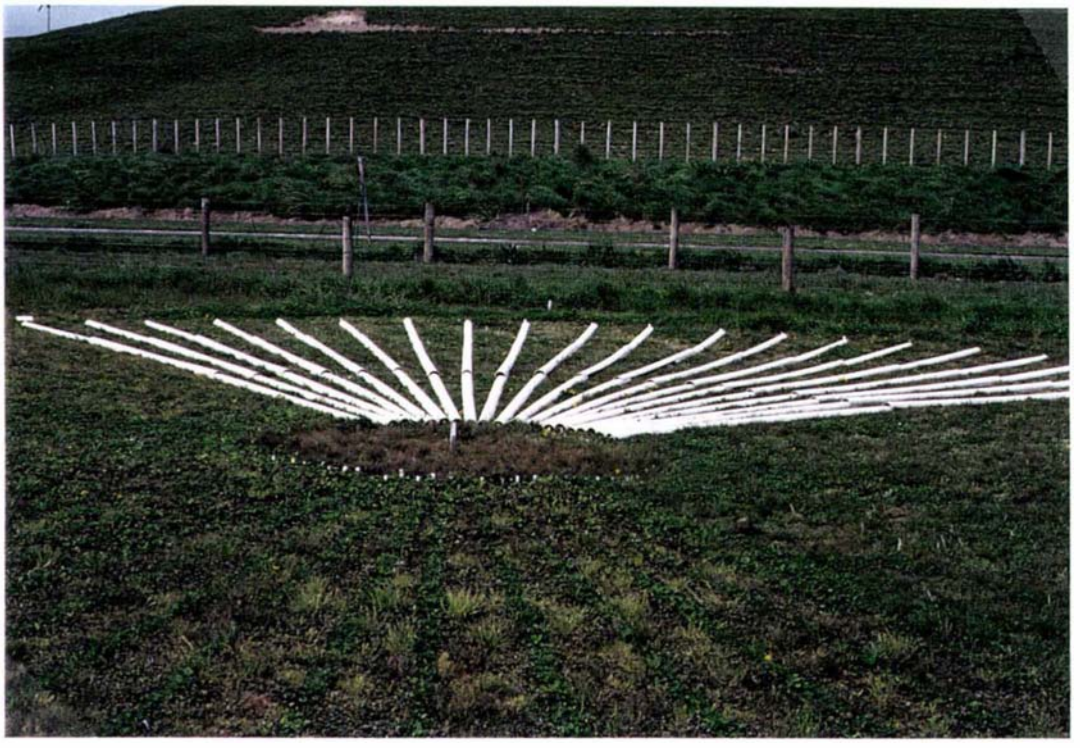


Plate 5-2. Inter-row weed control with Buster herbicide in two radial experiments.

early December respectively). Irrigation was applied at about 10 day intervals, starting from the middle of October to the middle of December. Each time the irrigation took one hour with the same garden sprinkler that delivered 600 litres of water h^{-1}. Nitrophoska (N:P:K:S = 12:10:10:2) was applied at 200 kg ha^{-1} before inflorescence buds were visible on 25 November. Climate data are shown in Appendix 6.

Sample plants were taken five times from the seed sown radial trial from early December 1995 to late February 1996. Five densities (3.1, 5.8, 10.8, 20.3 and 38.2 plants m^{-2}) as selected in the first season were used and 3 replicate plants from each of the five densities were taken. Plant height (from ground level to the highest canopy level) was measured at each sampling, before the plants were cut at ground level. In total, fifteen plants at each sampling were taken and each of these plants was sealed in a separate plastic bag. In the laboratory, leaf and stem numbers and inflorescence number were counted, and leaf area for each plant (cm^2 $plant^{-1}$) measured using a LI 3100 Li-Cor meter (Li-Cor Inc., Nebraska, USA). Leaf area index (LAI) was calculated from plant leaf area multiplied by the number of plants m^{-2}. The dissected components were dried in an air oven at 80° C for 3 days to determine aerial dry weight of each plant.

Mature inflorescences were counted and collected from three sample plants from each of five densities at the final harvest on 25 February 1996. The inflorescences from each individual plant were placed in a paper bag. All inflorescences in these bags were air dried at room temperature (about 24° C) for three months. After this, florets per inflorescence were counted by taking all the florets from each single inflorescence, and seed number per inflorescence was counted from five inflorescences taken randomly from the final harvest by rubbing those florets on a stippled rubber pad, and removing light chaffy material with air blast. Thousand seed weight and germination data were obtained using the methods described in the ISTA Rules for seed testing (ISTA, 1996). Seed yield $plant^{-1}$ was recorded at a constant moisture content after seed cleaning and seed yield m^{-2} was calculated from seed yield $plant^{-1}$ multiplied by the number of plants m^{-2}.

In the transplant (genotype) radial trial, seed yield and seed yield components of each of the twelve genotypes were measured from plants grown at the same five densities (3.1, 5.8, 10.8, 20.3 and 38.2 plants m^{-2}) as used for the seed sown radial trial. Mature inflorescences were harvested by hand on 10 and 23 January, respectively. These inflorescences from each plant of each density were held separately in paper bags at room temperature (about 24° C) for three months. The number of inflorescences per plant was recorded from the total harvested inflorescences. Floret number per inflorescence, seed number per inflorescence, thousand seed weight, germination percentage, seed yield per plant and seed yield m^{-2} were obtained using the same procedures used in the seed sown radial trial. However, not every genotype produced inflorescences, and/or some of plants in some of the genotypes did not produce inflorescences. This resulted in a shortage of samples and no data were available for some fixed densities.

The yield-density function of Holliday (1960) was used to fit inflorescence production and final harvested seed yields per plant and per unit area for both seed sown and seedling transplanting radial trials. The equation used was:

$$y = a + bd + cd^2$$

where y = seed yield (g) or inflorescence number, d = plant density (plants m^{-2}) and a, b, and c are parameters of the model. This yield-density model was chosen in preference to other models because the estimates of the parameters have been shown to be less biased (Gillis and Ratkowsky, 1978; Phetpradap, 1992). The rest of data were processed using the analysis of variance and Fisher's LSD test at P = 0.05 of the SAS package (SAS Institute Inc., 1990).

5.3.1 Vegetative and reproductive development in the seed sown radial trial

Leaf number

In the seed sown radial trial, leaf numbers per plant increased as plant density decreased, with the highest leaf number per plant (approximately 140) produced from plants grown at the lowest selected density (3.1 plants m^{-2}) (Figure 5-3a). Plants from the five densities all reached maximum leaf number in early January. In contrast, at the peak, leaf number m^{-2} was significantly lower for the lowest plant density but did not differ for the three highest densities (Figure 5-3b).

Leaf area

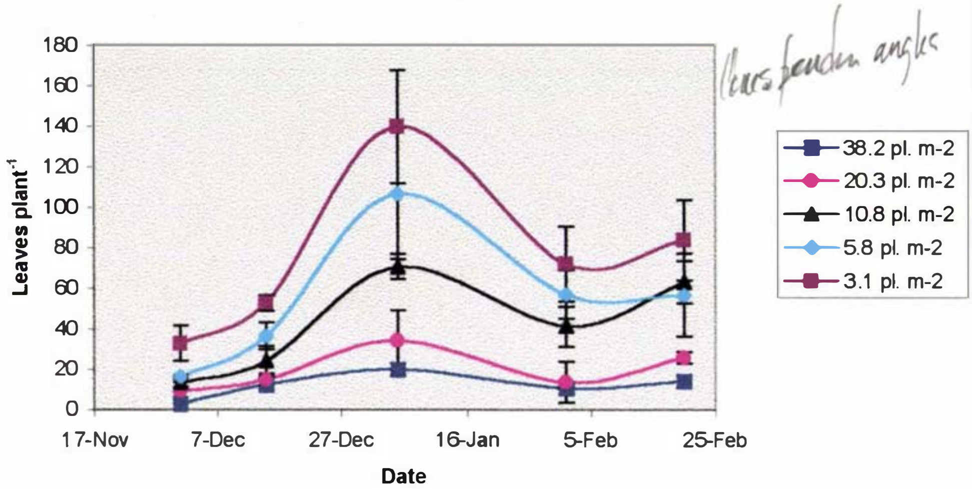
Total leaf area per plant increased rapidly in the three lowest densities (3.1, 5.8 and 10.8 plants m^{-2}) from the middle of December, and all plants in the five densities reached a maximum leaf area in the middle of January (Figure 5-4a). After that, leaf area per plant dropped faster at lower plant density than at higher density, and remained stable from early February. Leaf area index (LAI) increased with time and attained maximum values for all densities in early January (Figure 5-4b). The LAI ranged from 1.3 to 2.3, but plants at the density of 10.8 plants m^{-2} had the highest value after early January. The subsequent decline in LAI was faster in high densities than in low densities.

Plant dry matter

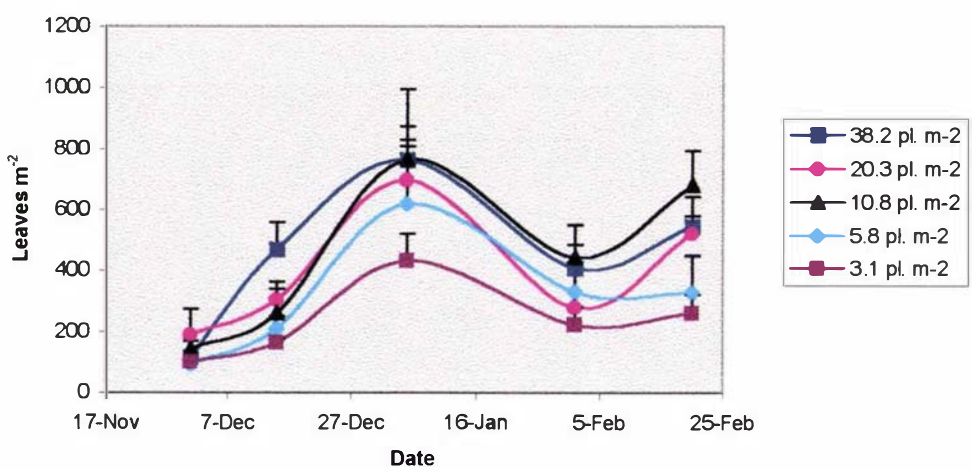
Plant aerial dry weight increased slowly during early vegetative growth but from mid December there was a rapid increase because of reproductive growth before flowering, particularly in the low-density plants (Figure 5-5). However, plant dry

a

Figure 5-3. Effect of plant density on (a) leaf number plant^{-1} and (b) metre^{-2} in Caucasian clover cv. Monaro during the 1995-1996 season. Vertical bar represents standard error.

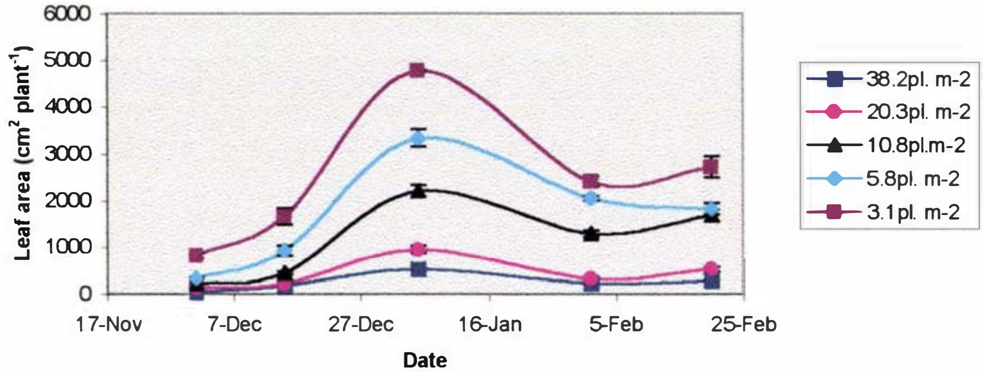


b



a

Figure 5-4. Effects of plant density on leaf area plant^{-1} (a) and leaf area index (b) in Caucasian clover cv. Monaro during vegetative and reproductive development in the 1995-1996 season. Vertical bar represents standard error.



b

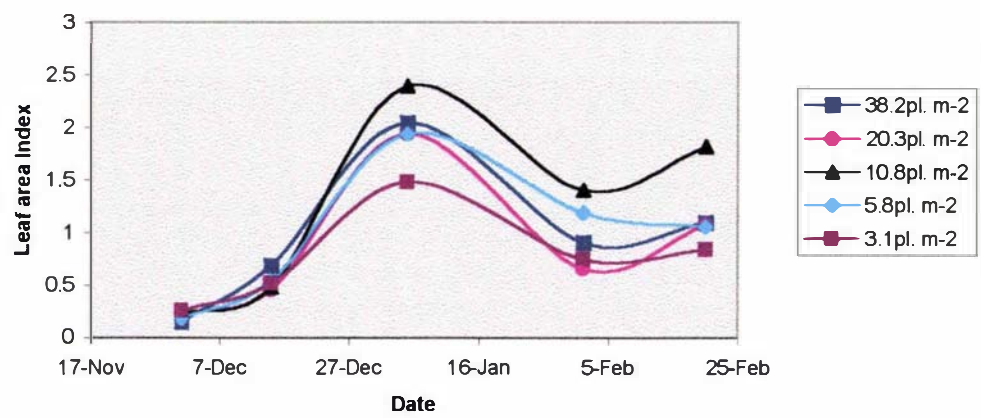


Figure 5-5. Effect of plant density on dry matter plant^{-1} in Caucasian clover cv. Monaro during the 1995-1996 season. Vertical bar represents standard error.

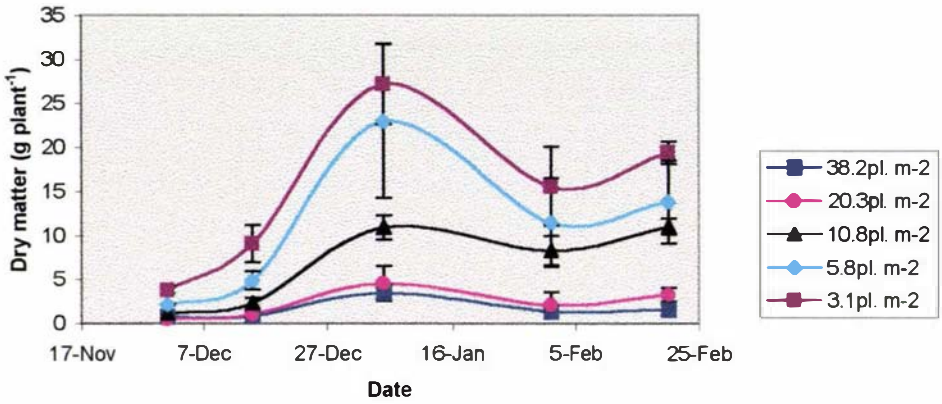
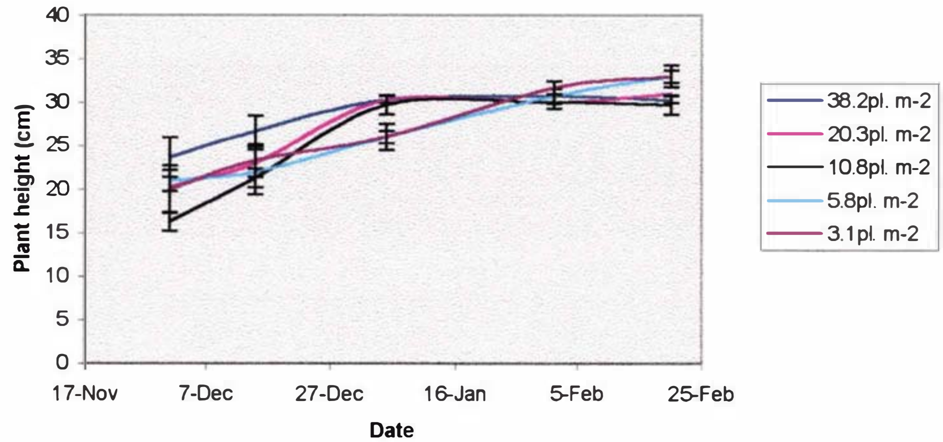


Figure 5-6. Effect of plant density on plant height in Caucasian clover cv. Monaro during the 1995-1996 season. Vertical bar represents standard error.



weight at the two highest densities did not increase. At the maximum value of plant dry weight for the lower density plants, dry weight was approximately four to five times greater than that of the higher density plants.

Plant height

Plants in the high density planting were higher than those plants in the low density planting during vegetative growth. However, there was no difference in height among plants from low and high densities during reproductive development (Figure 5-6).

Reproductive stems, inflorescence production and seed yield response

Reproductive development began as reproductive stems appeared from the middle of December. The number of reproductive stems was influenced by plant density. Low-density plants produced more stems than high-density plants. Plants in the lowest density planting produced the highest number of stems per plant (about 7). The number of stems per plant decreased significantly as plant density increased (Figure 5-7).

Inflorescence production was similar to the production of reproductive stems. Plants at the lower densities produced more inflorescences than plants at the higher densities. The highest number (about 16) of inflorescences per plant was attained from the lowest density (3.1 plants m^{-2}), followed by the densities of 5.8 plants m^{-2} and 10.8 plants m^{-2} (Figure 5-8). The total inflorescences per plant fell from about 16 at the lowest plant density to about 1 at the highest plant density, which represented a reduction of over 90%. There was a negative relationship between plant density and inflorescence number per plant (Figure 5-9a), i.e. the number of inflorescences per plant decreased as plant density increased (R^2 = 0.59). However, on a per unit area basis, the middle density (10.8 plants m^{-2})

Figure 5-7. Effect of plant density on reproductive stem number plant^{-1} in Caucasian clover cv. Monaro during the 1995-1996 season. Vertical bars represent standard error.

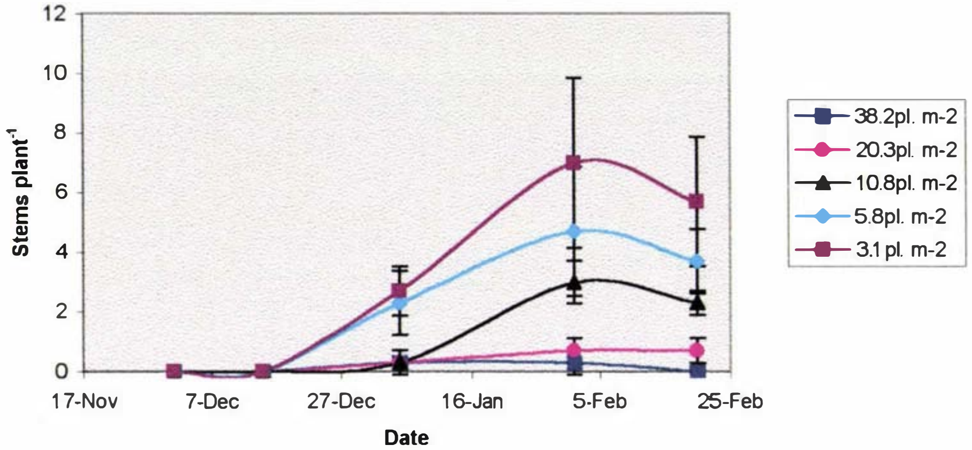
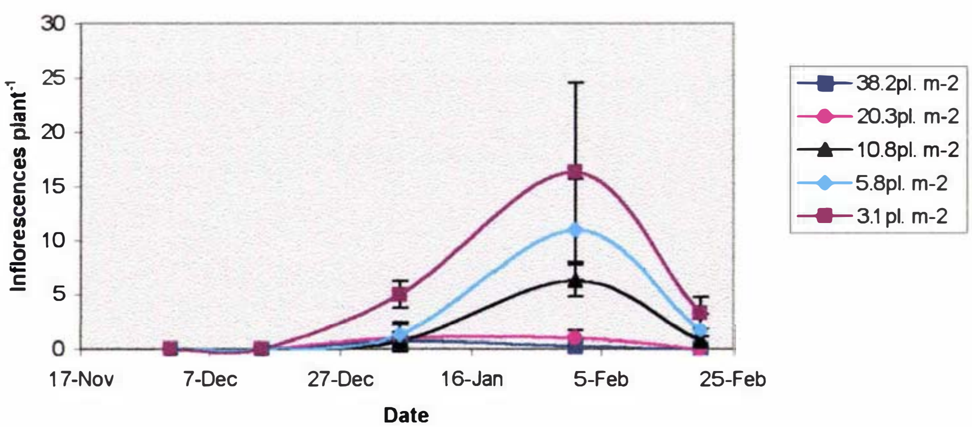
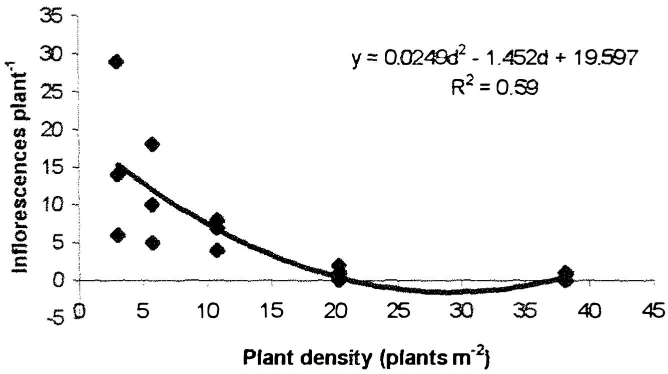


Figure 5-8. Effect of plant density on inflorescence number plant^{-1} in Caucasian clover cv. Monaro during the 1995-1996 season. Vertical bars represent standard error.

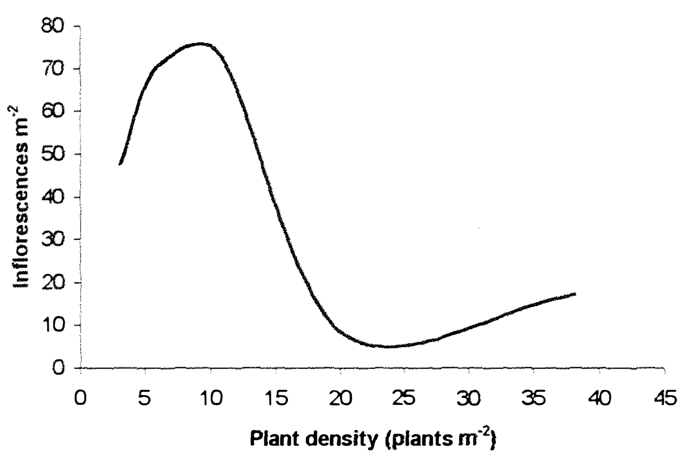


a

Figure 5-9. Relationship between plant density and inflorescence number plant^{-1} (a) and m^{-2} (b) in Caucasian clover cv. Monaro in the 1995-1996 season



b



produced the highest number of inflorescences per square meter (Figure 5-9b). Inflorescence number declined rapidly when plant density increased to 20.3 plants m^{-2} and over. Seed yield per plant and per square meter had a similar response to plant density. There was a negative relationship between plant density and seed yield per plant (Figure 5-10a), i.e. seed yield per plant decreased as plant density increased (R^2 = 0.89). Based on a per unit area, the highest seed yield was produced by the plants at the middle density (10.8 plants m^{-2}) (Figure 5-10b).

5.3.2 Relationships between plant density and aerial components

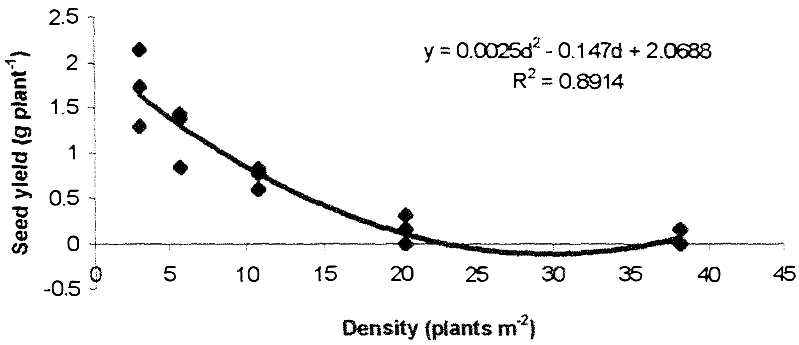
In the seed sown radial trial, plant density was significantly correlated with leaf number per plant (R^2 = 0.77), leaf area per plant (R^2 = 0.57), plant dry matter (R^2 = 0.72) and reproductive stem number per plant (R^2 = 0.61). These parameters increased as plant density decreased (Figure 5-11, 5-12, 5-13, and 5-14). Height of low-density plants was lower than that of high-density plants, and plant height increased as plant density increased during vegetative growth (R^2 = 0.59, Figure 5-15).

5.3.3 Relationship between aerial components as affected by plant density

Leaf number per plant was highly and positively correlated with aerial dry matter per plant (R^2 = 0.96, Figure 5-16), but leaf number was not correlated with inflorescence number per plant (R^2 = 0.34, Figure 5-17) or per square meter (R^2 = 0.25, Figure 5-18). Seed yield of cv. Monaro was highly correlated with inflorescence number per plant (R^2 = 0.95, Figure 5-19) and per square meter (R^2 = 0.91, Figure 5-20).

a

Figure 5-10. Relationship between plant density and seed yield plant^{-1} (a) and m^{-2} (b) in Caucasian clover cv. Monaro in the 1995-1996 season



b

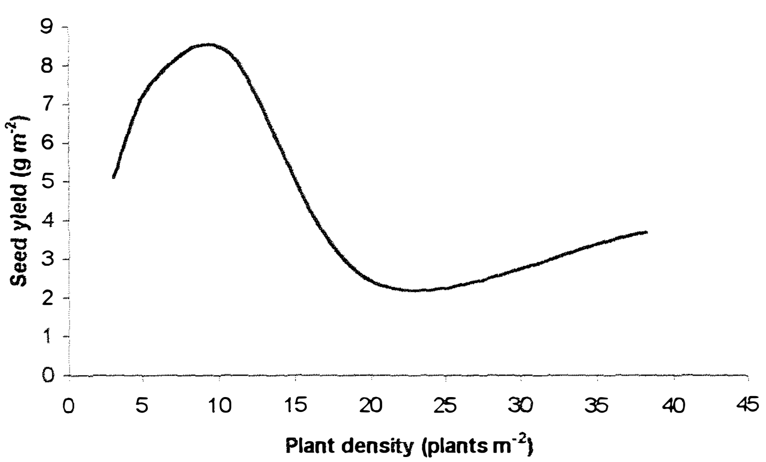


Figure 5-11. Relationship between plant density and leaf number plant^{-1} in Caucasian clover cv. Monaro during reproductive development in the 1995-1996 season

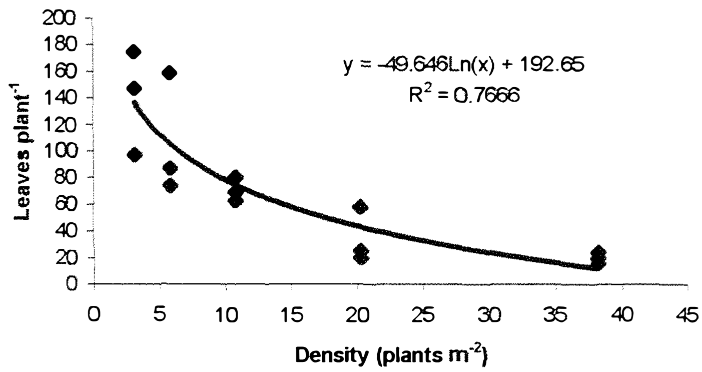


Figure 5-12. Relationship between plant density and leaf area plant^{-1} in Caucasian clover cv. Monaro during reproductive development in the 1995-1996 season

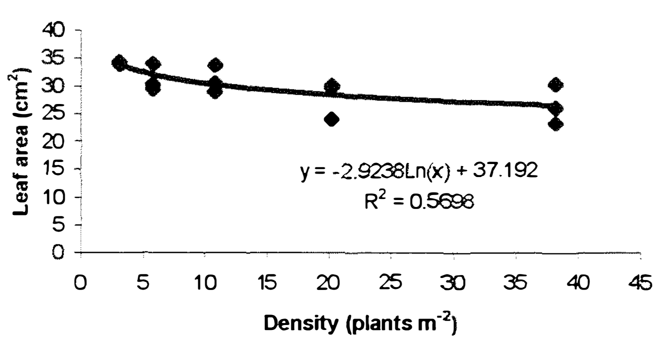


Figure 5-13. Relationship between plant density and above ground dry matter plant^{-1} in Caucasian clover cv. Monaro during reproductive development in the 1995-1996 season

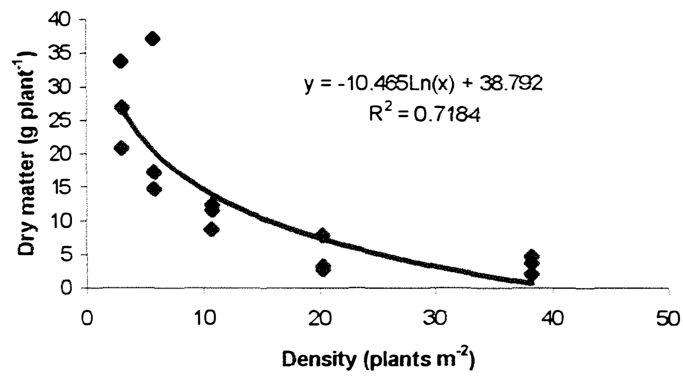


Figure 5-14. Relationship between plant density and reproductive stem number plant^{-1} in Caucasian clover cv. Monaro in the 1995-1996 season

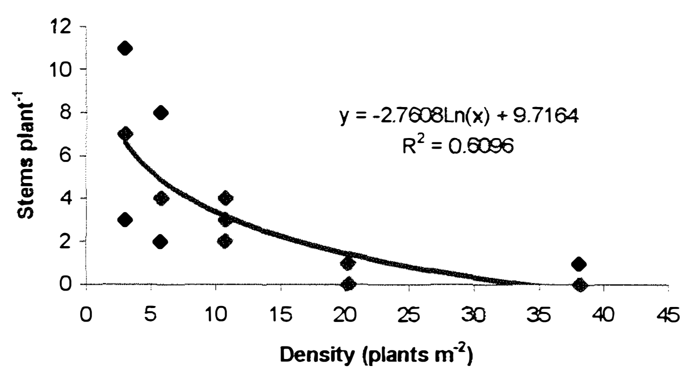


Figure 5-15. Relationship between plant density and plant height in Caucasian clover cv. Monaro in early reproductive development in the 1995-1996 season

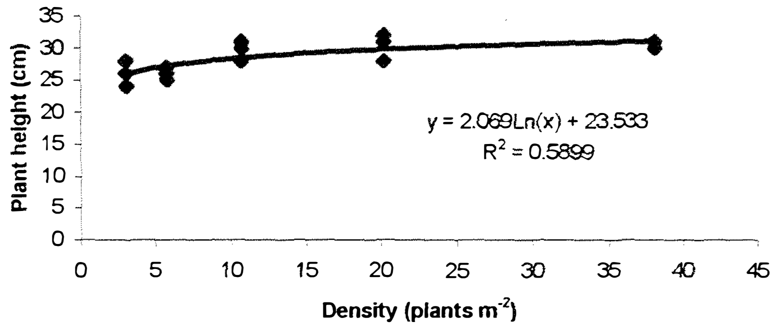


Figure 5-16. Relationship between leaf number and above ground dry matter plant^{-1} in Caucasian clover cv. Monaro in early reproductive development in the 1995-1996 season

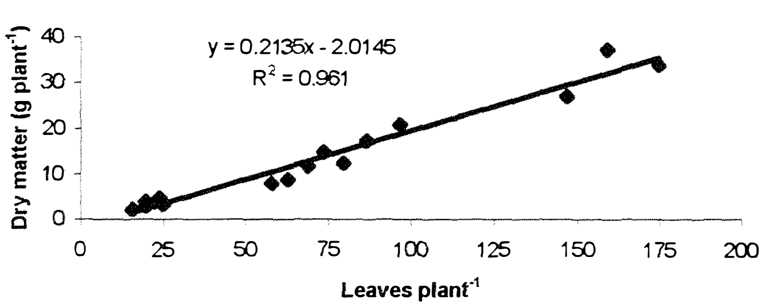


Figure 5-17. Relationship between leaf number plant^{-1} and inflorescence number plant^{-1} in Caucasian clover cv. Monaro in the 1995-1996 season

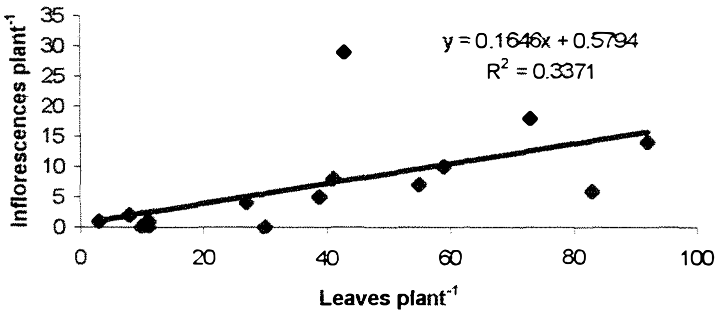


Figure 5-18. Relationship between leaf number m^{-2} and inflorescence number m^{-2} in Caucasian clover cv. Monaro in the 1995-1996 season

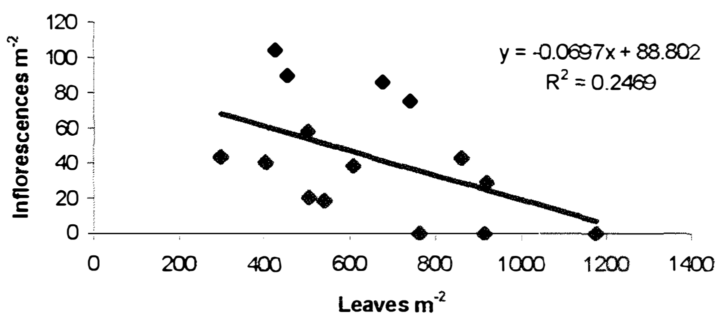


Figure 5-19. Relationship between inflorescence number plant^{-1} and seed yield plant^{-1} in Caucasian clover cv. Monaro in the 1995-1996 season

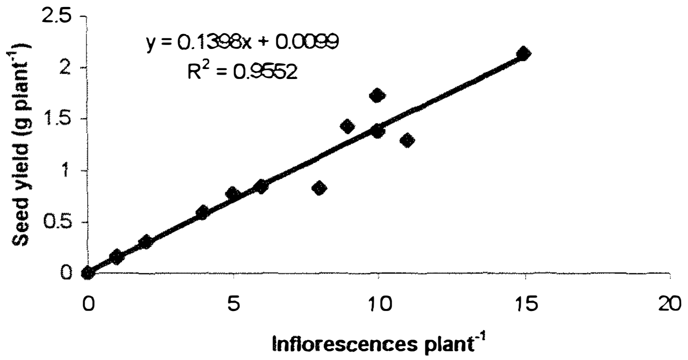
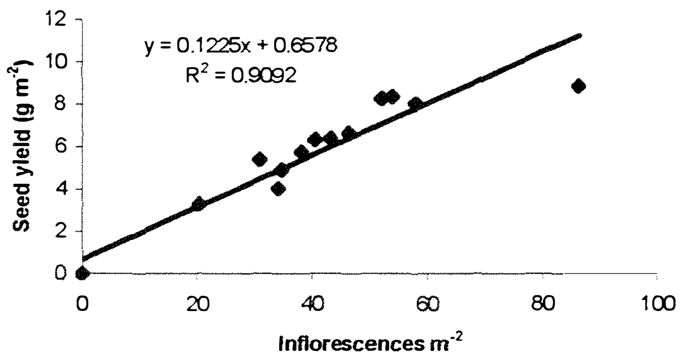


Figure 5-20. Relationship between inflorescence number m^{-2} and seed yield m^{-2} in Caucasian clover cv. Monaro in the 1995-1996 season



5.3.4 Seed yield and yield components in seed sown radial trial

There was a 35 fold difference in seed yield per plant between the highest density plants and the lowest density plants. Seed yield per plant increased (from 0.05 g to 1.72 g) as plant density decreased (Figure 5-10; Table 5-2) due to increases in inflorescence number (from 0.3 to 12) per plant (Table 5-3). Plant density had a significant effect on total inflorescence production per plant, but there was no difference between the densities of 5.8 plants m^{-2} and 10.8 plants m^{-2}, or 20.3 plants m^{-2} and 38.2 plants m^{-2}, respectively. The highest seed yield per square meter was attained from the density of 10.8 plants m^{-2} (Figure 5-10) but this did not differ significantly from that of the two lower densities (Table 5-2). Seed yield at the density of 10.8 plants m^{-2} was 4 fold higher than seed yield at the highest density (Table 5-2).

There were no significant differences in floret number per inflorescence or seed number per inflorescence among the different plant densities (Table 5-3). Thousand seed weight differed slightly among the different densities, and tended to decrease as plant density increased. There was no difference in seed germination and hard seed percentage, which ranged from 96-98% and 83-89%, respectively (Table 5-3).

5.3.5 Seed yield and yield components in the seedling transplant (genotype) radial trial

Reproductive development differed greatly among the twelve genotypes of Caucasian clover cv. Monaro. There was a significant difference for inflorescence number per plant and seed yield per plant at densities of 3.1, 5.8, 10.8 and 20.3 plants m^{-2} among these genotypes, but not for the density of 38.2 plants m^{-2} (Table 5-4). At the optimum plant density of 10.8 plants m^{-2} recorded from the seed sown radial trial, four of twelve genotypes failed to flower and all others showed a

Table 5-2. Effects of plant density on seed yield per plant and per square meter in Caucasian clover cv. Monaro for the seed sown radial trial in the 1995-1996 season.

Plant density (plants m^{-2})	Seed yield (g $plant^{-1}$)	Seed yield (g m^{-2})
3.1	1.72 a	5.33 abc
5.8	1.22 b	7.04 ab
10.8	0.73 c	7.85 a
20.3	0.16 d	3.18 bc
38.2	0.05 d	1.91 c
LSD (0.05)	0.47	4.29

Means with the same letter do not differ at $P \leq 0.05$

Table 5-3. Effects of plant density on inflorescence number (Infl. no.) per plant, floret number per inflorescence ($infl.^{-1}$), seed number per inflorescence, thousand seed weight (TSW), seed germination (Germ.) and hard seed (HS) in Caucasian clover cv. Monaro for the seed sown radial trial in the 1995-1996 season.

Plant density (plants m^{-2})	Infl. no. ($plant^{-1}$)	Floret no. ($infl.^{-1}$)	Seed no. ($infl.^{-1}$)	TSW (g)	Germ. (%)	HS (%)
3.1	12.0 a	121 a	66 a	2.257 a	98 a	86 a
5.8	8.3 b	116 a	83 a	2.267 a	98 a	83 a
10.8	5.7 b	98 a	69 a	2.059 c	96 a	89 a
20.3	1.0 c	111 a	70 a	2.219 b	96 a	89 a
38.2	0.3 c	108 a	65 a	2.120 c	97 a	87 a
LSD (0.05)	3.35	NS	NS	0.0245	NS	NS

Inflorescences were harvested at the final sampling on 25 February.

Means with the same letter do not differ at $P \leq 0.05$

Table 5-4. Number of inflorescences per plant (Infl. plant^{-1}) and seed yield per plant (SY g plant^{-1}) for 12 genotypes (G1-G13) in Caucasian clover cv. Monaro at five densities (plants m^{-2}) in the seedling transplant radial trial in the 1995-1996 season.

Items	Density Plants m^{-2}	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G12	G13	LSD (0.05)
	3.1	26.7	23.7	2.7	13.0	5.3	24.0	10.0	0	0	10.7	13.0	4.0	9.19
	5.8	15.3	12.7	1.3	8.0	7.3	8.7	5.7	0	0	4.7	7.5	3.3	8.43
Infl. p^{-1}	10.8	7.0	7.0	1.7	5.7	0	3.7	1.7	0	0	1.3	4.0	0	4.02
	20.3	1.7	1.3	0.7	1.3	0	2.0	0	0	0	0	1.5	0	1.94
	38.2	1.0	0.7	0.7	1.0	0	0	0	0	0	0	1.0	0	NS
LSD (0.05)		5.3	3.6	NS	4.2	NS	8.8	6.1	0	0	8.8	3.5	4.9	
	3.1	1.53	1.88	0.16	0.98	0.67	1.49	1.0	0	0	0.44	0.45	0.34	1.00
	5.8	1.39	1.11	0.15	0.62	0.64	0.47	0.49	0	0	0.35	0.75	0.13	0.74
SY g p^{-1}	10.8	0.89	0.58	0.09	0.55	0	0.44	0.10	0	0	0.09	0.23	0	0.39
	20.3	0.16	0.17	0.07	0.19	0	0.24	0	0	0	0	0.07	0	0.23
	38.2	0.13	0.08	0.06	0.06	0	0	0	0	0	0	0.05	0	NS
LSD (0.05)		0.51	0.71	NS	0.80	NS	0.93	0.66	0	0	NS	0.20	0.20	

marked reduction in flowering ability (often 50-60%) compared with 3.1 plants m^{-2}. Doubling plant numbers from 10.8-20.3 m^{-2} resulted in all genotypes producing 2 or less inflorescence per plant. For genotype 8 and genotype 9, no inflorescences were produced at all in the 1995-1996 season. For genotypes 5, 6, 7, 10 and 13, inflorescences were produced only from the plants in lower or middle density plantings. Only genotypes 1, 2, 3, 4 and 12 produced inflorescences at all five densities. For seed yield per plant a similar situation occurred for the twelve genotypes. However, only genotypes 1, 2 and 4 were used to study the effects of plant density on inflorescence number, seed yield and seed yield components in this radial trial because only these genotypes provided sufficient seed material for measurements across all five densities.

Plant density markedly influenced the number of inflorescences per plant for all three genotypes. The highest number of inflorescences per plant was produced from the plants at the lowest density (Table 5-4), but it did not differ between the densities of 20.3 and 38.2 plants m^{-2} for all three genotypes. The relationship found between plant density and inflorescence number per plant for genotypes 1, 2 and 4 was similar to that which occurred in the seed sown radial trial (Figure 5-21). The number of inflorescences per plant decreased with the increases in plant density, and there were high correlations between density and inflorescence number for genotype 1 (R^2 = 0.88), genotype 2 (R^2 = 0.89) and genotype 4 (R^2 = 0.82), respectively. The highest number of inflorescences m^{-2} were from the 10.8 plants m^{-2} density for all three genotypes (Figure 5-22).

Plant density greatly affected seed yield per plant in all three genotypes. The highest seed yield per plant (1.53 g for genotype 1, 1.88 g for genotype 2 and 0.97 g for genotype 4) was produced by the plants at the lowest density of 3.1 plants m^{-2} in all three genotypes. The lowest seed yield (0.13 g for genotype 1, 0.08 g for genotype 2 and 0.06 g for genotype 4) was produced by the plants at the highest density of 38.2 plant m^{-2} (Table 5-4). Although both genotypes 1 and 2 had higher

Figure 5-21. Relationship between plant density and inflorescence number plant^{-1} for genotype 1 (G1), genotype 2 (G2) and genotype 4 (G4) of Caucasian clover cv. Monaro

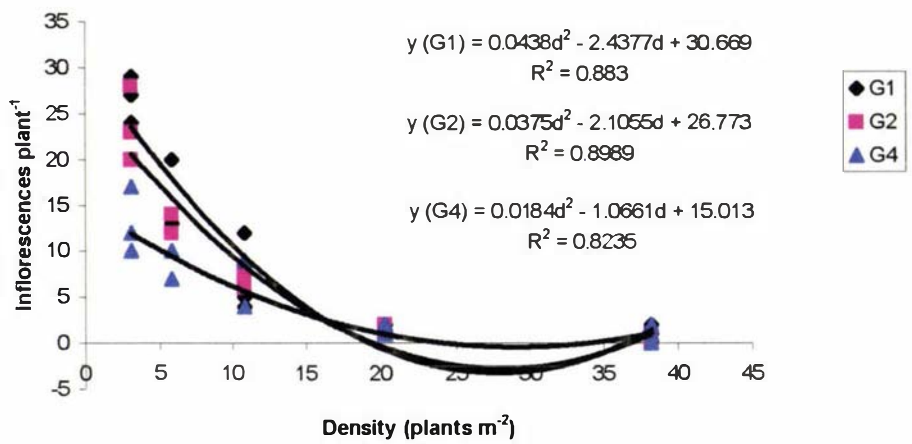
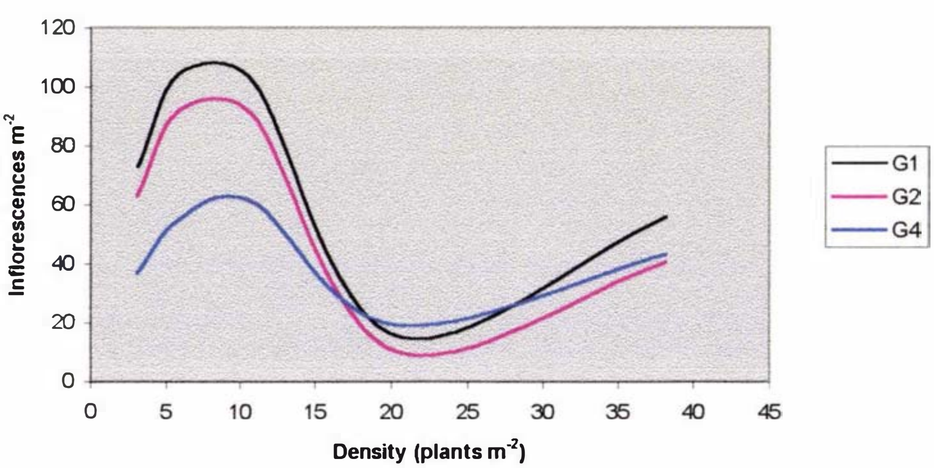


Figure 5-22. Effects of plant density on inflorescence number m^{-2} for genotype 1 (G1), genotype 2 (G2) and genotype 4 (G4) of Caucasian clover cv. Monaro



seed yield per plant than genotype 4 at lower densities, there was no significant difference among the three genotypes at all five densities. There was a significant relationship between plant density and seed yield per plant for two of the genotypes where seed yield per plant declined when plant density increased from a density of 3.1 plants m^{-2} to a density of 38.2 plants m^{-2} (Figure 5-23). The correlations were high for both genotype 1 ($R^2 = 0.80$) and genotype 2 ($R^2 = 0.77$), respectively. For genotype 4, no significant correlation was detected. Highest seed yields m^{-2} tended to be attained from the density of 10.8 plants m^{-2} for all three genotypes (Figure 5-24). This was the same optimum density as found in the seed sown radial trial.

There was no difference in floret number per inflorescence among the five densities for genotypes 1, 2 and 4 and among these genotypes. Floret number per inflorescence ranged from 73-87 for genotype 1, 85-90 for genotype 2 and 75-91 for genotype 4 (Table 5-5). Also, there was no significant difference in seed numbers per inflorescence among five densities for all three genotypes and among genotypes (Table 5-6). Seed number per inflorescence ranged from 39-54 for genotype 1, 38-59 for genotype 2 and 35-46 for genotype 4, respectively.

Thousand seed weight differed significantly among the plant densities for all three genotypes. The differences ranged from 2.50-2.78 g for genotype 1, 2.14-2.82 g for genotype 2 and 1.84-2.51 g for genotype 4 but there was no obvious plant density effect (Table 5-7). Also, differences occurred among the three genotypes. Seed germination ranged from 95-98% and did not differ among the densities or genotypes (Table 5-8). Hard seed percentage was high in all three genotypes, ranging from 82-92%, but there was no difference among the five densities and genotypes (Table 5-9).

Figure 5-23. Relationship between plant density and seed yield plant^{-1} for genotype 1 (G1), genotype 2 (G2) and genotype 4 (G4) of Caucasian clover cv. Monaro

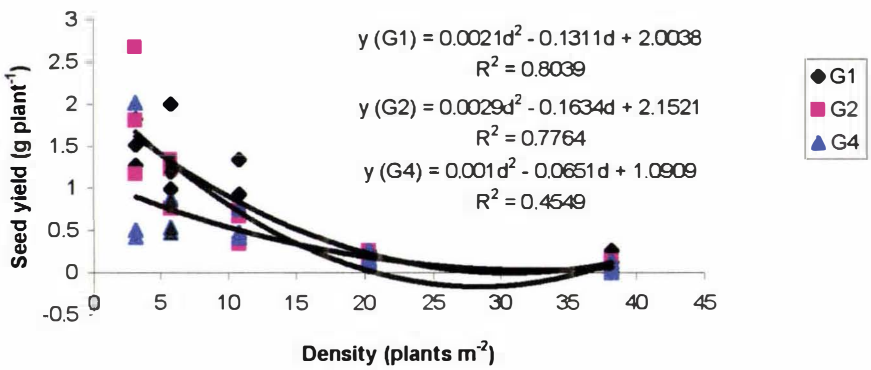


Figure 5-24. Effects of plant density on seed yield m^{-2} for genotype 1 (G1), genotype 2 (G2) and genotype 4 (G4) of Caucasian clover cv. Monaro.

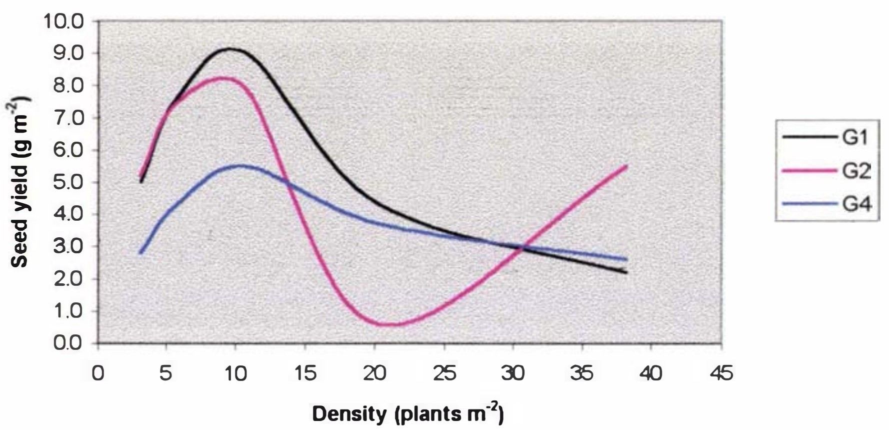


Table 5-5. Effects of plant density on number of florets per inflorescence in genotypes 1, 2 and 4 of Caucasian clover cv. Monaro.

Plant density (plants m^{-2})	Genotype 1	Genotype 2	Genotype 4	LSD (0.05)
3.1	87.0	85.0	78.8	NS
5.8	80.3	85.0	90.7	NS
10.8	73.7	88.3	78.7	NS
20.3	82.7	90.7	75.7	NS
38.2	80.0	88.3	91.0	NS
LSD (0.05)	NS	NS	NS	NS

Table 5-6. Effects of plant density on number of seeds per inflorescence in genotypes 1, 2 and 4 of Caucasian clover cv. Monaro.

Plant density (plants m^{-2})	Genotype 1	Genotype 2	Genotype 4	LSD (0.05)
3.1	39.3	41.3	37.3	NS
5.8	45.3	47.0	35.7	NS
10.8	38.7	41.0	46.0	NS
20.3	39.7	59.3	42.7	NS
38.2	48.3	54.0	41.0	NS
LSD (0.05)	NS	NS	NS	

Table 5-7. Effects of plant density on thousand seed weight (g) in genotypes 1, 2 and 4 of Caucasian clover cv. Monaro.

Plant density (plants m^{-2})	Genotype 1	Genotype 2	Genotype 4	LSD (0.05)
3.1	2.719	2.822	2.506	0.007
5.8	2.505	2.329	1.837	0.005
10.8	2.723	2.138	2.365	0.005
20.3	2.500	2.803	2.466	0.003
38.2	2.779	2.239	2.443	0.003
LSD (0.05)	0.050	0.045	0.034	

Table 5-8. Effects of plant density on germination percentage in genotypes 1, 2 and 4 of Caucasian clover cv. Monaro.

Plant density (plant m^{-2})	Genotype 1	Genotype 2	Genotype 4	LSD (0.05)
3.1	96	99	96	NS
5.8	95	98	95	NS
10.8	96	95	96	NS
20.3	99	98	96	NS
38.2	96	96	98	NS
LSD (0.05)	NS	NS	NS	

Germination percentage data were obtained after hard seed was removed by cutting the seed coat with a scalpel (ISTA, 1996).

Table 5-9. Effects of plant density on hard seed percentage in genotypes 1, 2 and 4 of Caucasian clover cv. Monaro.

Plant density (plant m^{-2})	Genotype 1	Genotype 2	Genotype 4	LSD (0.05)
3.1	84	88	82	NS
5.8	83	84	82	NS
10.8	91	86	84	NS
20.3	91	92	88	NS
38.2	82	88	90	NS
LSD (0.05)	NS	NS	NS	

5.4 DISCUSSION

5.4.1 Vegetative and reproductive development under different plant densities

Differing plant densities changed morphological characteristics in Caucasian clover cv. Monaro in this study. The changes were similar to those reported in lupin (Herbert and Hill, 1978), soybean (Chanprasert, 1988) and China aster (Phetpradap, 1992). Plant density initially had little effect on plant morphological characteristics of Caucasian clover cv. Monaro during early vegetative growth (before the middle of December). But differences in leaf number and leaf area per plant, plant height, reproductive stem and inflorescence number per plant began appearing among plants from low density to high density as plant growth continued (Figures 5-3, 5-4, 5-5, 5-6, 5-7, 5-8). There was a rapid increase in leaf production (both leaf number and leaf area) from the middle of December and the increase reached a maximum in early January. Plants grown at lower densities produced more leaves. At the maximum in early January, leaf number was around 7 times higher at the lowest density compared with the highest density (Figure 5-3). This indicated that low-density plants had more space for development and less competition for light, water and nutrients. However, plants grown at high density produced the highest leaf number per unit area (Figure 5-3b). This compensated for the low number of leaves per plant. At high density leaves occupied all the space per unit area. Plant density significantly affected leaf number per plant. Because of the significant difference in leaf number per plant, leaf area per plant was also significantly different (Figure 5-4a). Greater leaf number per plant resulted in a greater leaf area per plant, while more leaves per unit area produced a higher leaf area index. Leaf area index was relatively high at the high planting density. Changes in leaf area with plant density subsequently affected plant dry matter accumulation for individual plants.

The peak of leaf production occurred one month before the peak of reproductive growth in Caucasian clover in this study. Agronomically, this is important as it allows the plant to produce more carbohydrate and store reserves in its root system before the plant switches over to reproductive development. These reserves can be important for the subsequent production of reproductive shoots and inflorescences, seed formation and development (Chapter 3).

Large number of leaves per plant for those plants grown at low density resulted in rapid dry matter accumulation from the middle of December to early January, indicating accelerated growth under warmer summer conditions (21.9° C - Appendix 6) and also that plants were switching over from vegetative growth to reproductive development. In this study dry matter accumulation of above ground parts and leaf number per plant reached their maximum in early January (Figure 5-3, 5-5). This indicated that above ground plant dry matter mainly consists of leaves in Caucasian clover. Dry matter accumulation, however, was obviously inhibited in high density plants presumably because competition for light and nutrients became a limiting factor, as reflected by smaller plant size and a reduced number of leaves. These high density plants had a slow increase in leaf number per plant, slow increase in leaf area per plant and slow dry matter accumulation as well, although leaf area index was relatively high (Figure 5-4). Plant density influenced dry matter accumulation through affecting leaf number per plant in Caucasian clover.

Donald (1963) suggested that of all the factors influencing competition in crops, light becomes the major limiting factor to production. Similarly, Herbert and Litchfield (1984) suggested that competition for light is the major factor inducing morphological changes in plants when plant density is increased. In this study, the greatest development of above ground parts was from low-density plants as more light would be available for photosynthesis by both upper and lower leaves of a canopy. Therefore, potential seed production sites could be increased as a result because leaves, reproductive stems and inflorescences would increase. Plant density also has a significant effect on light distribution in plant canopies (Kasperbauer and Karlen, 1986). In cereals, 30% of the light reached the lower layer of leaves and soil surface in a low density crop; however, less than 10% reached the lower leaves as about 60% of the light was intercepted by the upper layer of leaves in the crop at high density (Kirby, 1976). Also, Steiner and Snelling (1994) reported that in an intercropped trial companion- and relay-cropped wheat reduced the percentage of photosynthetic photon flux density (PPFD)[1] available to Caucasian clover growing in the wheat under-canopy by approximately 60 to 20% of full sunlight PPFD from the time of wheat crop establishment until the wheat harvest and straw removal. Light availability to the clover beneath all wheat intercropping treatments increased after the wheat canopy began to senesce during grain maturation. Density influenced light availability. In China aster, plants with a more open canopy had a more even leaf distribution from the upper to the lower

[1] Photosynthetic Photon Flux Density (PPFD): incident photon flux density of photosynthetically active radiation ((PAR): the number of photons (400-700 mm) incident per unit time on a unit surface (Pearcy, 1989).

levels at low plant density, and more light was available to lower leaves as light was able to penetrate to basal branches. A dense upper canopy formed in high density plants could cause some degree of both mutual and self-shading (Phetpradap, 1992). Similarly, the growth of Caucasian clover could be affected by light distribution in the same way, as more leaves, stems and inflorescences per plant were recorded at low density in this study.

Plant height can be an indicator of light competition. Plants were taller in the high-density planting than those plants in the low density planting before the peak of reproductive growth in this study. Correlation analysis also showed that plant height was positively correlated with plant density (R^2 = 0.59) although it was not a strong correlation (Figure 5-15). However, the difference disappeared progressively with time and no difference was recorded once plants had developed to the late reproductive stage. Tamaki *et al.* (1973) suggested that competition for light at different degrees of mutual shading stimulates cell elongation. This increases plant height by an increase in internode length. Caucasian clover does not have a strong stem during vegetative growth. Plant height, in fact, is the height of a leaf from the base of the petiole to the tip of the blade. At high plant density, because the plants had longer petioles, plants were obviously higher than those plants at low plant density. Presumably, cells of petiole tissues of the clover were stimulated and elongated as a result of light competition. The petioles of the plants were lighter green in color and thinner than petioles in the low-density plants. This phenomenon was visible although the petiole tissues were not measured in this study.

Plant density greatly affected the number of reproductive stems and inflorescences per plant. During reproductive growth at high plant density, reproductive development was inhibited, and only a few reproductive stems and small numbers of inflorescence were produced from the small plants due to strong competition among plants. These differences were significant between low density and high-density plants (Figure 5-7, 5-8). High plant density therefore prohibited reproductive development of Caucasian clover; this clover is obviously sensitive to plant density. As a result the highest density plants produced about 7 times fewer reproductive stems and over 15 times fewer inflorescences per plant compared with plants at the lowest density, although temperature and sunshine were considered optimal for reproductive growth (Appendix 6).

There was a relationship between plant density and inflorescence number per plant. Inflorescence number increased as plant density decreased in the seedling (genotypes) transplanted radial trial (R^2 = 0.82-0.89, Figure 5-21) for genotypes 1, 2 and 4, although the relationship was not as strong in the seed sown radial trial (R^2 = 0.59, Figure 5-9). These results verified that plant density largely affected reproductive development in cv. Monaro. The lowest density plants (3.1 plants m^{-2}) had the greatest reproductive growth per plant. However, plants grown at the lowest density were not capable of creating the highest reproductive production per unit area. The current results showed that plants grown at the density of 10.8 plants m^{-2} produced the highest inflorescence number per unit area both in the seed sown and seedling transplant radial trial (Figure 5-9b, 5-22). As plants grown at this density had the highest leaf number per unit area (Figure 5-3b) and leaf area index (Figure 5-4b), presumably photosynthetic capability was also greater and therefore assimilate production greater, allowing the production of a greater number of inflorescence.

Plant density had a large effect on the production of aerial components of individual plants in cv. Monaro. The current study showed that plant density was negatively correlated with leaf number per plant (R^2 = 0.77), leaf area per plant (R^2 = 0.57) and above ground dry matter per plant (R^2 = 0.72). These parameters decreased as plant density increased. Plant size therefore greatly depends on plant density, but reproductive development is dependent on plant size. Plant density therefore indirectly affected reproductive production.

5.4.2 Relationship between vegetative and reproductive development as affected by plant density

Competition between vegetative and reproductive organs is recognised as an important phenomenon and, in many instances, can affect the agricultural yield of a crop (Williams and Joseph, 1976). Compared with the now cosmopolitan white clover, leaf production of Caucasian clover was slow in early development; this resulted in a slow increase of above ground dry matter, as above ground dry matter was highly and positively correlated with leaf production (R^2 = 0.96, Figure 5-16). Leaf growth rapidly increased later in the season as temperature increased. Leaf number per plant reached a maximum before the plants initiated reproductive development. During this period, Caucasian clover produced a large amount of leaf and strong photosynthesis could therefore occur. But this does not mean that the photosynthate from the leaves will be used directly for reproductive growth. It could be transported into the root system and accumulated there. Then, assimilates in the root could be remobilised and used for reproductive development. Coolbear *et al.* (1994) have suggested that root reserves rather than local photosynthesis are likely to drive reproductive development.

Leaf production was not correlated with inflorescence production per plant (R^2 = 0.34) or per square meter (R^2 = 0.25) in Caucasian clover in the current study. But it was highly correlated with root dry matter (R^2 = 0.98, Chapter 3). Caucasian clover had a high root to shoot ratio (above 2.36) compared with white clover which has a root to shoot ratio of only 0.15 for cv. Huia and 0.18 for cv. Tasmania (Spencer *et al.*, 1975). A large number of leaves per unit area are required to produce nutrients directly for reproductive development in white clover. However, it is different for Caucasian clover. Morphologically, Caucasian clover produces leaf and petiole growth above ground until reproductive stems are initiated and elongate in spring (Steiner, 1992). Because of a much higher root to shoot ratio in Caucasian clover, a high proportion of photosynthate actually had been used for root development or reserved in the root system before reproductive development is initiated. Accumulation of root dry matter was positively correlated with both leaf number (R^2 = 0.98) and reproductive production (R^2 = 0.89 for shoot, R^2 = 0.83 for inflorescence) (Chapter 3). Strong competition for nutrients could therefore exist between leaves and reproductive development above ground, if the root system drives and provides nutrients for reproductive development.

Plant density considerably affected reproductive development of Caucasian clover in this study. One of the reasons could be that plant density influences root development by increasing competition for developing space, water and nutrients among individual root systems under high density. Because of this competition, root development could be inhibited, although it was not measured in this study. Results in Chapter 3 have shown that reproductive development was positively correlated with root development and root dry matter accumulation. Reproductive development would be affected by root development if it in turn was affected by plant density. This implies that an indirect relationship exists between plant density and reproductive development in Caucasian clover. Therefore one hypothesis could be that plant density affects reproductive development through affecting root system development in Caucasian clover. The highest reproductive production per unit area would therefore be obtained when a suitable plant density allows the production of a well-developed root system.

5.4.3 Seed yield and yield components

Plant density significantly affected seed yield per plant, responses being similar in both the seed sown (Table 5-2) and seedling transplant radial trial (Table 5-4). As plant density increased from 3.1 plants m^{-2} to 38.2 plants m^{-2}, seed yield decreased. The difference was significant between the lower density and higher density both in the seed sown trial and in the seedling transplant trial. The results in the present study show that the plants grown at the lowest density produced the highest seed yield per plant, as has also been reported in many other crops including lucerne (Kowithayakorn, 1978), soybean (Chanprasert, 1988), *Salvia officinalis* L. (Macchia *et al.*, 1990), *Hyssopus officinalis* L. (Macchia *et al.*, 1990) and China aster (Phetpradap, 1992). This highest seed yield per plant at low planting density was due to a greater number of reproductive stems and inflorescences.

Cultivar Monaro showed a parabolic relationship for reproductive production per unit area with plant density, both for inflorescence number and seed yield m^{-2}. The highest seed yield m^{-2} was obtained from plants at the density of 10.8 plants m^{-2} (middle density) in the seed sown trial, although seed yield m^{-2} did not significantly differ between the high and middle densities. However, the difference was significant between the middle and lower densities (Table 5-2). This indicated that in the densities compared, 10.8 plants m^{-2} produced the highest seed yield per square meter. Similar results for seed yield were also obtained from the seedling transplant trial. The results in this study also suggested that Caucasian clover did not favour high density planting for seed production purposes. On this point, Steiner and Snelling (1994) have reported that when Caucasian clover cv. Rhizo was grown with intercropped (companion-planted) wheat for seed production, seed yield was 117.3 kg ha^{-1} in the second year from a 30 cm row spacing compared to a 15 cm row spacing which produced a seed yield of 73.9 kg ha^{-1}. When the clover was grown with relay-planted wheat, a 40 cm row spacing produced a seed yield of 145.5 kg ha^{-1} compared to a 20 cm row spacing, which produced a seed yield of 90.9 kg ha^{-1}. Guy (1996) also demonstrated that plants at a 45 cm row spacing produced more inflorescences per unit area (448 m^{-2}) than those at a 30 cm row spacing (288 m^{-2}) for a new hexaploid Caucasian clover cv. Endura.

In the current study, inflorescence number (around 80 m^{-2}) and seed yield (around 75 kg ha^{-1}) per unit area were low compared with the results reported by Guy (1996). Environmental factors are a likely cause of this difference. The site was not optimal for seed production, as the soil dried out rapidly and solidified, and tended to flood when it rained. The plants grown at this site were much smaller than those of the same cultivar when grown in a sand bed, which produced a seed yield of 690 kg ha^{-1} (from about 350 inflorescences per plant) in the first year and around 600 inflorescences per plant in the second season (Fu, unpublished data). However while in the sand bed trial (Chapter 3) the majority of the seed came from secondary crowns, in the radial trials seed was produced mainly from primary crowns. This was because firstly the accidental grazing by sheep in the first year of the trials removed many secondary crowns (or did not allow their development because regrowth efforts went back into the primary crowns), and secondly because in the second year, difficulties with inter row weed control tended to restrict secondary crown growth and development. As primary crowns produce only around 15% of the total seed yield (at least in the sand bed environment, Chapter 3), this probably explains why seed yield was substantially lower than that reported by Guy (1996). Reported seed yields from plot trials have been variable, within the range of 40-900 kg ha^{-1} (Bryant, 1974; Voloshenko *et al.*, 1979; Stewart and Daly, 1980; Efendi, 1993; Guy, 1996). Presumably, environmental factors and inherent properties of the cultivar could both influence seed yield.

Plant density significantly affected inflorescence number per plant in both the seed sown (Figure 5-9) and seedling transplant trials (Figure 5-23). The inflorescence number decreased with the increase in plant density. The number of inflorescences $plant^{-1}$ in fact, was the most important seed yield component determining final seed yield (Table 5-3), as the numbers of florets and seeds per inflorescence were not influenced by plant density in both the seed sown (Table 5-3) or seedling transplant radial trials (Table 5-5, 5-6). This indicated that plant density affected seed yield only through reducing inflorescence number. Seed yield per plant and per unit area was highly and positively correlated with the number of inflorescences, indicating that the response in Caucasian clover does not differ from that reported for other herbage legumes such as white clover (Van Bockstaele and Rijckaert, 1988; Hollington *et al.*, 1989; Marshall *et al.*, 1989; Nordestgaard, 1989) and birdsfoot trefoil (Albrechtsen *et al.*, 1966; Mos, 1983; Stephenson, 1984; McGraw *et al.*, 1986; Li and Hill, 1988; 1989).

Although thousand seed weight did differ ranging from 2.06 to 2.27 g among the different densities in the seed sown trial (Table 5-3) and from 1.84 to 2.82 g in the seedling transplant trial (Table 5-7), it did not change regularly, i.e. change with plant density. This suggested that plant density did not influence thousand seed weight. However, this flexible change seems one of the characteristics of this species as Dear and Zorin (1985) reported that thousand seed weight ranged from 2.11-2.63 g, and can be up to 3.2 g for hexaploid Caucasian clover (Khoroshailov and Fedorenko, 1973). Thousand seed weight also differed among genotypes 1, 2 and 4 at the same density, but no pattern was found i.e. one was not always higher than others at the different densities. Genotype 1 had a higher average thousand seed weight, followed by genotype 2, and genotype 4. The difference in thousand seed weight could be related to different genotypes. Plant density did not affect seed germination capacity in this clover. All plant densities produced seeds with germination percentage ranging from 95% to 99%, and there was no significant difference in germination among densities both in seed sown and seedling (genotype) transplant radial trials. This result was similar to those reported for soybean by Chanprasert (1988) and China aster by Phetpradap (1992). Plant density did not influence hard seed percentage as no significant difference occurred among the five densities in both the seed sown (Table 5-3) and seedling (genotype) transplant radial trials (Table 5-9). Also, there was no difference among the three genotypes at the same density.

5.4.4 Genotype effects on reproductive capability

Twelve genotypes demonstrated different reproductive capability in the seedling transplant radial trial (Table 5-4). The results obtained from this study indicated that genotypes 1, 2, 4 and 12 represented a high reproductive capability group compared with the others, and these genotypes produced inflorescences across all five densities. However, genotypes 3, 5, 8, 9, 13 represented another group which was regarded as having poor reproductive capability. Inflorescence production per plant was low even at the lowest density. Genotypes 6, 7 and 10 were in the middle. These results were similar to the results obtained in 1993-1994 (Appendix 4) and in 1994-1995 (Appendix 8). This indicates that differences in reproductive ability exist among the genotypes or individual plants in Caucasian clover cv. Monaro. This has been discussed in Chapter 4.

All three genotypes (1, 2 and 4) indicated a similar response to plant density. They produced a maximum seed yield at the density of 10.8 plants m^{-2}. However, this density was only optimum for the five densities selected. It is possible that a greater yield may have been obtained from a slightly lower density (around 9 plants m^{-2}) although this was not determined. It is also possible that seed yield would not differ significantly for a range of densities between 5 to 10 plants m^{-2}, as suggested by the data in Table 5-2.

The conclusion that can be drawn from this study is that both plant density and genotype affect Caucasian clover seed yield, but not seed quality. The optimum plant density in this study was 10.8 plants m^{-2}, at which seed yield per unit area was maximal. Increasing the plant density did not increase seed yield per unit area. Of the seed yield components, inflorescence number is the most important component determining seed yield per unit area in Caucasian clover cv. Monaro, and this character could be used to improve seed yield per unit area. Plant density (or inter plant competition) greatly influenced plant growth and development of

Caucasian clover. Large plants at low density produce more inflorescences than small plants at high density.

Daly *et al.* (1993) suggested that a population of 20,000 plants ha^{-1} was sufficient to produce a high seed yield potential in Caucasian clover in the year after establishment. This population is substantially lower than the 108,000 plants ha^{-1} at which maximum seed yield was recorded in the present trials, which were also conducted on a second year crop. However a direct comparison may not be possible, because of the previously discussed low secondary crown development in the radial trials. Uninhibited secondary crown development, as presumably occurred in the trial reported by Daly *et al.* (1993) may well have allowed high seed yield potential from a lower initial plant population. This requires further investigation, but the evidence from Chapter 3 strongly suggests that this is possible.

Chapter 6

THRIPS AND THEIR EFFECTS ON SEED YIELD AND QUALITY OF CAUCASIAN CLOVER CV. MONARO

6.1 INTRODUCTION

Many insect pests can cause damage to agricultural seed crops. They can be classified into those which attack the roots or foliage, and those which cause direct injury to inflorescences, florets, ovules, seeds and flowering stems (Chapman, 1984). In a preliminary investigation of insect pests on Caucasian clover cv. Monaro in 1995 at Massey University, several were identified. The most commonly recorded were red clover thrips (*Haplothrips niger* Phlaeothripidae), onion thrips (*Thrips tabaci* Lindeman, Thripidae), New Zealand flower thrips (*Thrips obscratus* Thripidae) and *Apterothrips secticornis* Thripidae (no common name). Australian crop mirid (*Sidnia kinbergi*, Hemiptera: Miridae) and clover case bearer moth (*Coleophora spissicornis,* Lepidoptera: Coleophoridae; *C. frischella*, Lepidoptera: Coleophoridae) were also found (Pearson, MAF Lincoln NZ, personal communication, 1995).

Thrips are commonly found in flowers, and have been reported to seriously damage seed yield and quality, particularly in red clover (Chapman, 1984). Red clover thrips are also fairly common on white clover, strawberry clover and lucerne (Yates, 1952). Thrips (order Thysanoptera) possess mouthparts that are like an abbreviated form of the true piercing/sucking pattern. Stylets are present but they are much shorter than in the Hemiptera. Thrips are therefore able to pierce and remove cell contents (including chlorophyll) only from superficial cells of the plant. This usually results in minute white flecks appearing, which eventually coalesce leading to a bleached or bronzed appearance. Thrips also inject saliva as they feed, and with some species this produces marked distortion of the plant parts attacked (Fenemore, 1982).

Most research on Caucasian clover has been mainly focused on agronomic evaluation, genetic differences, plant development and cultivation technology (Kannenberg and Elliott, 1962; Townsend, 1970; Bryant, 1974; Spencer *et al.*, 1975; Stewart and Daly, 1980; Duke *et al.*, 1981; Spencer and Hely, 1982; Speer and Allinson, 1985; Allinson *et al.*, 1985; Dear and Zorin, 1985; Alconero *et al.*, 1986; Daly and Mason, 1987; Sheaffer *et al.*, 1992; Yates, 1993; Strachan *et al.*, 1994; Moorhead *et al.*, 1994; Coolbear *et al.*, 1994; Widdup *et al.*, 1996; Virgona and Dear, 1996; Guy, 1996). As there was no information available on the direct effects of insect pests on seed yield and quality of Caucasian clover, the objective of this study was to determine the effect of changing thrips population during Caucasian clover reproductive growth and development on seed yield and quality.

6.2 MATERIALS AND METHODS

6.2.1. Experimental site and treatments

This experiment was carried out on the Pasture Research Unit at Massey University, Palmerston North beginning in April 1997. The site was chosen because thrips had been found at this site in the previous year. The previous crop in the area was sulla (*Hedysarum coronarium*). The experiment was conducted as a randomised block design and it was maintained in an area of 10 x 7 m^2 which held 12 plots (1 x 2 m^2) (Plate 6-1, Appendix 9). The border between plots was one meter wide. The soil type was an Ohakea silt loam (see Chapter 4). Climate data during July 1997 to May 1998 are provided in Appendix 10.

Plate 6-1. Layout of insect pest trial.

An adult plant of Caucasian clover cv. Monaro supplied by Dr. Keith Widdup (AgResearch Lincoln) was the source of the plants used for this experiment. 150 secondary crown segments were cut from this plant on 20 April 1997. Each crown segment was planted in a 12-cm diameter pot filled with general potting mixture and kept in a glasshouse at 30/20 ± 5° C at the Seed Technology Center, Massey University. Segments were watered once a week. Snail and Slug bait (Methiocarb 20 g kg^{-1} bait) was applied (about 7-8 pellets per plant) five days later after plant leaves were released and again in early June. On July 15 1997, plants were moved to an unheated plastic house. One month later, they were repotted into bigger pots (diameter 30 cm) containing a general mixture (bark and pumice 80:20 v/v; and added fertiliser (3 kg Dolomite, 3 kg agricultural lime, 3 kg Osmocote (N:P:K slow release 8-9 months), 1 kg Osmocote (N:P:K fast release 3-4 months), 0.6 kg Micromax (trace elements), 0.5 kg Iron sulphate per m^3). Pots were placed outside on 25 August.

Before the plants were transplanted into the field plots, sulla and weeds growing in the experimental site were mowed to ground level in early November and the site sprayed with Buster (Glufosinate-ammonium 200 g l^{-1}, 5 l ha^{-1}) on 22 November. One week later, 120 seedling plants were transplanted as single spaced plants (50 x 50 cm) into twelve plots. Each plot contained ten plants. Four of the ten plants in each plot were retained for seed harvest, and six were used to provide inflorescence samples during the flowering period. Water was applied for six hours immediately after transplanting with a small spray irrigating system (600 liters hr^{-1}). Subsequently, plants were irrigated for four hours every 7-10 days until the middle of January. Fertiliser (Cropmaster 15 (N:P:K:S = 15:10:10:8)) was applied by hand spreading at 400 kg ha^{-1} on 14 January. During plant development, hand weeding was done every 10 days. Sulla growing outside the experimental site was also mown and plant material removed on 15 January 1998.

The insecticide Mavrik (a.i. taufluvalinate 240 g l^{-1}), a contact poison that controls thrips, clover case bearer and aphids was used in this study. The insecticide plus a wetting agent (Raingard, approximate 5 ml in 1 liter solution) was sprayed during the evening or early morning when bees were not active. There were three treatments replicated four times. They were Control (C): no application of insecticide; Treatment 1 (T1) treated with Mavrik (a.i. taufluvalinate 240 g $litre^{-1}$) once at 150 ml ha^{-1} (recommended rate, Anon., 1996) on 28 December before the plants reached peak flowering; Treatment 2 (T2) treated with Mavrik at the same rate every 14 days on 30 Nov., 14 Dec., 28 Dec., 11 Jan., 25 Jan., 8 Feb., 22 Feb., 8 Mar., 22 Mar. and 5 April, respectively.

6.2.2. Plant measurement and statistical analysis

The first sample of inflorescences was taken during early flowering (on 9 December 1997). Afterward, samples were taken at 5 days intervals until the middle of April 1998. Four fully opened inflorescences were removed at random from four individual plants in each plot, and were sealed in a small plastic vial containing 10 ml of 95% ethanol. A total of 48 inflorescences (4 x 4 x 3) were picked at each sampling. These inflorescences were brought immediately to a laboratory at the Seed Technology Center and all of the florets of each inflorescence were dissected under a binocular microscope (2 x 10x) to record numbers of thrips adults and larvae per inflorescence. At peak flowering, representative pest samples were fixed in a small bottle containing ethanol. They were sent to an entomologist (Dr. Pearson, AgResearch Lincoln) for identification to at least species level. Then, photographs of adult and larvae thrips were taken at Massey University when the identified samples were returned (Plate 6-2, 6-3).

Flowering pattern was determined by counting inflorescence numbers per plant on four individual plants from each plot at five day intervals, starting in late November 1997 and continuing until late April 1998. Insect pest population

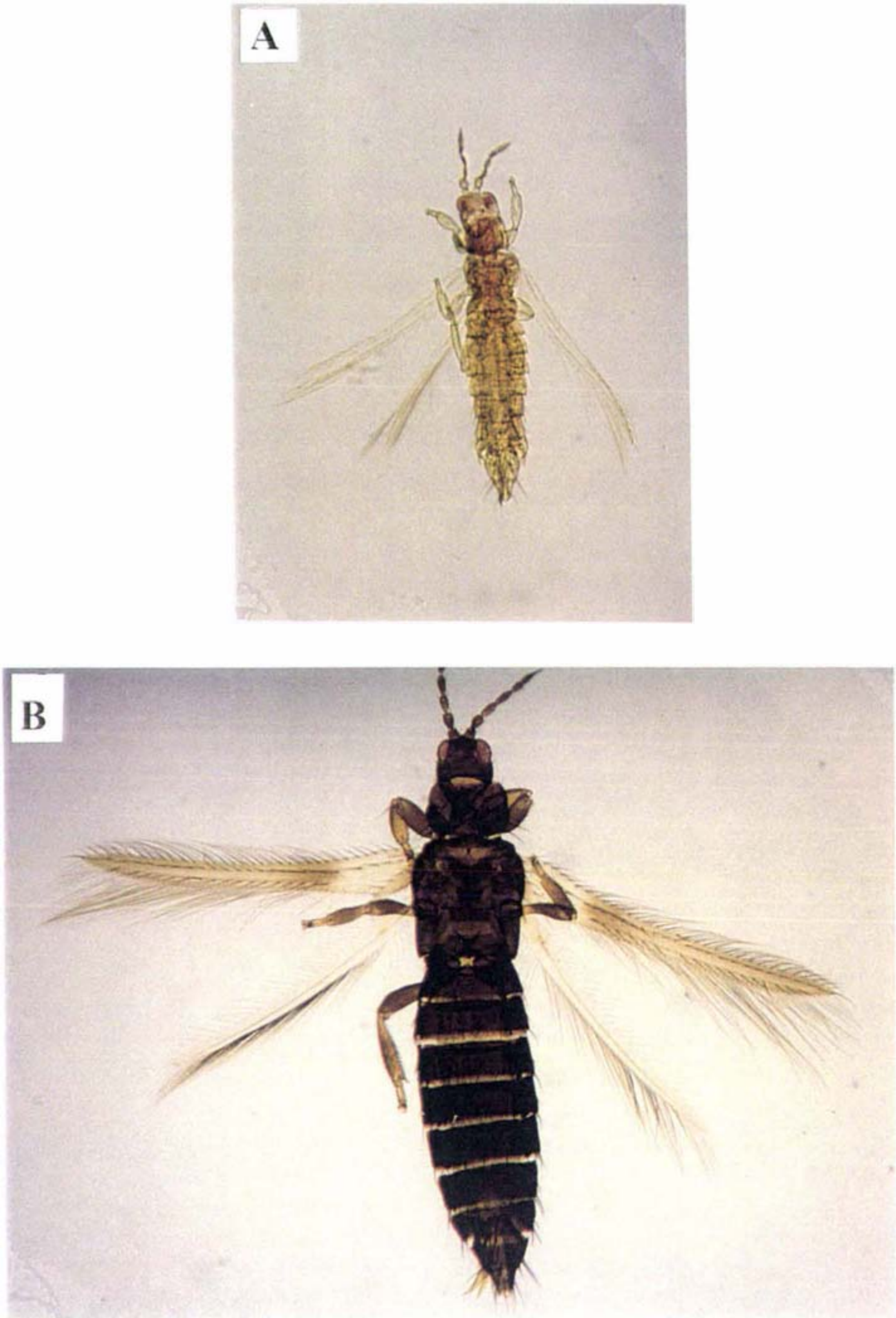


Plate 6-2. (A) Adult onion thrips (*Thrips tabaci*) and (B) adult New Zealand flower thrips (*Thrips obscratus*). (Both 40x).

Plate 6-3. Adult red clover thrips (*Haplothrips niger*). (40x).

change was determined by recording the number of thrips adult and larvae at each sampling from all twelve plots.

Hand harvesting was carried out by removing ripe inflorescences from the four retained plants in each plot in the middle of February and again in late February. The harvested inflorescences of each plant were kept separately in a paper bag. The rest of the mature inflorescences in each plot were also harvested separately at the same time. These bags with harvested inflorescences were kept in the laboratory at the Seed Technology Centre for air drying at room temperature (about 24° C) for five months. After drying, one hundred inflorescences were randomly sampled from each bag that held mature inflorescences harvested from each plot (100 x 12) and the number of florets per inflorescence was counted by removing florets from five of each 100 inflorescences. Then, all 100 inflorescences were threshed by rubbing between stippled rubber. Straw and light material was removed by sieving through 1 mm and 2 mm sieves. The remaining material was purified with an air blower at 345 rpm for 30 seconds (Burrows. Model 1836-4). Seed number per inflorescence and seed weight per inflorescence was recorded. Proportions of normal seeds (seeds which were not shrivelled and without brown spots on the seed coat), seeds with brown spots, and shrivelled seeds, were recorded separately for the four replicates. The remainder of the inflorescences from each bag were threshed in the same way. Seed yield per plant was measured by processing all inflorescences harvested from a single plant in the same way, too. Germination of bulk samples, germination for normal, brown spotted and shrivelled seeds and thousand seed weight of bulk samples, and normal, brown spotted and shrivelled seeds were tested using the method prescribed in the rules of the International Seed Testing Association (ISTA, 1996).

All data were processed using a SAS package (SAS Institute Inc., 1989) for analysis of variance and Fisher's LSD test at $P = 0.05$.

6.3.1 Insect pests identified

Several insect pests were identified in the Caucasian clover inflorescences in this study. They were onion thrips (*Thrips tabaci,* Lindeman Thysanoptera: Thripidae), New Zealand flower thrips (*Thrips obscuratus,* Thripidae), red clover thrips (*Haplothrips niger,* Thysanoptera: Phlaeothripidae), Australian crop mirid (*Sidnia kinbergi*, Hemiptera: Miridae) and aphids (Pearson, personal communication, 1997). The mirid and aphids were not common, (less than two per inflorescence), but the three thrips species (Plate 6-2, 6-3) were consistently found, particularly the onion thrips and red clover thrips. The different stages of red clover thrips (*Haplothrips niger*) development were recorded in Caucasian clover florets as larva I (yellow), larva II (bright red) and adult (shiny black) (Plate 6-4).

6.3.2 Flowering pattern and pest patterns

The first inflorescence appeared in the middle of November 1997. Peak flowering occurred from late December to mid January (Figure 6-1). During this period plants produced up to 8 inflorescences per plant. Open inflorescence number then declined rapidly, averaging less than 2 per plant from February on.

Populations of the three thrips species were recorded. These thrips (adults and larvae) reached around 25 per inflorescence during peak flowering and about 30 per inflorescence just after peak flowering, eventually increasing to a maximum of nearly 40 per inflorescence by the middle of March (Figure 6-2). The three thrips species populations differed during the season. Populations of adult thrips for all three species were less than 5 per inflorescence until just after peak flowering (Figure 6-3). Then, onion thrips dramatically increased from the middle of January and reached a maximum at about 12 adults per inflorescence. Meanwhile, red

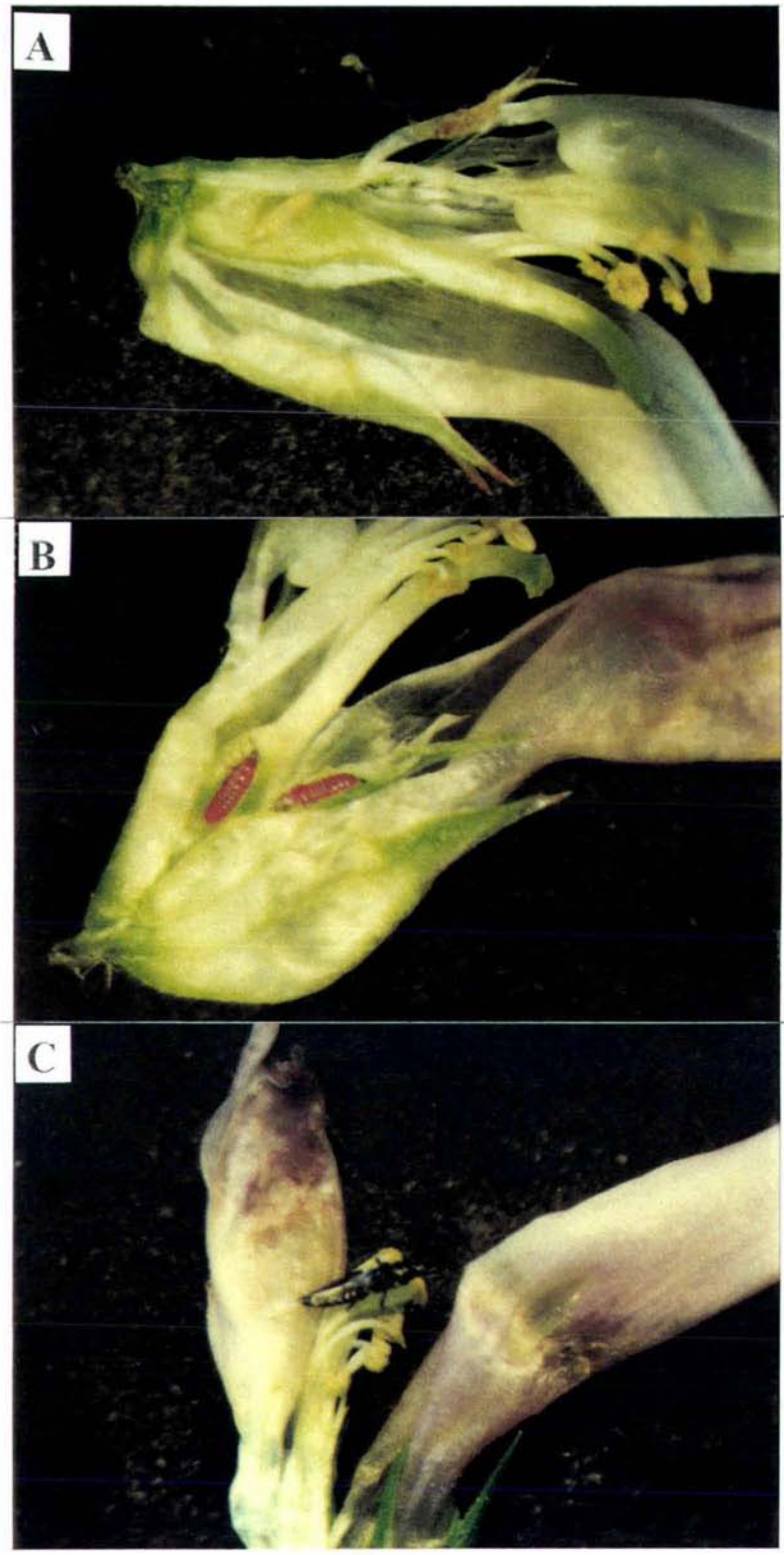


Plate 6-4. Stages of development of red clover thrips (*Haplothrips niger*) in Caucasian clover florets (A. Larva I, B. Larva II and C. Adult). (All 7x).

Figure 6-1. Flowering pattern in Caucasian clover cv. Monaro in the 1997-1998 season. Vertical bars represent standard error.

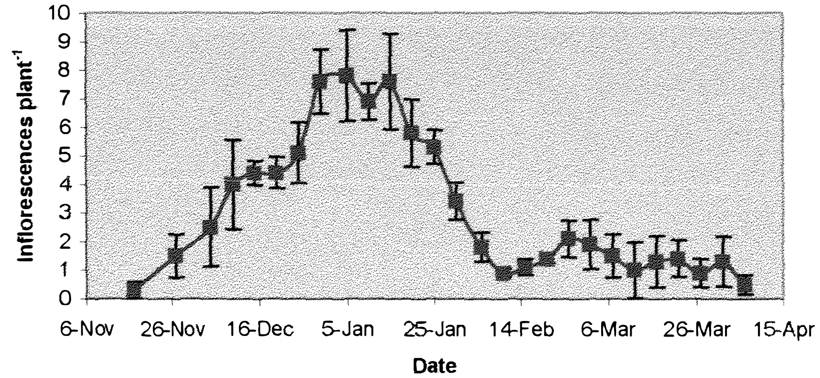


Figure 6-2. Thrips numbers per inflorescence in Caucasian clover cv. Monaro during the 1997-1998 season. Vertical bars represent standard error.

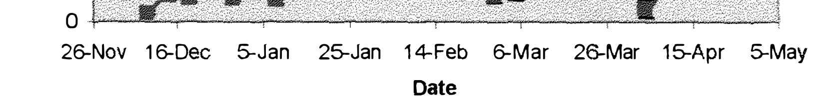


Figure 6-3. Patterns of adult onion thrips, adult red clover thrips and adult flower thrips in inflorescences of Caucasian clover cv. Monaro in the 1997-1998 season. Vertical bars represent standard error.

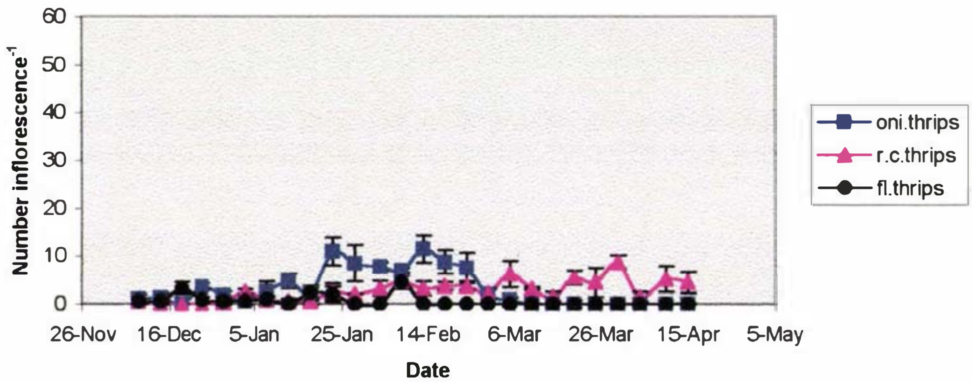
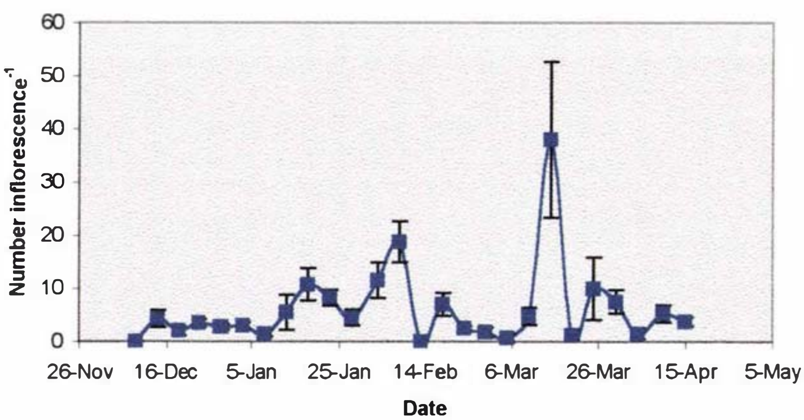


Figure 6-4. Larval thrips population in inflorescences of Caucasian clover cv. Monaro in the 1997-1998 season. Vertical bars represent standard error.



clover thrips increased gradually but flower thrips did not increase, and remained at around 2 per inflorescence. Onion thrips and red clover thrips mainly appeared after peak flowering when seeds in pods were developing. Larvae thrips pattern was recorded for the three thrips together (Figure 6-4). These larvae were not distinguished among species since there is no key for identification. The pattern of population development of larvae thrips showed that larvae were active from the middle of January to the middle of February when plants were in late peak flowering and seeds were developing. However the maximum number of larvae (around 40 per inflorescence) was recorded after peak flowering (in mid March (Figure 6-4)).

6.3.3 Seed yield and quality

Insecticide application did not significantly affect inflorescence number per plant or floret number per inflorescence (Table 6-1). However, treatment 2 (fortnightly application of Mavrik) significantly increased seed number per inflorescence, seed yield per inflorescence, seed yield per plant and thousand seed weight although the single application did not.

The insecticide application affected seed appearance (Plate 6-5) as reflected by different proportions of normal and shriveled seeds (Table 6-2). The multiple application of insecticide increased the percentage of normal and decreased the percentage of shriveled seeds, but there was no difference for the percentage of brown spotted seeds among the three treatments. Thousand seed weight differed significantly among the three types of seeds (normal, brown spotted and shriveled seeds) within a treatment and among different treatments (Table 6-3). Germination and hard seed percentages were significantly higher than the control for the multiple insecticide application and dead seeds were significantly lower (Table 6-4). There were no differences in fresh ungerminated seeds.

Table 6-1. Effect of insecticide application on seed yield and yield components of Caucasian clover cv. Monaro in the 1997-1998 season.

Treatment	Infl. Plant^{-1}	Floret Infl.$^{-1}$	Seed Infl.$^{-1}$	SY (g) Infl.$^{-1}$	SY (g) Plant^{-1}	TSW (g)
C	34.3	73	34.9	0.083	2.79	2.367
T1	35.5	79	37.1	0.089	3.20	2.467
T2	36.5	76	41.1	0.105	3.86	2.586
LSD (0.05)	NS	NS	6.0	0.016	1.01	0.117

C = control, T1 = treated once with Mavrik, T2 = treated with Mavrik fortnightly.

Table 6-2. Effect of insecticide application on the percentage (%) of normal, brown spotted and shriveled seeds harvested in Caucasian clover cv. Monaro.

Treatment	Normal seeds	Brown-spotted seeds	Shriveled seeds
C	75.0	5.3	19.8
T1	74.9	5.3	19.6
T2	81.6	4.9	12.8
LSD (0.05)	6.6	NS	6.7

C = control, T1 = treated once with Mavrik, T2 = treated with Mavrik fortnightly.

Plate 6-5. Three types of seeds harvested from cv. Monaro. (A. seeds with brown spots, B. shriveled seeds and C. visually normal seeds.) (All 10x).

Table 6-3. Effect of insecticide application on the thousand seed weight of normal, brown spotted and shriveled seeds in Caucasian clover cv. Monaro.

Treatment	Normal (g)	Brown spotted (g)	Shriveled (g)	LSD (0.05)
C	2.614	2.313	1.426	0.054
T1	2.628	2.347	1.478	0.049
T2	2.749	2.531	1.609	0.054
LSD (0.05)	0.045	0.059	0.057	

C control, T1 treated once with Mavrik, T2 treated with Mavrik fortnightly.

Table 6-4. Effect of insecticide application on the germination capacity of seeds in Caucasian clover cv. Monaro.

Treatment	Normal* (%)	Abnormal (%)	Hard seed (%)	Fresh ungerm. (%)	Dead (%)
C	82	5	79	0	13
T1	88	1	81	0	11
T2	96	1	90	1	2
LSD (0.05)	8.8	3.2	8.7	NS	8.1

* Germination percentage was obtained after hard seededness was removed by cutting the seed coat with a scalpel (ISTA, 1996).

6.3.4 Germination and hard seed percentages of normal, brown spotted and shrivelled seeds

Germination capacity did not differ among the treatments for the normal, brown spotted and shriveled seeds, but significant differences occurred among the normal, brown spotted and shriveled seeds within the same treatment (Table 6-5). A similar result was also obtained for hard seed percentage (Table 6-6).

6.3.5 Effect of insecticide treatments

The multiple insecticide application controlled the population of thrips. There was a significant reduction in the number of adult thrips following this treatment, but not between the control and the single application except in early January, as the application was on 28 December. The number of adult thrips declined after each Mavrik spray (Figure 6-5), but the population had increased two weeks after each application. Mavrik reduced the number of larval thrips. The number of larvae decreased significantly when the insecticide was applied on 28 December in treatment 1 compared with the number in the control. A near zero population of larval thrips was recorded when plants were insecticide treated fortnightly (Figure 6-6). In the 1997-1998 season, adult and larvae thrips appeared in early December and the population gradually increased from the middle of January to a peak at the middle of February, before declining slightly until the middle of April. An evident peak occurred for larval thrips in the middle of March.

6.4 DISCUSSION

6.4.1 Thrips and their population in Caucasian clover

Onion thrips, red clover thrips and New Zealand flower thrips were the major insect pests commonly found in Caucasian clover in this study. They dominated

Table 6-5. Differences in germination percentage (%) of normal, brown spotted and shriveled seeds in Caucasian clover cv. Monaro.

Treatment	Normal	Brown spotted	Shriveled	LSD (0.05)
C	99	77	47	12.5
T1	100	86	44	9.3
T2	100	81	53	13.7
LSD (0.05)	NS	NS	NS	

C = control, T1 = treated once with Mavrik, T2 = treated with Mavrik fortnightly.

Table 6-6. Differences in hard seed percentage (%) of normal, brown spotted and shriveled seeds in Caucasian clover cv. Monaro.

Treatment	Normal	Brown spotted	Shriveled	LSD (0.05)
C	98	48	38	13.5
T1	97	57	32	10.3
T2	97	51	41	15.4
LSD (0.05)	NS	NS	NS	

C = control, T1 = treated once with Mavrik, T2 = treated with Mavrik fortnightly.

Figure 6-5. Effect of insecticide application on adult thrips population in Caucasian clover cv. Monaro during the 1997-1998 season. Vertical bars represent standard error.

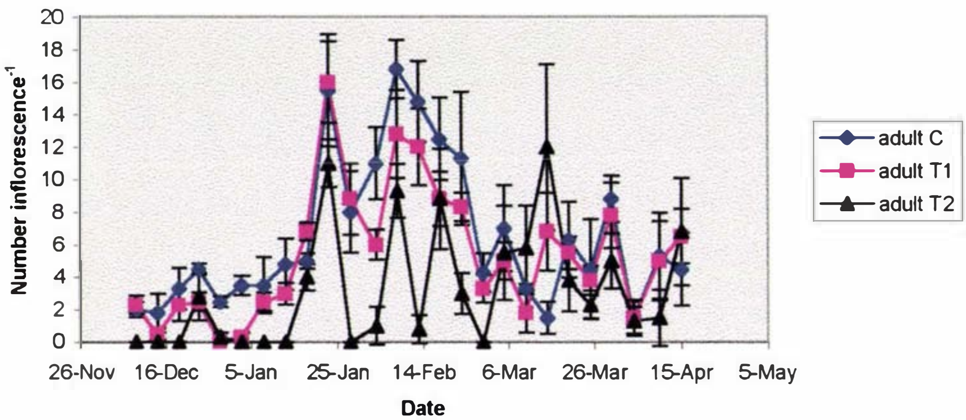
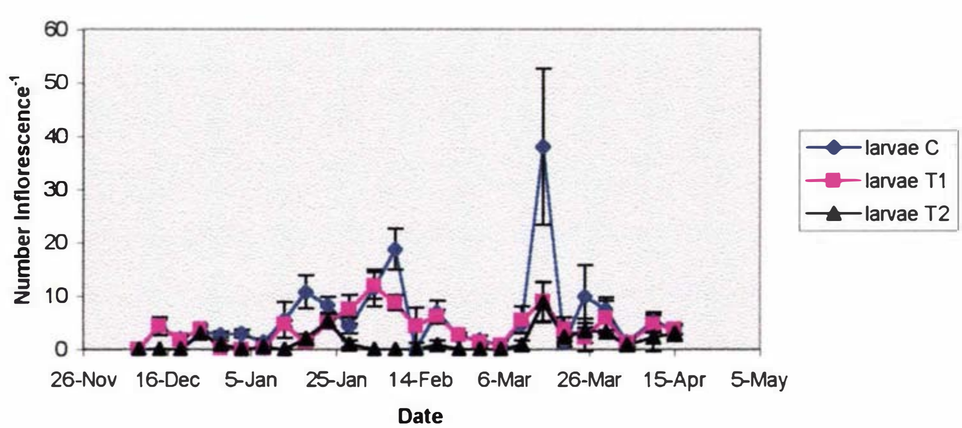


Figure 6-6. Effect of insecticide application on larval thrips population in Caucasian clover cv. Monaro in the 1997-1998 season. Vertical bars represent standard error.



the population of pests during the peak flowering period, although Australian crop mirid and aphids also occurred in low numbers. Thrips have been reported to cause considerable damage in pasture legumes such as white clover and red clover (Chapman, 1984). Adult onion thrips appeared most frequently (over 10 per inflorescence), followed by red clover thrips and New Zealand flower thrips (each about 2 per inflorescence) during peak flowering (Figure 6-3). These thrips also exhibited different occurrence patterns. Adult onion thrips occurred in higher numbers during seed development compared with the two other thrips. The numbers of onion thrips then dropped rapidly from late February, while the number of adult red clover thrips gradually increased to reach a peak in March. New Zealand flower thrips were present in low numbers only from the beginning to the end of flowering. The patterns of these thrips suggested that the population and number of adult thrips were dynamic and quite variable. Onion thrips and red clover thrips formed the main part of the thrips population in this clover during seed development. They are reported to cause damage to seed yield and seed quality by feeding on the floral tissues, which can interfere with normal fertilisation and reduce the amount of seed set, and by feeding on the ovaries and enclosed seeds, thereby scarring the seed coat which reduces viability (Yates, 1952; Chapman, 1984).

The pattern of larval thrips development showed that there were several peaks during the season (Figure 6-4). The first peak appeared towards the end of peak flowering when the number of the larvae increased to over 10 per inflorescence. The second peak appeared in early February during seed development. The two peaks in the thrips population at peak flowering and after peak flowering indicate a potential risk to seed yield and quality, as they cause similar damage to that of adult thrips. They also feed on the green seed pods, causing the developing seed to shrivel (Yates, 1952). The third and fourth larval peaks occurred well after conventional seed harvest, so, they were not important except possibly as an overwintering source for reinfection of the seed crop in the following season. In this trial, larvae of the three thrips could not be identified, as no key is available.

Peak flowering of cultivar Monaro occurred from approximately 25 December to 20 January in this study (Figure 6-1). Towards the end of peak flowering, the thrips population (adults and larvae) reached its first peak at around 25 per inflorescence. During seed development, the population of thrips reached a second peak of over 30 per inflorescence (Figure 6-2). Thus two peaks of thrips population occurred during seed development. This would appear to be the most important time to control thrips by insecticide application in order to reduce seed damage caused by both adult and larval thrips. Most adults and larval thrips appeared in those inflorescences or florets that had begun to wither or had already withered.

6.4.2 Seed yield and quality as affected by thrips

In this study the multiple insecticide application significantly increased seed yield per plant even though inflorescence number per plant (the most important determinant of seed yield in Caucasian clover; Chapter 3) did not differ significantly from that of the control (Table 6-1). Thrips certainly damaged seeds, and it is probable that the seed yield response to insecticide was as a result of reducing the thrips population, and that therefore thrips reduced seed yield. Seed yield was decreased because seed number per inflorescence and seed weight were reduced as a result of thrips damage, as suggested by Yates (1952). The 28% loss in seed yield was associated with a thrips population of 25 to 30 per inflorescence. Chapman (1984) suggested that 30-40 thrips per inflorescence caused a 43% seed loss in red clover.

Seed quality was significantly affected by thrips in this study. Thousand seed weight was significantly lower in the control and single insecticide application treatment compared with the multiple application (Table 6-1) because more shriveled seeds occurred (Table 6-2) in those treatments with a relatively high population of thrips, and thousand seed weight was also reduced. The thousand seed weight of the normal, brown spotted and shriveled seeds was significantly lower in the control and single insecticide application treatment compared with the multiple application treatment (Table 6-3). This indicated that lower thousand seed weights of the three types of seeds in the first two treatments were associated with the relatively high population of thrips. Presumably, nutrients for seed development were partly exhausted by the thrips, and as a result, thousand seed weigh of the three seed types was reduced.

Difference in seed quality also occurred in terms of seed germination between the control and multiple insecticide application treatment (Table 6-4). Plants in the control plots produced seeds with lower germination compared with that in the insecticide treatment. This can be related to the higher percentage of shrivelled seeds. Shrivelled seeds possessed the lowest germination (Table 6-5). Shrivelled seeds also had a lower hard seed percentage compared with normal seeds (Table 6-6), implying that shrivelled seeds suffered more damage during seed development. Poisonous saliva from thrips when they are feeding on ovaries may lead to distortion of seed tissue (Fenemore, 1982), probably also including the seed coat. This might be the reason why a lower percentage germination and hard seed occurred in shrivelled seeds. Presumably, this can be regarded as a poisoning procedure for seeds. Also, around 40% of shrivelled seeds were dead (Appendix 13). The seed coat of brown spotted seeds was damaged when adult and larvae fed on the ovaries and enclosed seeds thereby scarring the seed coat. The structure of the seed coat is modified in this area and allows access of water more readily than before (Yates, 1952). So, a lower percentage of hard seed also occurred in this type of seed (Table 6-6, Appendix 12). However, brown spotted seeds lost less germination capability than shrivelled seeds (Table 6-5) and the percentage of dead seeds was substantially lower ((8%) Appendix 12) because they had suffered less tissue damage.

Seed quality of Caucasian clover cv. Monaro depended on what proportion of the bulk consisted of shriveled or brown spotted seeds. This is important, since these two types of seeds determine bulk seed weight and germination capacity. Controlling the thrips population during seed development was necessary for keeping shriveled and brown spotted seeds at a low percentage. The results in the present study indicate that any damage to seed yield and quality is likely to be caused as seeds are developing, particularly as this coincides with the high population of thrips appearing at late peak flowering or during early seed formation.

6.4.3 Effect of different treatment times

The fortnightly application of insecticide significantly reduced the thrips population (Figure 6-5), particularly that of the larval thrips (Figure 6-6). Larval and adult thrips numbers were also reduced in early January following a single application of insecticide before peak flowering on 28 December. However, the thrips population then rose again to a level similar to that of the control 14 days after the insecticide application. This meant that the effect of Mavrik lasted no more than 14 days. The population will build up again if this spray interval is exceeded. In the control, the population of adult thrips initially increased slowly, before then accelerating during peak flowering, and reached a maximum of 15 per inflorescence in late January. During this period larvae also increased to about 10 per inflorescence. This coincided with the time that thrips became most active as seed was developing. The results suggest that the optimal time to use Mavrik is during late peak flowering or immediately after peak flowering when seed is developing (from mid January to mid February). A single Mavrik treatment before peak flowering only controlled thrips at the beginning of peak flowering. As a result seed development therefore was affected by the thrips, as there was sufficient time before seed harvest for the population of thrips to build up again. There was no significant difference in seed yield (Table 6-1) and seed quality (Table 6-2, 6-3, 6-4) between the control and the single insecticide treatment because a similar number of thrips was present in both treatments during seed development.

One application of Mavrik would be most effective if it was carried out at peak flowering rather than before peak flowering. Seed yield and quality would be less likely to be affected if the number of thrips was reduced at this time. Better control would be achieved by using two insecticide applications at peak flowering and 14 days later during seed development. This may be one way of increasing seed production and quality in Caucasian clover if thrips population warrant control. In contrast, multiple fortnightly insecticide applications, even though they controlled thrips numbers at a sufficiently low level (around 10 per inflorescence) to not affect seed yield or quality in Caucasian clover, would not be cost effective.

Chapter 7

GENERAL DISCUSSION AND CONCLUSION

7.1 PLANT DEVELOPMENT IN RELATION TO SEED PRODUCTION

Caucasian clover cv. Monaro initially developed slowly in the first season. With an increase of temperature, plant development accelerated (Chapter 3). During early vegetative growth the high root to shoot ratio (2.45-2.59) indicated that dry matter accumulation was faster underground than above ground, rapid root development being one of the characteristics of the species. Rapid accumulation of root dry matter was therefore one of the reasons causing the slow development of aerial components during early vegetative growth, because the root system enjoyed accumulative priority. A dramatic increase in leaf production occurred before the plant switched over to reproductive development, allowing more carbohydrate production for the development of aerial components as well as the continued root system development. Root dry matter increased while leaf production increased (Chapter 3). The rapid accumulations of root dry matter also indicates that the Caucasian clover root system is a reservoir for nutrients which are needed to support the subsequent production of reproductive shoots and inflorescences, seed formation and development. Reproductive development occurs only from crowns that mature early and have sufficient root reserves available to support reproductive growth.

Root dry matter was highly correlated with the number of leaves, reproductive shoots and inflorescences when plants reached peak flowering. All of these parameters increased when root dry matter increased. The number of secondary crowns was also correlated with root dry matter, increasing as root dry matter increased. Development of aerial components and secondary crowns is associated with a fast increase of root dry matter accumulation before peak flowering. In the absence of competition, secondary crowns produced reproductive stems and inflorescences in the first season and dominated the numbers of reproductive shoots and inflorescences per plant.

Cultivar Monaro demonstrated a protracted flowering behavior that can last four months. This protracted flowering was caused by an initial production of reproductive shoots from primary crowns and an extended production of reproductive shoots from secondary crowns when primary crown shoot production had ceased (Chapter 3). Individual plants that flower at different times also contribute to the protracted flowering of the cultivar (Chapter 4). Similar results were reported by Gurung (1991), Efendi, (1992), Hill *et al.* (1993) and Widdup *et al.* (1996).

7.2 SEED YIELD AND YIELD COMPONENTS

First year seed yields ranged from 83 kg ha^{-1} (Chapter 4) to 112 kg ha^{-1} (Chapter 6), but the second year seed yields from the density trial (Chapter 5) averaged only 75 kg ha^{-1} at the optimal density (10.8 plants m^{-2}). However the sand bed seed yields (Chapter 3) was the equivalent of 690 kg ha^{-1}. This came however from spaced plants with little competition, which were able to produce secondary crowns. These secondary crowns accounted for over 80% of the seed yield. In contrast in the radial trial, an agronomically poor site restricted plant growth and few secondary crowns were produced. Those that were present received herbicide damage during the inter-row spraying, and consequently failed to become reproductive. This meant that the radial trial seed yields came primarily from primary crowns. It is very obvious that secondary crowns are important for seed production in this cultivar.

Secondary crowns dominated both the number of reproductive shoots (71%) and inflorescences (73%) per plant, and produced nearly 85% of the total seed yield per plant in the first season in the sand bed (Chapter 3). Management should therefore be aimed at maximising secondary crown development. This will depend on early establishment, so that after winter, plants are big enough to make rapid vegetative growth, particularly of the root system, so that secondary crown development can be supported. It is also important that secondary crowns are not disturbed (by cultivation or herbicide application). The twin flowering peaks may cause some problems with deciding when to harvest, but it is probable that the risk of loss of seed from the earlier maturing primary crowns should be accepted in favour of the greater seed yield available from secondary crowns.

The number of inflorescence is the most important yield determinant in Caucasian clover, as seed yield increased with the increase of inflorescences per plant ($R^2 = 0.95$) and per unit area ($R^2 = 0.91$, Chapter 5). However, inflorescence number per plant differs among different genotypes and plant densities (Table 7-1). The most consistent yield component is floret number per inflorescence, which was not affected by density, genotype or pests (Table 7-1). Seed number per inflorescence changed when thrips infested plants, but will also depend on pollinator activity. However, there was no effect from plant density and genotypes (Table 7-1). There was potential for increasing seed number per inflorescence. From 43-133 seeds per inflorescence have been reported from a hexaploid cultivar of Caucasian clover (Kannenberg and Elliott, 1962) and this was similar to the number of seeds per inflorescence obtained in the present study. If an average of 80 florets is produced from a single inflorescence, potentially 160 seeds will form if everything is right during pollinating, fertilization and seed development. Increasing the population of pollinators may greatly increase seed number per inflorescence. Seed yield per plant would be greatly improved. However what is not known is whether the plant could actually continue to support all those seeds, particularly as root growth has begun to increase again at this time.

Table 7-1. Factors affecting seed yield components of Caucasian clover cv. Monaro.

Factor	Inflorescences Plant^{-1}	Florets Inflorescence^{-1}	Seeds Inflorescence^{-1}	TSW (g)
Prim. & Sec. Crowns	-	NS	*	*
Genotypes	*	NS	NS	*
Plant Density	*	NS	NS	*
Insect Pests	NS	NS	*	*

- Not applicable.

* Significant difference.

NS No significant difference.

Seed weights always differed in cv. Monaro (Table 7-1), ranging from 1.837 (Chapter 5) to 2.834 g (Chapter3). Khoroshailov and Fedornko (1973) have reported the variable thousand seed weight in hexaploid types and it has been confirmed in this study. Under the same environment, genotypes can produce significant differences in seed weight. At different densities, the same genotype produced different seed weights, but the difference did not change regularly. Genotypes may therefore be responsible for the variable seed weight of the cultivar.

7.3 SEED YIELD AS AFFECTED BY GENOTYPES IN CV. MONARO

Reproductive characteristics (flowering behavior, reproductive capability) differed in some of the genotypes within cv. Monaro. Different flowering times of individual plants are common in the cultivar and lead to a long flowering period, indicating variability among individuals within the cultivar (Gurung, 1991). This protracted flowering among individual plants made it difficult to produce high numbers of inflorescences over a short period. Such flowering behavior obviously is one of the factors which makes it difficult to produce a consistent seed yield. The genotypes with later flowering behavior and low reproductive capability (such as G9) affected seed yield per unit area in cv. Monaro, as many plants did not flower. This may imply that there is an obligate need for flowering induction, at least for some genotypes.

Twelve genotypes demonstrated great difference in reproductive capability in the seedling transplant radial trial (Table 5-4). Genotypes 1, 2, 4 and 12 represented a high or "normal" reproductive capability group compared with the others. These genotypes produced inflorescences across all five densities. However, genotypes 3, 5, 8, 9, 13 represented another group with a poor reproductive capability. Inflorescence production per plant was low, even at the lowest density. Some of them produced no inflorescences at all. Genotypes 6, 7 and 10 were in the middle. They produced inflorescences at low density. Differences in reproductive ability exist among the genotypes or individual plants, but may also vary from year to year in Caucasian clover cv. Monaro. The reproductive ability of genotypes 6, 7 and 10 was not satisfactory, but the potential was there as they had produced as many inflorescences as genotypes 1, 2, 4 and 12 produced in the previous year (Appendix 8). Why this seasonal variation occurred is not known.

7.4 SEED YIELD IN RELATION TO PLANT DENSITY

Caucasian clover is sensitive to high density planting. Plants grown at high density had their development restricted. Plants were smaller and produced fewer leaves. Dry matter accumulation was slow as development of aerial components was retarded presumably by competition for light, water and nutrients. Reproductive production was also significantly reduced when plant development was seriously restricted at high density. There was a large reduction of inflorescences per plant when plants were at a density that exceeded 10 plants m^{-2}. Although plants grown at the lowest density (3.1 plants m^{-2}) produced the greatest reproductive growth, they were not capable of creating the highest seed yield per unit area, as this clover showed a parabolic relationship both for inflorescence number and seed yield per unit area. Plants grown at the density of 10.8 plant m^{-2} produced the highest number of inflorescence per unit area. However, the optimal density may be lower than this but unfortunately this was not determined. Plant density affected seed yield only through affecting inflorescence number; this was highly correlated with seed yield per unit area (R^2 = 0.91). Increasing inflorescence number per unit area leads to an increase of seed yield per unit area.

There might be an indirect relationship between plant density and reproductive development. When plants grow at high density, competition for light, water and nutrients (developing space) is extreme among individual plants. Necessarily, plant development is inhibited and presumably root development is also impaired. Impaired development of the root system affects development of aerial components, and therefore the entire development would be retarded. High reproductive production is dependent on a good root system development (Chapter 3). If root development is retarded at high density planting sufficient inflorescences can not be produced from the crowns. The optimum plant density must allow good root system development so that good reproductive production per unit area can be obtained.

7.5 SEED YIELD AS AFFECTED BY INSECT PESTS

Thrips proved to be major insect pests in Caucasian clover in this study. They damaged seed yield by reducing seed number per inflorescence. Seed quality was also reduced through reducing seed weight (more shriveled seeds produced) and germination (more dead seeds occurred). Adult onion thrips had the highest population in this clover, followed by red clover thrips after peak flowering. They formed the main part of the thrips population. Two peaks of larval thrips population were recorded during peak flowering and after peak flowering when seed was developing. This coincided with two peaks of thrips population (adult and larvae) during peak flowering and seed development. Damage to seeds is mainly caused by larval thrips (Yates, 1952).

Controlling the thrips population is necessary during seed development, as seed yield was reduced by up to 28% when the thrips population was over 25 or 30 per inflorescence. Thirty to forty thrips per inflorescence were reported to cause a 43% seed loss (Chapman, 1984). At this population level, seed quality was significantly reduced. Thousand seed weight was significantly lower as more shriveled seeds occurred. Germination capacity also decreased as the development of individual seeds was affected. Seeds became shriveled and thrips biting resulted in the formation of brown spots on the seed coat, which also destroyed the seed coat. Seed viability therefore, decreased and a higher number of dead seeds appeared during seed germination. Controlling the thrips population during seed development is therefore necessary but multiple applications of insecticide are not recommended. Further work is necessary to determine the optimum application time for probably not more than two insecticide sprays.

7.6 GENERAL COMMENTS

One of the main objectives in this study was to investigate plant development, particularly the root system development of Caucasian clover and its relationship with seed production. In the sand bed, plants developed well and the root system distribution could be followed clearly. However, whether the relationships between the root system and aerial components in the sand bed also occurred in field conditions was not determined. This will require further work.

Because of the variable seed yield of cv. Monaro, it was decided to investigate any differences in morphological, flowering behavior and reproductive capabilities among the genotypes in the cultivar. The variable seed yield of the cultivar is strongly associated with genotypic differences in flowering time and reproductive performance and selection would greatly improve the cultivar's properties. A new cultivar (Endura) that is more uniform in its flowering and has a relatively higher inflorescence production has now been selected from cv. Monaro (Norriss, 1995), but it is possible that even greater improvement could be made.

The radial trial design is a good method for investigating the effects of plant density on plant vegetative and reproductive growth. However, the failure to obtain seed yield data in the first season (because of the accidental grazing) and the damage to secondary crowns by the application of herbicide in the second season meant that the true potential of the cultivar was not realized. What is required is a study of root system development in the field and in particular how root development is affected by density.

For insect pest control, a single insecticide application before peak flowering was not effective in controlling thrips during seed development while multiple applications were not cost effective. Further work is required to determine threshold thrips numbers and the best timing for a single insecticide application to protect seed yield and quality effectively in Caucasian clover.

7.7 CONCLUSION

Based on the results in the present study and the subsequent discussion, several conclusions can be drawn:

1. Slow aerial development of Caucasian clover in spring is due to fast development of the root system at that time. A characteristic of this clover is the accumulative priority of the root system during vegetative growth. Reproductive production greatly depends on root dry matter accumulation and root system development. Inflorescence production per plant is highly correlated with root dry matter accumulation.

2. In the first reproductive season, seed yield will be low if only primary crowns are present. The development of secondary crowns provides significantly increased opportunities for reproductive development, and therefore higher seed yield per plant.

3. Inflorescence number is the most important component determining seed yield per plant and per unit area in Caucasian clover cv. Monaro. Inflorescence number changes among different genotypes and plant densities. Floret number per inflorescence does not change with genotype or density. Seed number per inflorescence can be affected by pollinator activities, pest attack and presumably assimilate availability. Seed weight varies considerably.

4. Variable seed yield of cv. Monaro may be explained by genotypes that have different flowering behaviors and reproductive capability. Genotypes of the cultivar include early flowering and late flowering types, and this may contribute to the

protracted flowering period. Genotypes used in the current study had inflorescence production ranging from high to zero. Selection is necessary for improving seed yield.

5. Plant density greatly affects seed yield but not seed quality. The optimum plant density in this study was 10.8 plants m^{-2}, at which seed yield per unit area was maximal. Increasing the plant density did not increase seed yield per unit area. Plant density affects seed yield primarily through influencing inflorescence number per plant.

6. Thrips are responsible for reduction of seed yield and lower seed quality in Caucasian clover. Plants in which thrips were not controlled had a reduced seed number per inflorescence and smaller, lower quality seeds as a result of larval feeding.

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APPENDICES

Appendix 1. Climate data for Palmerston North, New Zealand from July 1994-March 1995.

Month	Temperature °C		Sunshine	Rainfall
	min	max	hours	mm
July	3.5	12.3	100	73.8
August	5.7	13.6	110	82.0
September	6.2	14.2	112	172.7
October	7.6	16.1	184	69.8
November	10.1	17.3	168	179.7
December	11.3	20.5	200	45.5
January	12.6	22.9	258	53.5
February	14.5	23.3	186	57.9
March	13.1	21.1	188	142.4

(Data obtained from AgResearch Grasslands)

Appendix 2. Layout of the experimental plots for genotype 2 (G2), genotype 9 (G9) and genotype 12 (G12) from cv. Monaro in 1994-1995.

G12	**G2**	**G12**
G2	**G2**	**G12**
G9	**G9**	**G9**
G9 ↑ 1.2 m ↓	**G2**	**G12**

← 3.0 m →

Appendix 3. Soil analysis for genotype characteristic trial, campus plot no. 21 of the Pasture Research Unit, Department of Plant Science, Massey University, completed on 24/11/94 by the Fertiliser and Lime Research Centre, Massey University.

Sample	pH	Olsen P	SO4	Exch. K	Exch. Mg	Exch. Ca	CEC	SVCF*
0-15 cm	5.5	18	6.0	0.23	1.30	5.4	17	1.08
15-30 cm	5.8	8	7.0	0.10	1.32	4.9	12	1.17

-Phosphate and sulphate values are expressed as µg/g (air-dry).

-Exchangeable cations and CEC values are expressed as meq/100g (air-dry).

-* The soil volume correction factor (SVCF) is a measure of the weight of air-dry soil (g) per volume (ml) and can be used to convert results to a volume basis.

Appendix 4. Seed yield harvested from individual plants of Caucasian clover cv. Monaro in the 1993-1994 season (cultivated in buckets since 1992).

Plant	Flowerheads			Total	Seed weight (g)			Total
No.	Nov.	Dec.	Jan.	$Plant^{-1}$	Dec.	Jan.	Feb.	(g) $plant^{-1}$
1	9	20	3	32	1.36	3.11	0.16	4.36
2	7	30	9	46	1.20	4.33	0.54	5.97
3	0	0	0	0	0	0	0	0
4	2	5	0	7	0.20	0.94	0	1.14
5	0	2	3	5	0	0.30	0.18	0.48
6	3	3	2	8	0.17	0.54	0.27	0.98
7	3	3	0	6	0.39	0.22	0	0.61
8	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
10	0	3	1	4	0	0.32	0.01	0.33
11	1	3	1	5	0.23	0.48	0.08	0.79
12	7	18	4	29	0.54	1.96	0.21	2.71
13	0	0	0	0	0	0	0	0

Appendix 5. Soil analysis for the seed sown and seedling transplant (genotypes) radial trials, campus plot number 21 of the Pasture Research Unit at Massey University completed on 10/06/94 by the Fertiliser and Lime Research Centre, Massey University.

Sample	pH	Olsen P	SO_4	Exch. K	Exch. Mg	Exch. Ca	Exch. Na	CEC
0-15 cm	5.2	19	13.2	0.31	1.05	5.4	0.2	16
15-30 cm	5.2	13	13.9	0.35	0.88	4.4	0.1	14

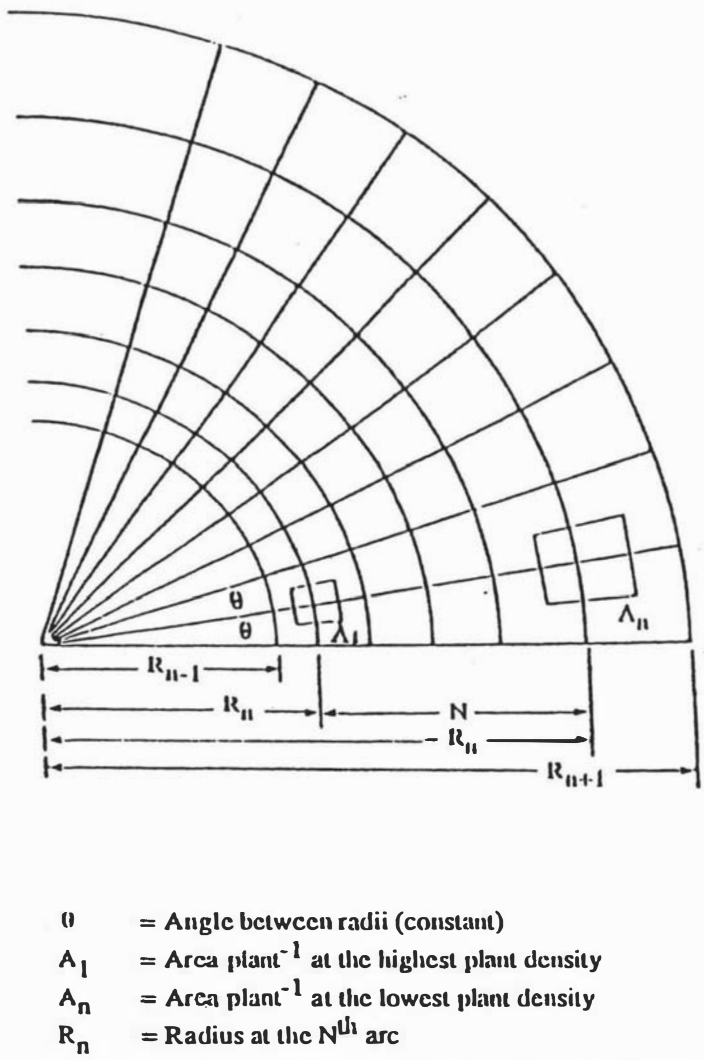
-Phosphate and sulphate values are expressed as μg/g (air-dry).

-Exchangeable cations and CEC values are expressed as meq/100g (air-dry).

Appendix 6. Climate data for Palmerston North, New Zealand from July 1995-March 1996.

Month	Temperature °C		Sunshine	Rainfall
	min	max	hour	mm
July	4.6	11.7	113	120.8
August	4.8	12.5	116	72.8
September	7.2	15.2	118	106.4
October	8.6	16.7	135	139.6
November	9.1	18.1	176	102.4
December	13.3	21.9	187	101.0
January	14.6	23.0	195	50.5
February	13.8	22.8	206	128.7
March	10.6	20.0	166	79.1

(Data obtained from AgResearch Grasslands)



θ	= Angle between radii (constant)
A_1	= Area plant^{-1} at the highest plant density
A_n	= Area plant^{-1} at the lowest plant density
R_n	= Radius at the Nth arc
R_{n-1}	= Distance of the inner guard row from the centre of the circle
R_{n+1}	= Distance of the outer guard row from the centre of the circle
N	= Number of arcs (or plant densities)

Appendix 7. A diagrammatic representation of Nelder's radial spacing design (type Ia).

Appendix 8. Comparison of total inflorescence production of 57 plants from density of 44.7 plants m^{-2} to density of 2.6 plants m^{-2} for genotype 1 to genotype 13 in the seedling transplant radial trial in the 1994-1995 season.

Genotype	Mean
1	143.7 b
2	139.7 bc
3	37.3 ef
4	152.0 ab
5	62.0 e
6	171.7 ab
7	188.3 a
8	16.3 f
9	73.3 de
10	103.3 cd
12	170.5 ab
13	58.3 e
LSD (0.05)	38.9

Appendix 9. Layout of the experimental plots for the insect pest trial with three treatments (Control (C), treated with Mavrik once (T1) and treated every two weeks (T2)) in 1997-1998.

T1-1	**C2**	**T2-3**
C1	**T1-3**	**T2-4**
T1-2	**T2-2**	**T1-4**
T2-1 ↑ 1.0 m ↓	**C3**	**C4**

← 3.0 m →

All treatments with 4 replicates.

Appendix 10. Climate data for Palmerston North, New Zealand from July 1997-May 1998.

Month	Temperature °C min	Temperature °C max	Sunshine hour	Rainfall mm
July	3.0	12.7	145.5	32.0
August	5.4	13.1	140.0	60.1
September	6.8	14.2	116.0	79.0
October	8.4	16.2	132.0	77.8
November	10.6	18.1	192.1	57.1
December	11.2	20.4	211.0	103.4
January	12.6	22.5	179.1	31.7
February	15.5	25.1	178.5	61.4
March	13.4	23.1	192.8	35.6
April	10.7	19.9	157.6	72.9
May	7.4	15.9	115.6	100.9

(Data obtained from AgResearch Grasslands)

Appendix 11. Germination of normal seeds harvested after treatment with or without Mavrik in cv. Monaro.

Treatment	Normal (%)	Abnormal (%)	Hard seed (%)	Fresh ungerm. (%)	Dead (%)
C	99	0	98	0	1
T1	100	0	97	0	0
T2	100	0	97	0	0
LSD (0.05)	NS	NS	NS	NS	NS

Germination percentage was obtained after hard seededness was removed by cutting the seed coat with a scalpel (ISTA, 1996).

Appendix 12. Germination of brown-spotted seeds harvested after treatment with or without Mavrik in cv. Monaro.

Treatment	Normal (%)	Abnormal (%)	Hard seed (%)	Fresh ungerm. (%)	Dead (%)
C	77	12	48	3	8
T1	86	7	57	3	4
T2	81	10	51	1	8
LSD (0.05)	NS	NS	NS	NS	NS

Germination percentage was obtained after hard seededness was removed by cutting the seed coat with a scalpel (ISTA, 1996).

Appendix 13. Germination of shrivelled seeds harvested after treatment with or without Mavrik in cv. Monaro.

Treatment	Normal (%)	Abnormal (%)	Hard seed (%)	Fresh ungerm. (%)	Dead (%)
C	47	8	38	5	40
T1	44	7	32	4	43
T2	53	11	41	4	32
LSD (0.05)	NS	NS	NS	NS	NS

Germination percentage was obtained after hard seededness was removed by cutting the seed coat with a scalpel (ISTA, 1996).