Evaluation of vegetatively reproductive red clovers (*Trifolium pratense* L.) for use in pastoral systems.

A thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Plant Science at Massey University, New Zealand

Mark Graham Hyslop

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This Thesis is dedicated to my parents, Brenda and Stewart,
for all their love and support over the years.

ABSTRACT

The persistency of red clover (*Trifolium pratense* L.) can be poor when grazed in mixed and pure stands, but it is still regarded as a valuable source of high quality summer feed in some farming systems. Vegetatively reproductive red clover selections offer promise to solve this persistency problem. The objectives of this research were to study the growth, perennation, reproductive ability and productivity of vegetatively reproductive red clover selections. A series of nine spaced plant and mixed and pure sward experiments were conducted at Hastings, Dannevirke, AgResearch Grasslands and Massey University, Palmerston North, New Zealand (latitude 40°23'S) from 1995 to 1998. The vegetatively reproductive red clovers evaluated were cv. Astred, F2419 and cv. Gualdo with Grasslands Pawera and Turoa used as controls.

Preliminary investigations into the incidence of rooted plantlet production by prostrate red clover selections under grazing found 37, 16 and 5 rooted plantlets per parent plant for Astred, F2419 and Turoa, respectively. Astred produced larger rooted plantlets which survived better than F2419 and Turoa.

Astred produced 57% of its plantlets on primary stems, or branches off these stems, that grew from the parent plant crown in September, whereas F2419 plantlets developed on a wider spread of stem ages. The majority of plantlets formed on quaternary branches at a range of distances (4cm - 55cm) from the parent plant crown. Astred produced less rooted plantlets per parent plant, had longer, wider leaves, thicker stems and was taller at 10 months of age than Gualdo.

More rooted plantlets were established per Astred parent plant under wet surface soil conditions than dry conditions (22.2% and 3.7% gravimetric soil surface water content, respectively), but when the dry soil surface was watered, there was a compensatory increase in the number of rooted plantlets. Removal or retention of flowers did not affect rooted plantlet formation.

There was no difference in the total herbage accumulated between Astred and Pawera in the first growing season, under 4, 6 and 8 week grazing frequencies, and lax and hard grazing intensities, but Astred produced more (P<0.001) herbage at the first spring grazing. Astred swards had significantly higher percentages of parent plants than Pawera in all treatments after two seasons grazing. Grazing intensity and frequency affected the number of rooted plantlets produced which ranged from 29 to 66 per m^2. The recommended grazing management for pure swards of Astred red clover is to graze every 4 to 6 weeks, or when pre grazing height reaches 30cm, whichever is later, with a minimum post grazing residual of 10 cm over the whole grazing season for effective plantlet production.

Pawera and Astred had contrasting total percentages of leaf and stem from 20/9/96 to 6/1/97 (P<0.001) with Pawera having thicker stems compared with Astred in all corresponding grazing treatments (P<0.001). The total percentage of regrowth for Astred or Pawera was not significantly different.

The persistence of tagged parent plants of Astred and Pawera in the 6 weeks hard grazed treatment was similar until after 2.5 years of age when (P<0.001) more Astred parent plants survived. Grazing intensity and frequency affected the number of all plantlets (plantlets with no roots, aerially rooted or rooted) produced, which ranged from 0 to 11 per m^2 for rooted plantlets after each grazing. In mixed swards on three farm trials at Hastings, Dannevirke and Palmerston North, Astred parent plant persistence decreased with increasing grazing pressure, but significantly more parent plants survived than for Pawera.

The vegetatively reproductive red clover selections performed at productive levels that could make a significant contribution to New Zealand agriculture as truly perennial, taprooted forage legumes capable of increasing or maintaining their parent plant population over time under grazing.

Key words: Astred, F2419, Gualdo, rooted plantlet, vegetatively reproductive, *Trifolium pratense*

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THESIS STRUCTURE

This thesis is based on a series of papers evaluating vegetatively reproductive red clovers. All chapters, except Chapters One (General Introduction) and Two (Review of Literature), have been structured as scientific papers and modified to thesis format. Chapters Three, Eight and parts of Chapter Nine (see Appendix I) have been published and Chapters Four to Seven are being prepared for submission to journals. Chapters Three to Eight deal with one or more specific experiments with a detailed discussion, conclusion and list of references at the end of each Chapter. References for Chapter One are merged into those at the end of Chapter Two. Chapter Nine contains an integrated general discussion linking the findings of all chapters. A summary of the main findings from the research in this thesis is also presented at the end of Chapter Nine.

Note: All references contained within this thesis referring to two authors have been cited as the first author *et al.*, instead of referring to both authors.

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1. INTRODUCTION

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Red clover (*Trifolium pratense* L.) is widely regarded as a legume of major importance in temperate agricultural systems (Fergus *et al.*, 1960; Taylor *et al.*, 1979; Smith, 1993; Hay *et al.*, 1989; Frame *et al.*, 1998) and is second only in use to white clover (*Trifolium repens* L.) or lucerne (*Medicago sativa* L.) in many temperate regions (Taylor *et al.*, 1995; Frame, 1990). The excellent nutritive quality (Hay *et al.*, 1978, Cosgrove *et al.*, 1985; Ussher 1986; Hay *et al.*, 1989), high yield potential (Hay *et al.*, 1978; Taylor *et al.*, 1979; Cosgrove *et al.*,1985; Ussher 1986; Laidlaw *et al.* 1988; Hickey *et al.*, 1989) and drought tolerance of red clover make it an important component in short term high quality pastures for fattening livestock and where herbage is conserved as silage or hay (Fergus *et al.*, 1960; Taylor *et al.*, 1979; Bowley *et al.*, 1984; Frame *et al.*, 1998). Red clover is an important component of New Zealand pasture seed mixes, despite its usual lack of persistence beyond four years, and a tendency to cause bloat in cattle (Waghorn *et al.*,1990; Bush *et al.*, 1994; Essig, 1985) and reproductive problems in sheep, especially when grazed as pure swards (Newton *et al.*, 1973; Thomson, 1975; Shackell *et al.*, 1984; Keogh *et al.*, 1996; Kramer *et al.*, 1996; Frame *et al.*, 1998). Its main contribution is to summer feed, particularly in dry areas (Rumball, 1983).

All red clover cultivars currently in use (with the exception of cv. Astred and cv. Gualdo) grow from woody crowns and predominantly reproduce from seed. Crowns, however, are susceptible to over grazing (Brougham 1959; Brougham 1960; Cosgrove *et al.*, 1985; Curll *et al.*, 1989), treading damage (Hay *et al.*, 1989), and fungal infection (Leath, 1989; Smith *et al.*,1985; Hay *et al.*, 1989; Skipp *et al.*,1990), particularly in Winter (Hay *et al.*, 1989), resulting in poor stand persistence (Brougham 1959; Brougham 1960; Cosgrove *et al.*, 1985; Curll *et al.*, 1989; Sheath *et al.*, 1989; Frame 1990). At best, red clover is regarded as a short-lived perennial and only persists for 1 to 4 years in mixed and pure swards (Brougham 1959; Brougham 1960; Fergus *et al.*, 1960; Taylor *et al.*, 1977; Rumball, 1983; Cosgrove *et al.*, 1985; Bowley *et al.*, 1988; Sheath *et al.*, 1989; Frame 1990). The reasons for the inevitable decline in the number of adult plant crowns has been the focus of much research (Fergus *et al.,* 1960; Taylor *et al.,* 1977; Rufelt, 1982; Bowley *et al.,* 1984; Cosgrove *et al.,* 1985; Lancashire,1985; Smith, 1981; Smith *et al.,* 1985; Smith *et al.,* 1988; Skipp *et al.,* 1990; Montpetit, *et al.,*1991; Taylor *et al.,* 1996). Many of these problems have been solved by conventional breeding methods, but persistence still remains a problem.

Red clover selections do exist that have stems which make contact with the soil surface and produce clonal plants (termed plantlets hereafter) at stem nodes (see Plate 1.1). The first such cultivar, Astred, was released in 1992 (Smith, 1992) by the Department of Agriculture, Tasmania and has shown an improvement in persistency (Smith, 1993; Smith *et al.,* 1993) relative to current cultivars in grazed pastures. Research with other taprooted perennial forage plants (crown type red clover, lucerne and birdsfoot trefoil) has shown that the frequency and intensity of grazing affects plant development and hence persistence and productivity (Nelson *et al.,* 1968; Leach 1969, Leach 1979; Volenec *et al.,* 1987; Bowley *et al.,* 1988). There is little information in the literature on plant development, the dynamics of vegetative reproduction, and the general growth of vegetatively reproductive red clovers with and without grazing.

Therefore, the objectives of this thesis are to:

i) Compare the general persistence of vegetatively reproductive red clovers with “crown” type red clovers, under different grazing conditions.

ii) Gain an understanding of the morphology of the vegetative reproductive red clover plant, and develop management recommendations on how to graze vegetative reproductive red clovers to promote the formation of vegetative offspring.

iii) Gain an understanding of what physical conditions promote vegetative reproduction in some vegetatively reproductive red clover selections.

iv) Gain an understanding of the general performance and usefulness of vegetatively reproductive red clovers as a forage type.

2. LITERATURE REVIEW

2.1 INTRODUCTION

Red clover in New Zealand is less important than white clover as a forage legume, it is less adaptable and less persistent in our pastures because it lacks the two main survival devices of white clover, vigorous stolons and the ability to produce seed even under frequent grazing conditions. The niche for current red clovers is, therefore, in systems for forage conservation and in short term pastures (Rumball, 1983).

Red clover selections do however exist that can vegetatively reproduce (Smith 1993; Smith *et al.,* 1993). Only a small number of experiments have been conducted using this plant type (Hamilton, 1993; Smith, 1993; Smith *et al.,* 1993; Orr *et al.,* 1996) mainly due to the recent availability of commercial cultivars (Astred in 1992 and Gualdo in 1998).

Because of the limited published research specifically based on vegetatively reproductive red clover and the distinctive differences between vegetatively reproductive red clover and conventional crown type red clover, this Chapter provides a brief overview of red clover use in New Zealand, its persistence, production and adaptations, but will mainly focus on vegetative reproduction in other comparable, taprooted and non taprooted forage plants, their management for persistence, vegetative reproductive mechanisms and morphology.

Several detailed comprehensive reviews of crown type (or conventional) red clovers have been published (Fergus *et al.,* 1960; Taylor *et al.,* 1979; Bowley *et al.,* 1984; Smith *et al.,* 1985; Smith, 1993; Taylor *et al.,* 1996; Frame *et al.,* 1998), and it is not the intention here to review or repeat any of these reviews in detail, but rather to present topics pertinent to vegetatively reproductive red clovers.

2.1.1 Definition of terms for vegetatively reproductive red clovers (Trifolium pratense L.)

Due to the relatively recent release of vegetatively reproductive red clovers and some confusion associated with their taxonomy and morphology (Mirzaie-Nodoushan, 1993), a definition of plant parts precedes the review of literature.

Stem: An elongation of a crown bud coming from the axil of the cotyledons, at or above the soil surface (Frame *et al.*, 1998). Stems are always aerial in early growth with some becoming positively geotrophic (bending) and resting on the soil surface as they mature. Some selections have stems which act more like stolons (e.g. F2419), but these are always termed stems in this study.

Plantlet: A non-elongated axillary growth from a stem node meristem that remains in the rosette form for several months or more. These are classified as either having no roots, roots but not in the soil (aerially rooted), or rooted and fixed firmly in the soil (rooted plantlets). Plantlets in general terms could also be termed ramets (Grime *et al.*, 1986), leaf-bud propagules (Cummings *et al.*, 1961), clonal offspring, and daughter plants (Smith *et al.*, 1993).

Parent plant: In most cases the originally sown plant (genet) (Grime *et al.*, 1986), or a plant that has been generated by vegetative means and is one year or more in age and capable of vegetative reproduction. That is, refers to any plant that can generate plantlets. Each parent plant may have several crowns.

2.2 HISTORY AND PLANT PERSISTENCE

2.2.1 The history, role and importance of red clover in New Zealand

Red clover (*Trifolium pratense* L.) has been utilised in many different ways and in all types of farming systems since its introduction into New Zealand by early European settlers. These have ranged from over-sowing in bush burn mixtures, biennial pastures sown with Italian ryegrass, under-sown with grain crops and used as a stubble crop, temporary pastures of 3 to 6 years duration, and permanent pasture (Campbell, 1931).

Initially (pre 1900) all strains were the early flowering, rapid growing, short lived, tall, open, stemmy types, commonly known as "cow grass" or New Zealand broad red clover (Hilgendorf, 1936). By 1941 the first selection was made from locally produced seed, certified in 1946, and renamed Grasslands Hamua in 1964 (Rumball, 1983). Because of the poor persistence of this early flowering type which was considered almost an annual, a later flowering, dense, prostrate and slower growing type was introduced to New Zealand from Britain (Rumball, 1983). In the late 1920s a breeding programme started with this later flowering type, and Montgomery red clover (Grasslands Turoa) was released in 1937 (Rumball, 1983). The use of Turoa, and later the tetraploid Grasslands Pawera (1972), became more common in permanent pasture mixtures because of their increased persistency and disease resistance compared to the early flowering types (Rumball, 1983).

The two principal reasons for legume use in farming systems that do not extensively apply fertiliser nitrogen, are the advantages in the quality and bulk of feed over the Spring and Summer period, and the ability to fix atmospheric nitrogen (Suckling, 1959; Levy, 1970; Langer, 1973; Grant *et al.,* 1978). Fixed nitrogen is immediately available for host plant growth and ultimately available for associated grass growth (Langer, 1973; Taylor, 1985). Reported yields have ranged from 9-18,000 kg DM/ha (Taylor *et al.,* 1979; Cosgrove *et al.,* 1985; Ussher, 1986; Laidlaw *et al.,* 1988; Hickey *et al.,* 1989) in pure swards to 1-14,000 kgDMha in mixed swards (Hay *et al.,* 1978; Frame, 1986; Hickey *et al.,* 1989; Roberts *et al.,* 1990).

Legumes contain low ratios of soluble carbohydrates to protein (Aman *et al.,* 1983), 60-80% digestible dry matter (Taylor, 1985), and higher levels of protein and minerals than grasses (Miller, 1984) and, as a result, animal growth rates are high (1966; Gibb *et al.,* 1976; Day *et al.,* 1978; Thomas *et al.,* 1981; Hunt *et al.,* 1990; Fisher *et al.,* 1991; Niezen *et al.,* 1991; Barry *et al.,* 1993; Niezen *et al.* 1993). Swards with red and white clovers do, however, promote bloating in cattle and sheep (Waghorn *et al.,* 1990; Bush *et al.,* 1994; Essig, 1985). Red clovers of high formononetin content can also affect the reproductive performance of ovulating sheep and cattle if in high proportions of a mixed sward or when grazed as a pure sward (Newton *et al.,* 1973; Thomson, 1975; Shackell *et al.,* 1984; Keogh *et al.,* 1996; Kramer *et al.,* 1996; Frame *et al.,* 1998).

2.2.2 Persistency of crown type red clover

There has been no evidence to suggest that the early importations of red clover (mainly from Britain) have gradually evolved or adapted into local ecotypes, even though our pastures in New Zealand are of much longer duration than those of Europe (Rumball, 1983). No New Zealand literature reports vegetatively reproductive ecotypes being found in New Zealand from this early introduced material.

The lack of persistence of red clover (*Trifolium pratense* L.) is a major limitation to the acceptance of this forage by farmers (Cosgrove *et al.,* 1985; Lancashire 1985; Bowley *et al.,* 1988; Frame *et al.,* 1998). While it is the second most commonly sown legume in New Zealand, its content is generally low in permanent mixed pasture swards after 2 to 3 years (Lancashire, 1985). It generally reaches maximum production in its first or second season from sowing and then declines thereafter (Langer, 1973; Smith *et al.,* 1985; Frame *et al.,* 1998). Persistence is severely limited in areas of less than 500-800 mm annual rainfall (Greenwood *et al.,* 1982; Gramshaw *et al.,* 1989; Sheath *et al.,* 1989). The loss of production is from the death of plants which has been attributed to disease, primarily crown (*Selerotinia trifoliorum*) and root rots (*Fusarium* and *Rhizoctonia* spp.) (Fulton *et al.,* 1959; Leath, 1989; Smith *et al.,* 1985; Hay *et al.,* 1989; Skipp *et al.,* 1990), insect attack (Stem eelworm, Nematodes, slugs, pea aphid, grass grub, porina and the lesser clover leaf weevil) (Graham *et al.,* 1959; Leath, 1985; Smith *et al.,* 1985; Watson *et al.,* 1989; Via, 1991; Barratt *et al.,* 1992) and internal breakdown, a physiological disorder degrading the centre of the taproot and crown area (Graham *et al.,* 1960; Newton *et al.,* 1960; Cressman, 1967).

As plant numbers in the sward decrease, compensatory levels of production per plant have been measured for short periods of time (Frame *et al.,* 1998), similar to that of lucerne before these plants deteriorate and die (Pulli, 1980). For this reason, production loss can appear to happen in a very short time period, when it has been occurring over a much longer period of stress.

Frequent and hard grazing can also promote the death of large numbers of plants from depletion of carbohydrate reserves in the taproot (Bowley *et al.,* 1984; Hay, 1985; Curll *et al.,* 1989; Hay *et al.,* 1989). Grazing when soil moisture levels are high, particularly in late Autumn, Winter and early Spring, can cause plant death by burial and treading damage to crowns, allowing crown rotting micro-organisms to enter (Hay, 1985; Hay *et al.,* 1989).

2.3 VEGETATIVELY REPRODUCTIVE PLANT STRATEGIES

2.3.1 Vegetative replacement

It was previously suggested (Rumball, 1983) that there was little prospect of breeding red clovers with stolons as a way of improving persistence as this would offset the high levels of production for which red clovers were mainly grown. This assumption would be true if plant types were very prostrate and partitioned a high proportion of growth to stolon or root production. Erect and open plant types are considered necessary to achieve high production levels and are considered more suitable to the lax grazing systems of dairy farms (Rumball, 1983; Curll *et al.*, 1989; Smith, 1993).

The two main mechanisms plants use to ensure their genes are passed on to future generations (and so their form of plant persists in that environment) are sexual reproduction (flowering) and asexual reproduction (cloning or vegetative replacement) (Sarukhan, 1974; Jones *et al.*, 1989). In a natural, infrequently grazed environment the majority of red clover types rely on sexual reproduction (Fergus *et al.*, 1960; Frame *et al.*, 1998) and hence the production of large quantities of seed, some of which is hard seed that will stay dormant for many years (Bowley *et al.*, 1984; Puri *et al.*, 1984; Frame *et al.*, 1998). In a grazed situation plants either never get a chance to mature and flower because of grazing frequency and grazing intensity (Curll *et al.*, 1989; Hay *et al.*, 1989; Taylor *et al.*, 1996), or are managed so they are kept in the vegetative state to maintain herbage quality (Frame *et al.*, 1998). Sexual reproduction is unsuitable as a means for currently available cultivars to maintain persistency in grazed swards. Very prostrate, low dry matter producing selections of red clover may be able to maintain populations though sexual reproduction (Smith, 1993), but this plant type is not widely used at present.

One concept to consider is whether plants can be grouped as having either an open or closed reproductive systems. Development of red clover plant types that could produce stems which grow rooted plantlets at stem nodes (as found by Smith *et al.,* 1993) would change red clover from a closed to an open plant system. The advantage of an open system is the possibility of parent plant replacement by cloned offspring, rather than the specified life cycle (or expectancy) of a closed system guaranteeing the decline of the parent plant population, unless there is recruitment from seed (Taylor *et al.,* 1996). Vegetative replacement by cloning of the parent plant offers a feasible solution to the persistency problems of red clover (Smith, 1993; Taylor *et al.,* 1996).

2.3.2 Vegetative, reproductive strategies

Some species (e.g. *Rubus* spp.) rely equally on both seed and vegetative reproduction to maintain or increase their population size (Sarukhan, 1974), whereas others depend almost exclusively on vegetative reproduction (e.g. *Pteridium* spp.). The type of vegetative, reproductive structures involved (stem, rhizome, tuber, bulb or bud) affect the vegetative, reproductive strategy of the plant type (Van Groenendael *et al.,* 1996).

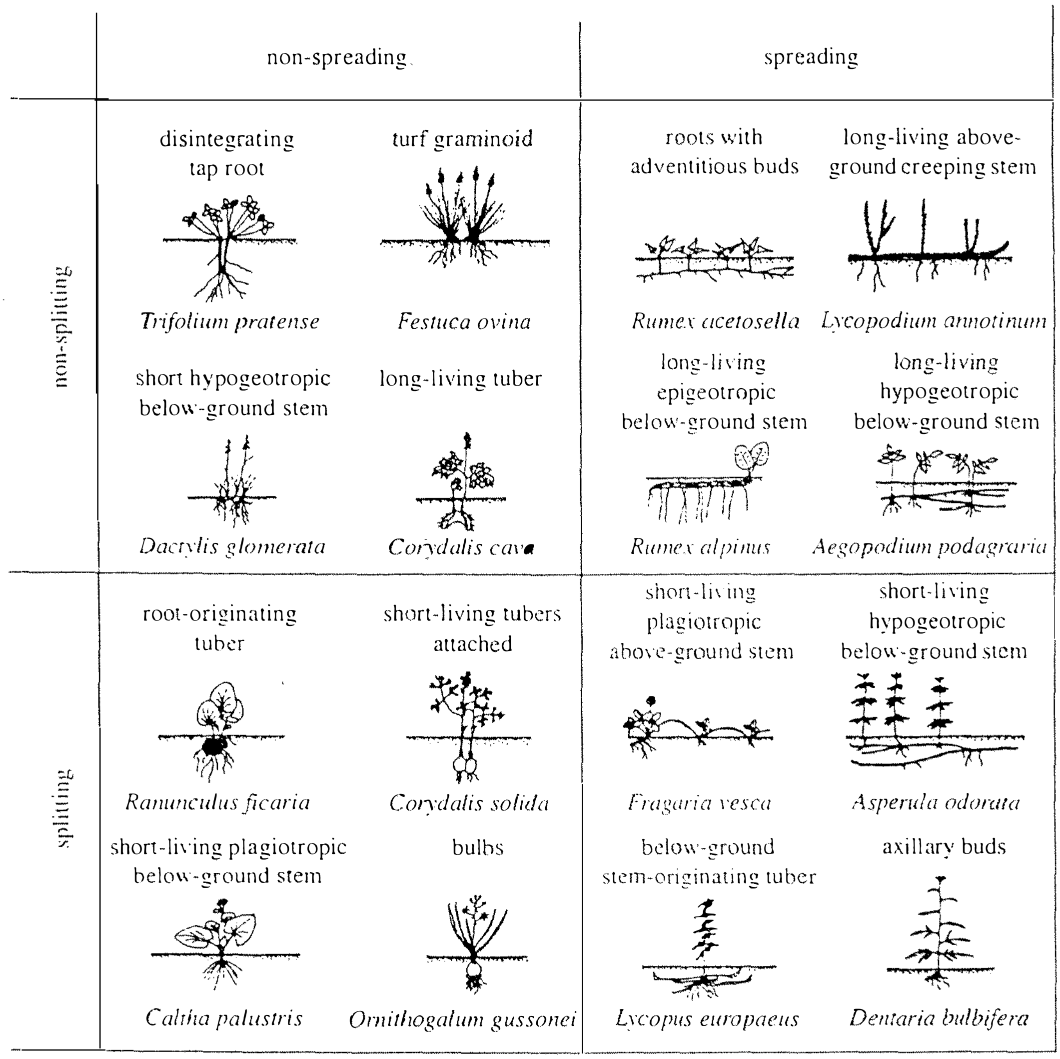

Figure 2.1 Various forms of clonal structures categorised according to their capacity to spread and the longevity of their connection. Origin of clonal structure and examples of species are given for each of the four categories (Van Groenendael et al., 1996)

The modes of vegetative reproduction are many (see Figure 2.1), but the red clover plants within this study primarily fall into one of two categories:

- plants with short lived, above ground stems (cultivars Astred and Gualdo)
- long lived, above ground creeping stems, but still with a central taproot (F2419).

Because there is no published information on the vegetative reproductive habit of Astred, Gualdo or F2419, or any vegetatively reproductive red clover selection, a brief description of the reproductive mechanisms of *Ranunculus repens*, *Fragaria vesca* and *Rubus fruticosus* is useful to review likely mechanisms. For example:

- With *Ranunculus repens*, there is little inter-dependence of the daughter plants along stolons when they are being produced (Ginzo, 1970) and the connecting stolons decay rapidly (within a month), providing full independence to the established daughter plants. Stolons are produced from the end of May (Northern Hemisphere) with the highest number observed by mid-July and very few after. The physical link between the rooted nodes begins to break down from July onwards, with most new ramets fully independent before the end of September (Sarukhan, 1974).

- Strawberry (*Fragaria vesca*) plant regeneration is mainly from long, above ground stolons which allow ramification over large areas. Vigorous clonal spread is frequently observed, but the production of above ground, plantlet producing stolons are highly seasonal. Turnover of the rosettes (parent plants) is relatively slow (Angevine, 1983), and plantlets detached as a result of disturbance may also play a role in the spread of the species (Grime *et al.,* 1986).

- During short days in Autumn, *Rubus fruticosus* (blackberry) stem apices become positively geotrophic (bend to the ground) where they make contact with the soil and produce roots and a resting bud, which later becomes active in the early Spring as the connection from the parent plant degenerates. Seedlings appear to be more vulnerable to competition from established plants than new vegetatively produced offspring (Amor *et al.,* 1980).

2.3.3 Types of temperate perennial forage legumes

Temperate perennial forage legumes can be classified into four major classes, woody subshrubs, taprooted herbs, rhizomatous herbs and stoloniferous herbs (Forde, *et al.,* 1989), and perhaps into three groups with respect to their vegetative reproductive strategies which range from "barely clonal and fully, sexually reproductive" to "fully clonal". These are:

- Those plants that form a deep taproot system and woody crown, and in some instances support growth by the development of adventitious roots from the crown as the taproot degenerates. The main mechanism of reproduction is by seed. This group includes most of the major temperate forages, lucerne (*Medicago sativa* L.), red clover (*Trifolium pratense* L.) and birdsfoot trefoil (*Lotus corniculatus* L.).
- Plants that perennate and spread by the formation of rhizomes (nodal rooting stems), which then degenerate the connection from the parent or from adventitious shoots arising from the root system, all in addition to an initial, sometimes taprooted crown. These include zigzag clover (*Trifolium medium* L.), lotus (*Lotus pedunculatus*), creeping lucerne (*Medicago sativa* L.), red clover (*Trifolium pratense* L.) "Astred", (*Trifolium polymorphum*), and crown vetch (*Coronilia varia* L.). All also reproduce from seed to some extent.
- Plants that perennate by forming clonal patches of rooted stolons which can persist after the original plant has died. In some instances the stolon grows from one end and dies at the other, surviving only from nodal roots, but some plant species form small tap-like roots. This group includes plants such as white clover (*Trifolium repens* L.) and strawberry clover (*Trifolium fragiferum*) which also reproduce from seed.

Barely clonal red clovers rely on the production of adventitious roots from the cotyledonal area of the crown (Montpetit, *et al.,* 1991). This process should not be confused with that of vegetatively reproductive red clovers that form new offspring (plantlets) at stem nodes. As the plant ages, the deterioration of the taproot begins by internal breakdown (Graham *et al.,* 1960; Newton *et al.,* 1960; Cressman, 1967; Spedding *et al.,* 1972; Montpetit *et al.,* 1991), even without the presence of pathogens and grazing (Taylor *et al.,* 1996). Later, saprophytic or weakly pathogenic organisms invade these taproots and unless adventitious roots are strongly developed, which is not usually the case in sown stands (Taylor *et al.,* 1996), plant death occurs. The surviving plants may produce several new "pseudo crowns" around the disintegrated taproot by year three or four (Terekhova, 1956). Crop persistence in 4 year old swards was associated with adventitious rooting types of red clover plants when assessed by measuring the levels of Spring vigour (Montpetit *et al.,* 1991).

2.3.4 Demography of vegetatively reproductive plant populations

The vegetative spread of a species within its community has many genecological implications (Harberd, 1957), with a single genet (originally one seedling) possibly extending its ramets (or plantlets in vegetatively reproductive red clovers) over a considerable area. Perennial species which have the ability to both spread vegetatively and regenerate from seed, often shift away from reproduction by seed under certain management practices, such as continual mowing or frequent grazing (Grime *et al.,* 1986).

The most consistent feature in plants which possess regenerative, vegetative expansion mechanisms, such as stolons, suckers or persistent rhizomes, is the relatively low risk of mortality to the offspring (Sarukhan *et al.,* 1973; Callaghan, 1984). This is achieved through a prolonged attachment to the parent plant and the mobilisation of resources from parent to offspring (Grime *et al.,* 1986; Hutchings *et al.,* 1986). In general, newly produced daughter ramets are strong sinks for assimilates (Hutchings *et al.,* 1986). There is a definite competitive advantage in high disturbance environments (Klimes *et al.,* 1997), (e.g. grazing) where the parent plants capacity to sustain clonal ramets often enables them to out-compete and have a higher probability of survival (Sarukhan *et al.,* 1973; Callaghan, 1984) than offspring which have established from an independent propagule (seed), which is a lengthy and hazardous process. Once the connection from the parent plant declines, risk of mortality to the ramet increases (Sarukhan *et al.,* 1973; Callaghan, 1984). However, ramets from many clonal perennial species exhibit a constant mortality risk through time, whereas seedlings display a declining mortality risk as they age, especially over the first few weeks of life (Harper, 1977; Sarukhan *et al.,* 1973).

However, vegetative offspring are still comparatively small compared to established parents, and are therefore often exposed to more severe and different hazards than those experienced by established plants (Grime *et al.,* 1986). Successful regeneration in many species depends on the exploitation of locally favourable sites (Williams *et al.,* 1965, Grubb, 1977). Vegetative seasonal regeneration, as with vegetatively reproductive red clovers is a mechanism of gap exploitation, and survival is usually limited to those individuals which occur in gaps. Successive regeneration over time depends on the creation of suitable gaps within the sward each year (Grime *et al.,* 1986) at the time when seasonal vegetative reproduction takes place. Clonal reproduction also allows the long-term occupation of a site which is assumed to be an advantage when resources are scarce and need to be monopolised (de Kroon *et al.,* 1990).

When this vegetatively reproductive mechanism is present in a population, the dynamics of the population can be very different to that when sexual reproduction is the only means of parent plant replacement. The fluctuations in net population density of *Ranunculus repens* in grazed swards were small when compared with the numbers of plants which were recruited (as plantlets) and lost from the population (Sarukhan *et al*., 1973), even though vegetative reproduction was seasonal. There was a sequence of gain-dominated and loss-dominated periods during the history of the populations but after two years the percentage survivorship of plants compared to the original population was constant.

2.4 CROWN FORMED PLANTS AND VEGETATIVELY REPRODUCTIVE TYPES

2.4.1 Taprooted legumes compared to clonal plants

The main advantage taproot forming legumes such as *Medicago sativa*, *M. falcata*, *Trifolium hybridum, Hedysarum coronarium, Onobrychis viciifolia, Trifolium pratense*, and *Lotus coniculatus* have over highly clonal plants (clone-formers) such as *Trifolium repens, T. burchellianum, T. africanum, T. semipilosum, T. fragiferum*, and *Lotus pedunculatus*, is the taproot structure itself. It acts as an underground storage organ for water, starch, soluble carbohydrate, and protein (Bowley *et al.,* 1984; Smith *et al.,* 1985; Taylor *et al.,* 1996), and can access moisture at great depth in dry periods (an exceptional 39m for *Medicago sativa*, but more normally 2-4m, Angelini 1979), conferring differing degrees of drought tolerance. In many cases the growing points arise from the cotyledons at or below the soil surface (Forde *et al.,* 1989), and are protected from grazing (Fergus *et al.,* 1960; Thomas, 1980) or other above ground hazards (e.g. fire) to some extent. Adventitious roots are often formed from the cotyledonal/taproot area, and can form the major area of the root system as the plant ages (Fergus *et al.,* 1960; Spedding *et al.,* 1972; Forde *et al.,* 1989; Montpetit *et al.,* 1991).

Persistence of the original plant (genet) is achieved most successfully by deep taprooted crown-formers which monopolise environmental resources in their surrounding neighbourhood with their long lived structures (Snaydon, 1985). The taproot also provides good regrowth capabilities after grazing (Evans, 1973; Bowley *et al.,* 1984; Taylor *et al.,* 1996), and a high degree of cold tolerance in some species (Bula *et al.,* 1954; Therrian *et al.,* 1960; Bowley *et al.,* 1984). These taprooted crown-formers are however “spot bound” and only increase in size slowly by peripheral growth (Forde *et al.,* 1989) which severely limits their ability to inhabit new sites by vegetative means, via the production of new independent plants (ramets or plantlets in vegetatively reproductive red clovers case). Having both a taproot and the ability to spread by the production of ramets allows some taprooted species to spread vegetatively, for example, some selections of vegetatively reproductive red clovers, creeping lucerne, *Lotus corniculatus* and *L. pedunculatus.* Breeding initiatives with *Trifolium repens* for true taprootedness has been in progress for these very reasons (Caradus *et al.,* 1989).

2.4.2 Management and persistence of vegetatively reproductive forage plants

The management of other species of perennial forage legumes and pastures must relate to the growth characteristics of the individual species concerned, which indirectly also governs their persistence, production and suitability for cutting or grazing (Haynes, 1980; Marten, 1985). As the establishment of new plants from seed occurs infrequently in stable, mature swards, mechanisms of perennation via vegetative reproduction are of great importance under such conditions (Snaydon, 1985).

Conventional crown type forage legumes (e.g. *Trifolium pratense* L., *Medicago sativa* L.) almost always rely on the persistence of the originally established plants (Jones *et al.,* 1989). Grazing or cutting management of lucerne cannot prevent the inevitable decline in plant density, but can improve persistence (Leach, 1978). Forage plants that can recruit by vegetative means need to be managed so that asexual recruitment is maximised by timely defoliation at controlled intensity.

Grasslands Maku lotus (*Lotus pedunculatus*) initiates horizontal stems from an underground rootstock comprising a crown and taproot (MacDonald, 1946). These stems are predominantly underground, and for this reason are generally referred to as rhizomes (Barnard, 1969; Levy, 1970). However, such growth can also occur above ground, particularly within dense vegetation, and therefore these stems are sometimes referred to as stolons (Clapham *et al.*, 1962; Barnard, 1969; Healy, 1976) and runners (MacDonald, 1946). Axillary shoot growth can occur at above ground nodal positions (Sheath, 1980a) on these stems which also produce roots (termed secondary crown and taproot) (Sheath, 1980a), and a structure similar to that of a plantlet found in vegetatively reproductive *Trifolium pratense* selections. However, most of these secondary crown structures initiate from a true rhizome (underground stem) and therefore cannot be directly compared to the plantlets of vegetatively reproductive *Trifolium pratense* selections.

Persistence of *Lotus pedunculatus* is directly associated with rhizome growth and spread (Frame *et al.*, 1998) which peaks in Summer and Autumn under New Zealand conditions (Sheath, 1980a). Defoliation in mid-Summer reduces rhizome volume in Autumn (Sheath, 1980a; Sheath, 1980b) while defoliation during early Autumn almost eliminates rhizome growth (Wedderburn *et al.*, 1985). When the stand density of *Lotus pedunculatus* is sparse, avoidance of grazing during late Summer and Autumn enables a better plant density to develop (Frame *et al.*, 1998).

Effective management of creeping lucerne requires maintenance of root reserves. Creeping lucerne vegetatively reproduces by producing adventitious shoots from lateral roots below ground (Murray, 1957), which enables persistence and competition with other species in the sward (Heinrichs, 1954; Clark, 1960). Physiological investigations with cv. Rambler (Daday, 1962) showed short day length and low temperatures were both needed for the expression of the creeping habit. Within populations of creeping-rooted selections, not all plants expressed the creeping habit (Bray, 1970; Busbice *et al.,* 1969; Daday, 1962; Daday, 1968), however those that did had much higher survival rates under close grazing than all conventional control cultivars and non-creeping plants of the same selection (Daday, 1968), with no apparent association found between palatability preference and cultivar persistence. Another selection, cv. Alfagraze, selected for its grazing tolerance, better maintained its plant population at high forage allowances under continuous stocking by maintaining high levels of total non-structural carbohydrates and producing many stems and crown buds (Brummer *et al.,* 1991; Brummer *et al.,* 1992; Bates *et al.,* 1996).

2.5 CONCLUSIONS

Red clover has been widely used in New Zealand due to its nitrogen fixing ability, high yield, excellent nutritive value and adaptability to different soil types, but its persistence past 2, 3 or 4 years in mixed or pure swards continues to be a problem in many farming systems, even with the later flowering types such as Grasslands Pawera. Research on other vegetatively reproductive forages suggests those species with taproots and vegetatively reproductive mechanisms are persistent, but not as productive as those without the means to vegetatively reproduce. Very little information is available on vegetatively reproductive red clover, its growth, production in New Zealand, or its vegetatively reproductive mechanism. Red clover selections that can vegetatively reproduce would change red clover to an open system of reproduction, so that it can replace ageing parent plants and possibly maintain a stable parent plant population.

Some red clover selections capable of this open reproduction or vegetative reproductive mechanism are the focus of the research in this thesis, which will examine the growth, production, persistence, and vegetative, reproductive aspects of this plant type.

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3. THE INCIDENCE OF NODAL ROOTING AND ROOTED PLANTLET SURVIVAL IN SPREADING RED CLOVERS♣

Plate 3.1 4 year old F2419 parent plant in hard grazed hill country pasture.

♣This chapter has been published in *Proceedings of the New Zealand Grassland Association* **58**, 243-246 (Hyslop *et al.*, 1996).

3.1 ABSTRACT

Red clover (*Trifolium pratense* L.) can provide high quality Summer feed but has declined in popularity due to its poor persistency in temperate grazing systems. From 1991 to 1996 the incidence of nodal rooting of prostrate selections of red clover was quantified and the survival of the resulting rooted plantlets was tracked over time, under grazing conditions. Three field experiments were conducted using Astred, Grasslands Pawera, F2419 and Grasslands Turoa selections of red clover. The main experiment comprised three harvests (plants and rooted plantlets dug up) of three red clover selections, in a randomised complete block design with no grazing. The subsidiary rooted plantlet survival experiment followed the life and death of 160 rooted plantlets from the three selections under rotational grazing to 5 cm, over a period of 48 weeks. The numbers of rooted plantlets produced per parent plant of each selection, were 37, 16 and 5 for Astred, F2419 and Turoa, respectively ($P<0.001$). Astred produced significantly larger rooted plantlets ($P<0.001$, mean = 130.6 g/DM/plantlet) over seven months compared to F2419 and Turoa (means = 31.5 and 10 g DM/plantlet, respectively). At week 48 rooted plantlet survival ranged from 84% (Astred) to 27% (Turoa) ($P<0.001$). The experiment at Ballantrae consisted of oversowing 20 kg/ha of two clover selections (Pawera, creeping selection) into sprayed pasture in 1991. Plant density, pasture composition and herbage accumulation was measured until 1995 with all treatments continuously stocked at 16 ewes/ha. Pawera contributed only 1.1% to herbage accumulation during the second year when oversown on hill country and disappeared from the sward soon after.

On average, the creeping selection made up 3.2% of the total sward herbage accumulation from year 2 to year 5. It was concluded that the spreading red clovers represented by Astred and F2419 selections have the potential to be more persistent than crown red clovers under grazing.

Key words: Astred, creeping, nodal rooting, rooted plantlet, spreading red clover, *Trifolium pratense*

3.2 INTRODUCTION

Red clover (*Trifolium pratense* L.) is one of the least persistent clovers in temperate pastures, particularly where grazing pressure is high (Cosgrove *et al.,* 1985), yet it is a valuable source of high quality Summer feed in some farming systems. All red clover cultivars used in New Zealand grow from crowns and predominantly reproduce from seed. The crowns are susceptible to treading damage and fungal infection, particularly in Winter, resulting in poor persistence (Hay *et al.,* 1989). Pure stands of red clover usually only persist 3 to 4 years.

Red clover selections exist that have prostrate stems that produce clonal plants at nodes which may improve persistency in grazed pastures. This red clover type is termed spreading in this chapter. A cultivar of a vegetatively spreading red clover, Astred, was more persistent than cultivars Grasslands Turoa, Grasslands Hamua and Redwest when grazed with sheep over a three year period in Tasmania (Smith *et al.,* 1993). Nevertheless, the perennation and productivity of spreading red clovers requires further evaluation to determine their potential in New Zealand farming systems, and to develop appropriate management strategies.

The objectives of this research were to quantify the incidence of nodal rooting of prostrate selections of red clover (creeping and spreading), and to track the survival of the resulting rooted plantlets over time, under grazing conditions in New Zealand.

3.3 MATERIALS AND METHODS

Three field experiments were conducted using selections of red clover, cv. Astred, cv. Grasslands Turoa and F2419 (breeding material from AgResearch Grasslands). All three red clover selections have a prostrate growth habit and are known to produce plantlets. Experiments on morphological development and rooted plantlet survival were undertaken on the Pasture and Crop Research Unit, Massey University, Palmerston North, New Zealand (latitude 40°23'S) from May 1995 to May 1996. The soil type was a Tokomaru silt loam, with pH 5.7 and Olsen P 17 µg/g soil. The area has an annual rainfall of 995 mm (10 year average) and mean annual soil temperature at 100 mm of 12.8°C (10 year average). The long-term persistency trial was conducted at Ballantrae Hill Country Research Station, 20 km North west of Palmerston North on a 5° west-facing slope, from May 1991 to May 1996. The soil type of this site was a Ngamoka silt loam soil type, pH 5.5 and an Olsen P of 18 µg/g soil.

3.3.1 Morphological experiment

The experiment comprised three red clover selections x three harvests in factorial combination using a randomised complete block design with ten replicates. Blocks were 7 m x 5 m. Seedlings were randomly planted out within blocks in September 1994 and grazed four times to 10 cm over the Spring/Summer period by mature ewes. No grazing took place after February 1995. Parent plants and their surrounding rooted plantlets were dug up when the parent plants were 9, 13 and 16 months of age from sowing (Autumn, late Winter and Summer respectively) and washed by hand. Weeds and white clover between plants were controlled with Buster (200 g/l of glufosinate-ammonium as a water soluble concentrate), applied using a weed wiper at three parts water/one part herbicide.

Rooted plantlets per parent plant, rooted plantlet weight, rooted plantlet root weight, rooted nodes per parent plant, nodal root weight, and parent plant weight (leaf, stem, taproot weight) were measured. All samples were oven-dried at 80°C for dry mass.

3.3.2 Tagged plantlets

This experiment consisted of three selections of red clover compared using a completely randomised design. Seedlings were planted in September 1994. Parent plants were grazed by mature ewes four times to 10 cm over the Spring/Summer period of 1994 and then down to 5 cm in May, October, December, February and May 1995 to 1996 respectively. Fourteen parent plants from each selection (Astred, F2419, Turoa) were randomly chosen and their rooted plantlets tagged with sheep ear tags on wire pegs. Sixty-nine Astred, 48 F2419 and 43 Turoa rooted plantlets were tagged, and rooted plantlet survival was recorded in May, September, December, February and May. Total experimental area was 14 m x 10 m.

3.3.3 Long term persistency trial

There were three treatments (creeping selection, Grasslands Pawera, untreated resident pasture) in a randomised complete block design with four replicates. Plots were 2 m x 2 m. Red clover treatments were sprayed with 2 l Roundup (36% glyphosate) in 300 l water per ha on 2/5/91, and were either oversown with 20 kg/ha of Pawera or a spreading red clover selection including F2419 (hereafter termed the creeping selection). Germination (standard test) was approximately 90%. The experimental area was continuously stocked with 16 ewes/ha.

Red clover seedling density was measured at 37, 72, and 98 days after sowing. Herbage accumulation was measured with a cut-and-trim technique using 0.5 m^2 exclosure cages between 27/8/91 and 22/2/96. Pasture in the cage was cut to approximately 2 cm in height, six times per year, at an average mass of 2800 kg DM/ha above cutting height. Cages were moved after each cut. Botanical composition was determined from subsamples dissected into red clover, white clover and other species.

3.4 RESULTS

3.4.1 Morphological experiment

Total parent plant weight at harvest 1 (15/5/95) was not significantly different among the three selections, with means of 199.4, 148.5, 233.9 g DM/parent plant (s.e.m. ± 38.5) for Astred, F2419 and Turoa respectively. There were no significant differences between selections in the ratio of stem (stolon) to total parent plant weight (Astred 56:44, F2419 65:35, and Turoa 63:37).

Table 3.1 shows the degree of rooting at the stem nodes for the three different selections and whether a rooted plantlet was produced above these nodal roots, over time. There was a significant difference ($P<0.001$) in the number of rooted plantlets produced by each selection, the overall means being 37, 16 and 5 for Astred, F2419 and Turoa, respectively. Rooted nodes per parent plant included rooted plantlets and roots from nodes with no shoots above. Astred produced significantly more ($P<0.001$) rooted nodes than the other two selections. Significantly more rooted nodes ($P<0.001$) were present at the 15/5/95 harvest, (mean = 61) than at any other time.

Astred produced significantly larger rooted plantlets over the assessment period of seven months when compared to the other two selections ($P<0.001$, means of 130.6, 31.5 and 10 gDM/plantlet for Astred, F2419, Turoa, respectively) (see Table 3.2). Astred plantlets were significantly larger ($P< 0.001$) than F2419 and Turoa. There were no differences in the shoot:root ratio across the three selections, but there was a significant difference ($P<0.001$) over the harvest times with means of 12.2:1, 8.7:1, 5.2:1 for Summer, Autumn and Winter respectively (see Table 3.2).

Table 3.1 The number of rooted plantlets and rooted nodes per parent plant at each harvest from 15/5/95 to 11/12/95.

	Rooted plantlets per parent plant			Rooted nodes per parent plant		
Harvests:	May	Sept	Dec	May	Sept	Dec
Astred	43	25	42	107	36	42
F2419	16	14	16	25	40	37
Turoa	5	5	6	50	53	22
s.e.m.		5.1			11.5	

Table 3.2 Total rooted plantlet mass (g) and rooted plantlet shoot:root ratio.

	Total rooted plantlet mass (g)			Rooted plantlet shoot:root ratio		
Harvests	May	Sept	Dec	May	Sept	Dec
Astred	15.9	28.0	348.0	5.8:1	4.6:1	12.4:1
F2419	6.8	10.3	77.4	9.5:1	6.9:1	14.0:1
Turoa	1.6	2.3	26.2	10.8:1	4.0:1	10.2:1
s.e.m.		29.4			1.8	

3.4.2 Tagged rooted plantlets

Table 3.3 shows the percentage of the originally tagged rooted plantlets of the three red clover selections surviving at each assessment, under rotational grazing. At week 15 there were no significant differences across selections, but by week 27 and 37 significantly more Astred rooted plantlets remained than for the other two selections, and by week 48 the percentage of remaining rooted plantlets was greatest for Astred and least for Turoa ($P<0.05$).

Table 3.3 The percentage of tagged rooted plantlets of red clover selections remaining after 48 weeks under rotational grazing by mature ewes.

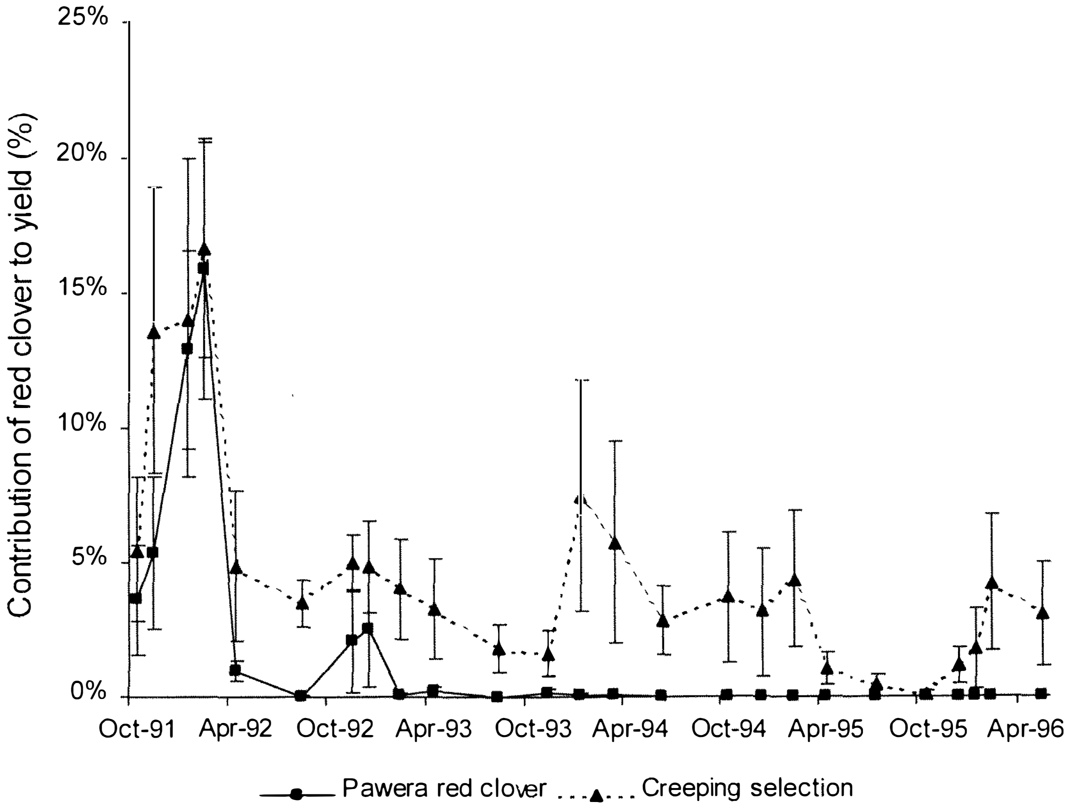
Recordings	Week 1	Week 15	Week 27	Week 37	Week 48
Astred	100	100	90.4	85.0	83.8
F2419	100	86.9	64.6	52.4	52.4
Turoa	100	87.8	62.6	40.1	26.9
s.e.m.			7.9		

3.4.3 Long term persistency trial

There was no significant difference in seedling density, measured over three dates, which averaged 343 and 564 seedlings m^{-2} for Pawera and creeping selection, respectively (mean s.e.m. ±138 seedlings m^{-2}). The contribution of red clover to total herbage accumulation was not significantly different between red clover treatments during the first year (8 and 11% for Pawera and creeping, respectively, see Figure 3.1). However, Pawera contributed only 1.1% to yield during the second year and disappeared from the sward soon after. On average, the creeping selection made up 3.2% of the yield from year 2 to year 5. There was seasonal variation in its contribution which was greatest in late Summer (7.5%) and least in Winter (2.1%).

White clover yield was not significantly different among the three treatments ($P>0.05$), and averaged 9.4, 9.8, and 9.5% of yield over four years for the control, Pawera and creeping selection, respectively. Annual total herbage accumulation of the control treatment (resident pasture) was significantly greater than that of the red clover treatments in year 1 ($P<0.001$) (16,000 vs 12,500 kg DM/ha/yr). Annual total herbage accumulation of the Pawera treatment was significantly greater than for the other two treatments in year 2 ($P<0.05$) (15,000 vs 12,900 kg DM/ha/yr). Annual yield did not vary across treatments in years 3 and 4 ($P>0.05$) (mean = 15 200 kg DM/ha/yr).

Figure 3.1 Contribution to yield (%) for Grasslands Pawera, a crown-type cultivar, and a spreading red clover, "creeping selection" over 5 years of continuous sheep grazing in well-fertilised hill country pasture. Vertical bars represent s.e.m.



3.5 DISCUSSION

All the spreading red clover selections, Astred, F2419, Turoa, and the creeping selection, developed independent rooted plantlets on stem nodes. Astred was the most prolific producer of rooted plantlets and these were larger and more likely to survive than those from F2419 and Turoa. Astred also spread more than the other selections with rooted plantlets up to 900 mm from the parent plant compared with 150 mm for Turoa rooted plantlets. It was observed in the field that vegetatively produced Astred rooted plantlets themselves developed rooted plantlets on their stems after one year.

The similar mass of the parent plants of Astred, F2419 and Turoa and the similar allocation of mass to stem tissue suggested that the growth potential of Astred was not disadvantaged by its abundant rooted plantlet production. A key difference between the selections appeared to be the capacity to produce plantlets at rooted nodes rather than the development of rooted nodes (see Table 3.1). A comparison of the morphological (no grazing) and tagged rooted plantlet (grazing) experiments suggested that grazing affects rooted plantlet survival, and the number of rooted nodes that develop rooted plantlets. Field observations have suggested that Astred parent plants are less persistent than those of some other selections of red clover. A demographic study of the development and lifespan of plantlets, rooted plantlets and parent plants is required.

Turoa has a long history of use in New Zealand and has poor persistency despite being able to develop rooted plantlets (Hay *et al.,* 1989). Turoa rooted plantlets were formed close to the parent plant crown, were small in size and number, short lived and therefore ineffective. Similarly, Smith *et al.,* (1993) showed that under set stocking with sheep in Tasmania, Turoa declined to 5% ground cover after 15 years, whereas Astred retained 55% ground cover.

The creeping selection of red clover used at Ballantrae (see Plate 3.1) was persistent under hill country grazing relative to Pawera. Although the contribution to herbage mass was highly seasonal, the creeping selection, based on F2419, appeared to have established a stable population after year 2. The persistence of the creeping red clover at Ballantrae was not at the expense of white clover in the sward. White clover content in the creeping treatment was similar to that of resident and Pawera treatments, and total legume content was greatest in the creeping treatment.

In conclusion, the Astred and the F2419 selections, owing to their capacity to maintain a plant population by vegetative reproduction, have the potential to be more persistent under grazing than red clovers restricted to growth from a crown. Nevertheless, further research on the performance under grazing of these spreading red clovers in pure and mixed swards is required.

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4. THE MORPHOLOGY AND DEMOGRAPHY OF ASTRED, F2419 AND GUALDO VEGETATIVELY REPRODUCTIVE RED CLOVER SELECTIONS (*Trifolium pratense* L.)

Plate 4.1 18 month old Astred parent plant 2m in diameter.

4.1 ABSTRACT

Some understanding of the demography and morphology of vegetatively reproductive red clovers is required before grazing management recommendations can be made to enhance sward persistency though vegetative plantlet production. Experiment 1 consisted of 15 plants of Astred and 15 of F2419, planted as spaced plants in a completely randomised design with no cutting or grazing, with the objective of quantifying the incidence and positioning of rooted plantlet and stem formation in Astred and F2419 under field conditions. Astred produced 57% of its rooted plantlets on primary stems that grew from the parent plant crown in September, or branches off these stems, whereas F2419 rooted plantlets developed on a wider spread of stem ages. F2419 produced significantly ($P<0.001$) more rooted plantlets than Astred per parent plant with no rooted plantlets produced on stems that grew from the crown after November for both cultivars. Significantly more ($P<0.001$) rooted plantlets formed on quaternary branches at a range of distances (4cm - 55cm) from the parent plant crown. Experiment 2 compared morphological characteristics and vernalisation of Astred and Gualdo seedlings in a completely randomised design with 30 plants of each cultivar. A destructive harvest took place when the plants were 10 months old. Astred produced significantly ($P<0.001$) less rooted plantlets per parent plant, had longer and wider leaves, thicker stems and was taller in height at 10 months of age than Gualdo. There was no significant difference in the number of rooted plantlets formed in Autumn on vernalised parent plants.

Key words: Astred, demography, F2419, Gualdo, morphology, rooted plantlet, *Trifolium pratense*, vernalisation

4.2 INTRODUCTION

Wild ecotypes of vegetatively reproductive red clovers have been growing in the greater Mediterranean area for, perhaps, many centuries but have remained unstudied until the chance discovery of vegetatively reproductive plant material (*Trifolium pratense* L.) by the Department of Agriculture, Tasmania, in seed accessions gathered from Crato, Portugal in 1968 by Dr C. Neale-Smith (Smith *et al.,* 1993). The first commercial cultivar cv. Astred was released from this material in 1992. A second cultivar, Gualdo, was released in 1998 from Italian based material. Other wild accessions of *Trifolium pratense* L. that exhibited vegetative reproduction were collected by Forde and Easton from south west Europe. F2419 came from this material.

A demographic understanding of where and on what stems rooted plantlets occur on the parent plant is required (Jones *et al.,* 1989) so that grazing management recommendations can be developed for vegetatively reproductive types of red clover to ensure the production of successive generations of rooted plantlets. The demographic growth analysis used in Experiment 1 focused on the rooted plantlets as index units (Hobbs *et al.,* 1987) to obtain specific information on rooted plantlet aspects, rather than on the demography of the parent plant and its plantlets.

Smith *et al.,* (1993) reported Astred produced strong stolons (here termed stems) in late Summer and daughter plants (here termed rooted plantlets) from nodes on these stems after flowering. The rooted plantlets usually appeared within 100mm of the parent plant and as these developed roots, the connection to the parent plant degenerated. However, there has been some confusion in the literature (Mirzaie-Nodoushan 1993) between adventitious roots, nodal roots and rooted plantlets arising from these vegetatively reproducing red clovers. Adventitious roots originate from the upper part of the crown or cotyledonal area (Montpetit *et al.,* 1991), and could be confused with nodal roots or roots from rooted plantlets that are very close to the parent plant crown, as a large mass of the three different root types can form.

The morphology of Astred red clover has been described in detail by Smith (1992) under Tasmanian conditions where leaf size was both shorter and narrower compared to Grasslands Hamua, Grasslands Colenso, Redquin and Redwest by 4-6mm and 2mm respectively. No data has been published on Astred's morphological growth habit in New Zealand. It is unclear whether there are distinct morphological differences between different selections of vegetatively reproductive red clovers.

The morphological development of the parent plants and rooted plantlets of Astred, F2419 and Gualdo was examined in two experiments. The objective of Experiment 1 was to quantify the incidence and positioning of rooted plantlets in relation to stem age and type (primary, secondary, tertiary and quaternary), and when stem formation occurred in Astred and F2419 under field conditions. The objective of Experiment 2 was to compare the morphological differences between Astred and Gualdo under New Zealand conditions, and to see if vernalisation had an effect on the number of rooted plantlets formed in Autumn.

4.3 MATERIALS AND METHODS

4.3.1 Experimental sites

Experiment 1 was conducted at the Pasture and Crop Research Unit, Campus block, Massey University, Palmerston North New Zealand (latitude 40°23'S) from 23/5/96 to 20/5/98. The soil type was an Ohakea silt loam, with pH 6.1 and Olsen P 16.7µg/g soil. Experiment 2 was at the Pasture and Crop Research Unit, Frewens block, Massey University, Palmerston North, New Zealand (latitude 40°23'S) from 28/1/98 to 5/5/98. The soil type was a Manawatu silt loam (Hewitt, 1992), with pH 5.7 and Olsen P 17µg/g soil. The area has an annual rainfall of 995 mm (10 year average) and mean soil temperature of 12.8°C at 100 mm (10 year average).

Both sites were cultivated by conventional methods, (plough, roll, power harrow) to form a firm seed bed and 150kg/ha of diammonium phosphate (D.A.P) was broadcast at planting and harrowed in. Both sites had been in Summer crops (barley for Experiment 1 and Pasja for Experiment 2) so no pre cultivation spraying for weed control was required. Seed for each experiment was germinated in the glasshouse and plants grown up in root trainers until eight weeks of age, then transplanted into the field.

4.3.2 Design and management

4.3.2.1 Experiment 1

Fifteen plants of Astred and 15 plants F2419 were transplanted on 23/5/96 at 3 x 3m spacings in a completely randomised design. Strips of weed mat 1.9m wide were pinned to the soil between rows for weed control and were rolled back as the plants grew out close to the mat edges (see Plate 4.1). All other weed control was by push hoe when required. Weed seedlings were all removed by hand as they appeared after the first Summer (1996/97).

4.3.2.2 Experiment 2

Fifteen plants of each cultivar were vernalised over a six week period (total chilling of 235hr) at a night temperature of 4°C from when they were two weeks of age, and each morning returned to the glasshouse for the day. The 15 remaining plants of each cultivar were left in the glasshouse 24hr a day with a minimum night temperature of 14°C. Both vernalisation treatments had the same photo period. All 30 plants (eight weeks of age) of each cultivar (Astred and Gualdo) were transplanted on 28/1/98 at 1.5m spacings within and between rows, in a completely randomised design. Plants were hand irrigated every day for three weeks until established, and all weed control was by push hoe when required. No grazing or cutting took place on either experiment.

4.3.3 Measurements

4.3.3.1 Experiment 1

A different coloured wire was twisted on each new stem that came from the crown (primary stems) at the beginning of each month from July 1996 to February 1997, after which no more new stems grew from crown buds. On 6/6/97, rooted plantlets were counted per parent plant and their position categorised as on a primary (stem from crown), secondary (branch off stem from crown), tertiary (branch off secondary stem) or quaternary branch (branch off tertiary stem) and then traced back to the crown to record what age primary stem (distinguished by coloured wire tag) they initiated from. The distance from the crown of each rooted plantlet was also measured. Where parent plants were large, random sample areas (% area of each plant) were measured instead of the whole plant.

4.3.3.2 Experiment 2

Plant diameter measurements were taken on 19/2/98 by measuring the length of the longest stem from crown to growing tip. All other measurements were taken on 5/5/98 (158 days from sowing) and included plant height, longest stem length, stem diameter, stem number per plant, number of rooted plantlets per plant, number of nodes per 300mm of stem, leaf length, leaf width, taproot mass, total leaf weight, total stem weight, and if plants had flowered. All measurements had one sample taken per parent plant (30 replicates/cultivar) except for stem length, stem diameter, leaf length and leaf width, which had two samples (60 replicates/cultivar). Taproot mass, total leaf weight and total stem weight were obtained by digging and dissecting each whole parent plant. All samples were oven dried at 80°C for 48 hours. Parent plant height measurements were made at the highest point of each plant using a sward stick. Height was recorded when the first contact was made with the plant.

In both experiments rooted plantlets were defined as plantlets that had produced roots that were firmly attached to the soil, but were still attached to their parent plant by a degrading stem and with a growing shoot directly above the nodal roots.

4.3.4 Statistical analysis

Due to the skewed distribution of the rooted plantlet data in Experiment 1, a generalised linear model with a Poisson distribution and log link-function was used to analyse it. Experiment 1 was treated as a completely balanced randomised design, and analysed using SAS/STAT Proc Genmod (SAS/STAT Institute Inc. 1998). Because of the log link-function, the standard errors for the means are symmetric on the log scale, but when transformed back to the original scale they are asymmetric, so the variation is presented as confidence intervals. The production of stems from parent plant crowns was analysed using the General Linear Model GLM procedure of SAS (SAS Institute Inc. 1988) for a balanced completely randomised design as this data set was normally distributed.

All data for Experiment 2 were evaluated by analysis of variance using the General Linear Model GLM procedure of SAS (SAS Institute Inc. 1990) for a balanced completely randomised design.

4.4 RESULTS

4.4.1 Experiment 1

4.4.1.1 Parent plant primary stem production

Astred grew significantly more primary stems in September (P<0.001), October and November (P<0.05) than F2419, which only produced more primary stems (2.7/parent plant, s.e.m. ± 0.37) than Astred's (1.1/parent plant, s.e.m. ± 0.29) in August. The average total number of primary stems produced per parent plant from June to February (after which no more primary stems came from parent plant crowns) was 38.9 (s.e.m. ± 2.6) and 29.6 (s.e.m. ± 2.8) for Astred and F2419, respectively (P<0.05). The main months for primary stem production were September, October and November (see Figure 4.1).

4.4.1.2 Frequency of rooted plantlet production

F2419 produced significantly (P<0.001) more rooted plantlets than Astred per 15 parent plants on stems of every age, and branches off those stems that rooted plantlets were found on. Differences in the number of rooted plantlets produced by stem age, per 15 parent plants, ranged from 26-330 in July to 213-392 in September for Astred and F2419, respectively (see Figure 4.2). No rooted plantlets were produced on stems or branches off those stems, that grew from the crown after November for both Astred and F2419 (see Plate 4.2).

Figure 4.1 Primary stems produced per parent plant from July to February for Astred and F2419 plant types. Vertical bars represent s.e.m. (Expt 1).

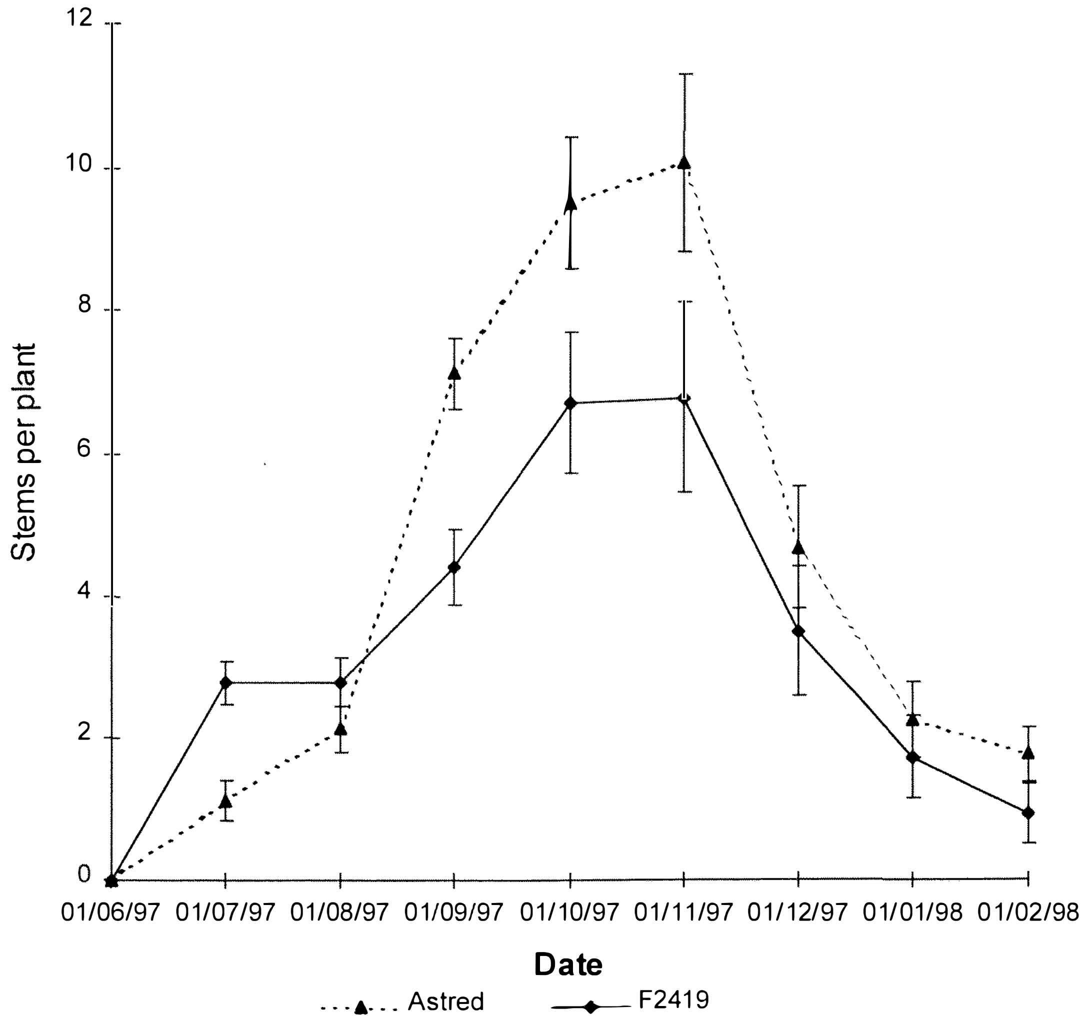
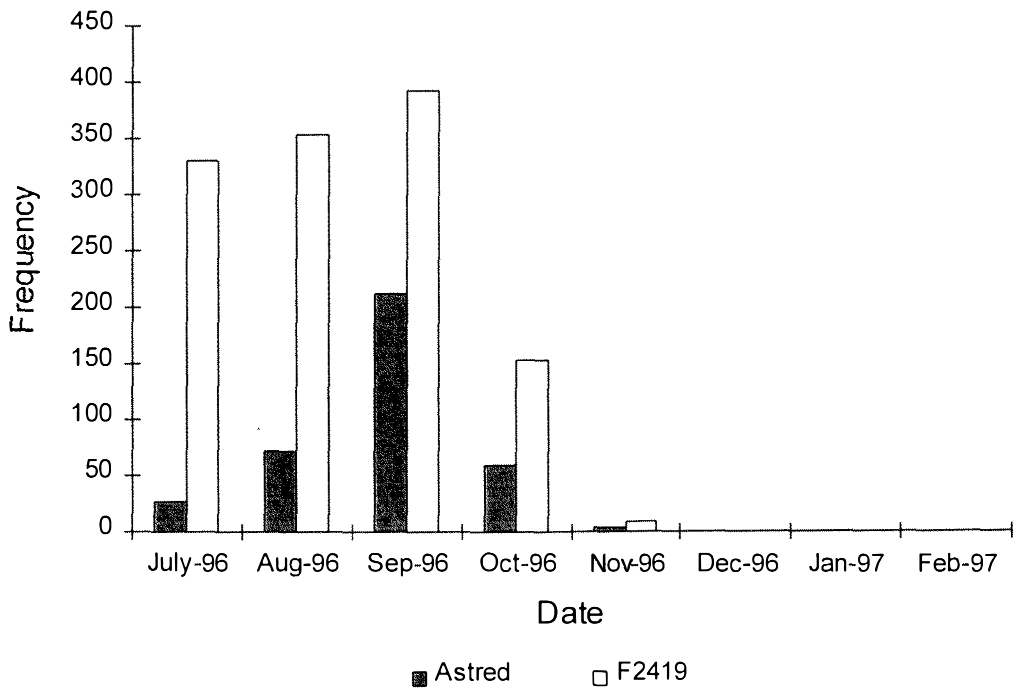


Figure 4.2 Frequency of rooted plantlets produced from 15 parent plants of Astred and 15 plants of F2419 on stems generated from July to February. (Expt 1).



4.4.1.3 Rooted plantlets produced by stem age

Astred produced 57% of its rooted plantlets on primary stems or branches off these stems that grew from the parent plant crown in September, whereas F2419 rooted plantlets mostly came from a wider spread of stem ages: July, August and September (see Table 4.1). F2419 produced significantly ($P<0.001$) more rooted plantlets per parent plant on different stem ages than Astred, but there was no interaction between cultivar and stem age ($P<0.121$).

4.4.1.4 Rooted plantlets produced by branch type

Astred and F2419 produced significantly ($P<0.001$) different numbers of rooted plantlets on all four different branch types. Both cultivars grew the majority ($P<0.001$) of their rooted plantlets on branches off tertiary stems, 40% and 74% for Astred and F2419 respectively (see Table 4.2). There was a significant interaction between cultivar and branch type where rooted plantlets were produced ($P<0.001$).

Plate 4.2 Plantlets beginning to form roots. Note root nodules.

Table 4.1 The number of rooted plantlets produced on different stem ages for Astred and F2419 (rooted plantlets per parent plant). (Expt 1).

Plant selection	Month stems grew from crown	Lower confidence level	Mean	Upper confidence level
Astred	July	0.3	**1.7**	8.2
F2419	July	14.0	**22.0**	34.5
Astred	August	1.8	**4.8**	12.2
F2419	August	15.2	**23.5**	36.4
Astred	September	8.2	**14.2**	24.4
F2419	September	17.2	**26.1**	39.5
Astred	October	1.4	**3.9**	11.0
F2419	October	5.3	**10.2**	19.9
Astred	November	0	**0.2**	19.4
F2419	November	0.03	**0.5**	9.4

95% upper and lower confidence levels

Table 4.2 The number of rooted plantlets produced on different stem branches for Astred and F2419 (rooted plantlets per parent plant). (Expt 1).

Plant selection	Stem order	Lower confidence level	Mean	Upper confidence level
Astred	Primary stems	0.5	**2.0**	7.2
F2419	Primary stems	0.04	**0.5**	6.8
Astred	Secondary stems	1.9	**4.5**	10.6
F2419	Secondary stems	2.2	**5.1**	11.7
Astred	Tertiary stems	4.4	**8.2**	15.5
F2419	Tertiary stems	9.3	**15.1**	24.5
Astred	Quaternary +	5.6	**10.0**	17.7
F2419	Quaternary +	48.6	**61.7**	78.3

95% upper and lower confidence levels

4.4.1.5 Distribution of rooted plantlets by stem type

Figures 4.3 and 4.4 show the distribution of rooted plantlets produced per parent plant, by stem type (either primary, secondary, tertiary or quaternary) on the stem ages where each cultivar produced most rooted plantlets (see Table 4.1). There was a consistent trend for Astred and F2419 to produce high numbers of rooted plantlets from quaternary branch sites. F2419 produced over 75% of rooted plantlets at the quaternary branch site compared to Astred's 45%. There was a high variation (see 95% confidence intervals) in the number of rooted plantlets produced between stem types.

4.4.1.6 Rooted plantlets distance from parent plant crown

Both Astred and F2419 rooted plantlets formed at similar distance extremes from their respective parent plant crowns (average minimum distance 4cm, average maximum distance 55cm). At all primary stem ages, except November, (shown by upper and lower qualities, Figure 4.5) Astred rooted plantlets were formed at greater distances from their parent plant crowns than F2419 rooted plantlets. F2419 rooted plantlets were formed at a greater distance from their parent plant crowns (17.9cm and 29.5 cm for Astred and F2419, respectively) across all stem ages.

Figure 4.3 Distribution of Astred rooted plantlets on different stem sites produced from primary stems that grew in August, September and October. Vertical lines are 95% confidence intervals. (Expt 1).

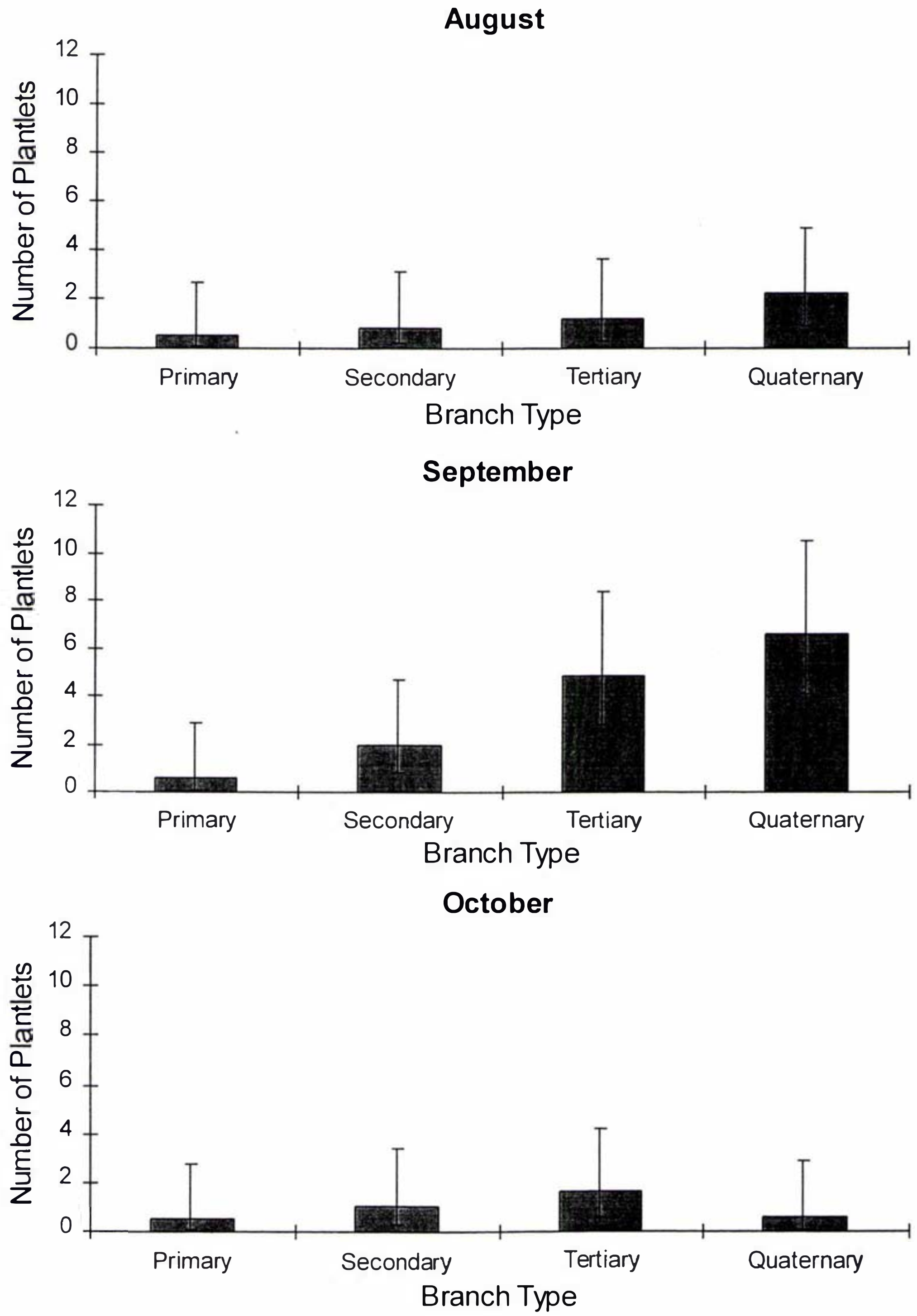


Figure 4.4 Distribution of F2419 rooted plantlets on different stem sites produced from primary stems that grew in July, August and September. Vertical lines are 95% confidence intervals. (Expt 1).

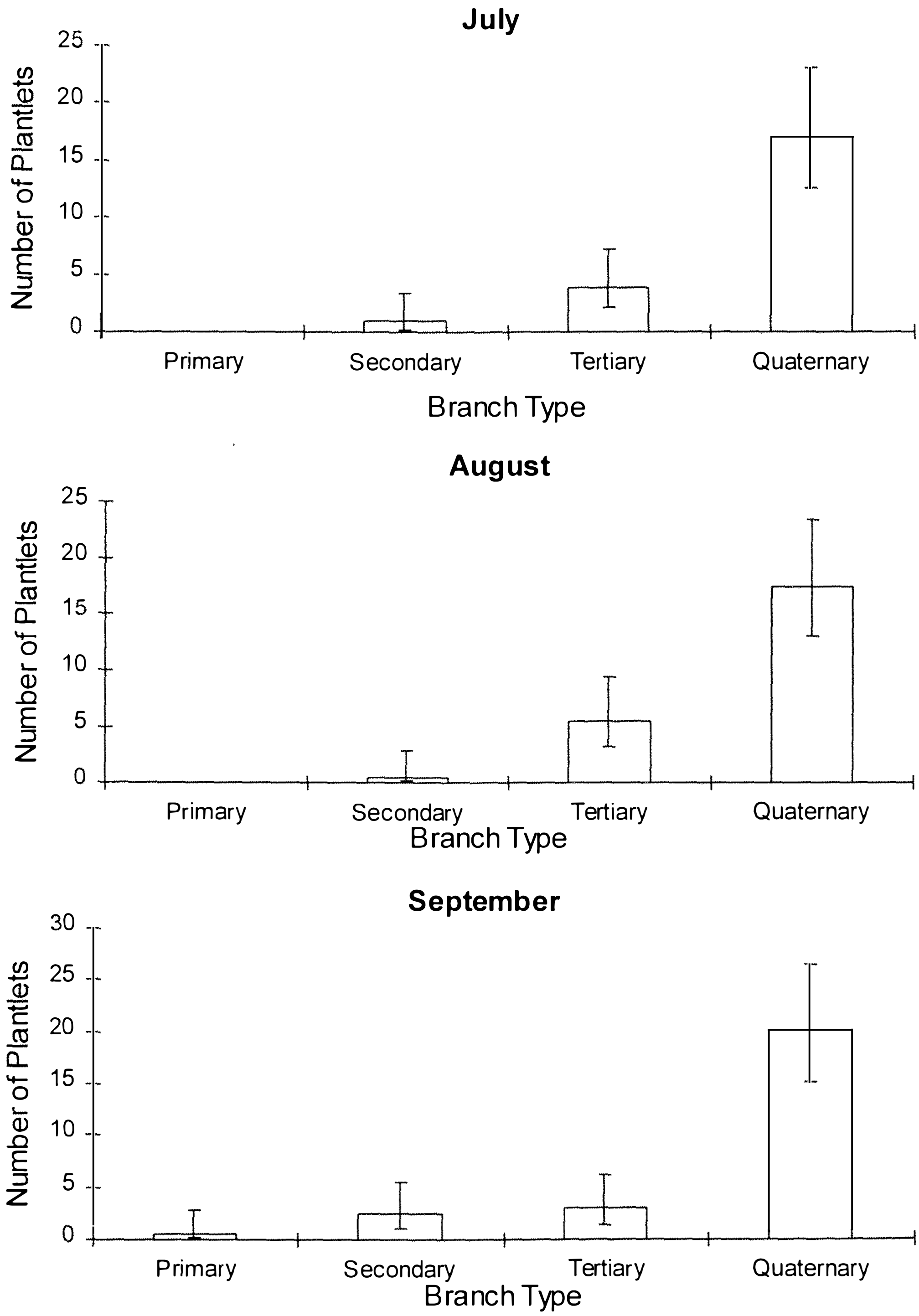
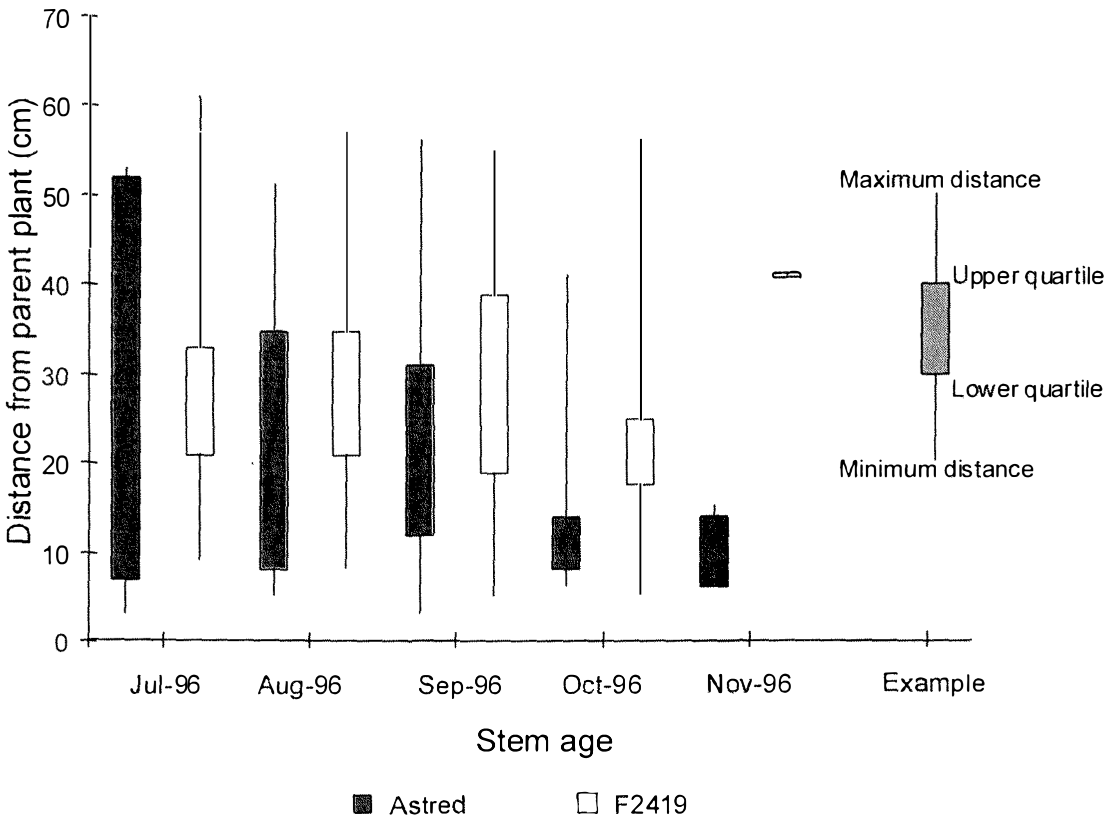


Figure 4.5 The distance of rooted plantlets from their parent plant crown (cm) by the age of stem that rooted plantlets grew on. (Expt 1).



4.4.2 Experiment 2

Astred and Gualdo had similar morphological characteristics, however Astred produced less rooted plantlets per parent plant, had longer and wider leaves, thicker stems and was taller at 10 months of age. There were no other significant differences in the other morphological characteristics measured (see Table 4.3).

When the morphological characteristics (see Table 4.3) were analysed by the vernalisation groupings, there were no significant differences between species or vernalisation treatments for any of the characteristics measured.

Table 4.3 Morphological characteristics of the vegetatively reproductive red clover cultivars Gualdo and Astred after one growing season. (Expt 2).

Character	Astred	Gualdo	s.e.m.	P
Plant height	15.5	12.1	0.59	0.001
Longest stem length 19/2/98	12.4	13	2.19	0.859
Longest stem length 5/5/98	58.9	55	3.49	0.440
Stem diameter	5.1	4.1	0.11	0.001
Stem number per plant	51	54.7	2.89	0.369
Rooted plantlet number per plant	23.5	32	2.87	0.040
Leaf length	46.6	40.4	0.90	0.001
Leaf width	28.6	26.2	0.60	0.008
Taproot mass	22	24.2	1.28	0.245
Total leaf weight	35.4	34	1.41	0.479
Total stem weight	42.4	41.7	1.49	0.748
Total plant weight	254.8	222.3	25.7	0.376
% of plants that flowered	53	33	9.01	0.122
Number of nodes per 1m of stem	39.4	42.5	4.35	0.614

4.5 DISCUSSION

Rooted plantlets of Astred and F2419 were mainly formed on quaternary stems that originated from primary stems produced in July, August and September, but this was under conditions without grazing or cutting. Under grazing, rooted plantlets are formed close to the parent plant (Smith *et al.,* 1993) and except in lax grazed swards, it is unlikely that they are formed on quaternary or tertiary stems. It is more probable that rooted plantlets form on primary or secondary stems due to the higher order stems being removed by the grazing animal. The positioning and dynamics of plantlet and rooted plantlet production under grazing would therefore be different from the findings of Experiment 1 because the sites available for plantlets to form would not be the same. Formation of plantlets is primarily limited by the number of viable nodes (axillary buds) on the stems that are near, or touching the ground. Removal of apical dominance of any stem (primary, secondary, tertiary or quaternary) may induce axillary buds to form into plantlets, or a combination of other factors such as light levels and photoperiod may be responsible (Newton *et al.,* 1990).

Crown type red clovers have been divided into two categories, either early (produce several successions of flowering stems from crown buds) or late (produce a single heavy flush of flowering stems from crown buds) flowering types (Forde *et al.,* 1989). Astred and F2419's main months of primary stem initiation were September, October and November, and both cultivars would fit somewhere between the early and late flowering categories, even though Astred is Winter active compared to Grasslands Pawera (Hyslop *et al.,* 1998). F2419 parent plants and some plants within the Astred population did not follow the standard model of Wintering over as a rosette (Christie *et al.,* 1991), instead Spring and Summer produced primary stems were still alive and elongating in

June. Mean flowering date for one year old, Autumn sown Astred spaced plants was 20th February.

Stems grown after November (until February) did not produce any rooted plantlets and, from field observation, the majority of these stems produced flowers. F2419 produced significantly ($P<0.05$) less primary stems (29.6, s.e.m. ± 2.8 and 38.9, s.e.m. ± 2.6, F2419 and Astred respectively), but grew a dense mat of secondary, tertiary and quaternary branches off the primary stems. F2419 would be more sensitive to a decrease in rooted plantlet production if some primary stems were lost by grazing because less crown buds were initiated as primary stems from each parent plant crown per growing season. However, this mechanism of dormant crown buds has been associated with better parent plant persistence in crown type red clovers (Hay *et al.,* 1989; Frame *et al.,* 1998). The lower order stems (tertiary and quaternary) in both Astred and F2419 produced rooted plantlets at a greater distance from the parent plant crown.

Flowering in crown type red clovers is initiated by long day length, without prior exposure to low temperatures (plants have no vernalisation requirement, Taylor *et al.,* 1996; Aitken, 1964). No difference was found in the number of rooted plantlets produced by Astred or Gualdo after 235hr chilling at 4°C. It is possible that plantlet formation from stem buds is triggered by short periods of exposure to temperatures below 4°C in the Autumn (frosts), but it is unlikely that any long period of chilling is required as the plantlets form on Spring grown stems in the proceeding Autumn and hence there is limited time for this chilling to take place.

Even though there was no difference ($P<0.614$) in the number of nodes per metre of stem between cultivars, the number of rooted plantlets produced from those nodes was significantly ($P<0.040$) different. It is not known why some nodes in vegetatively reproductive red clovers remain dormant while others become activated to produce a plantlet. Comparable studies in white clover on axillary stem buds found two states of dormancy, a combination of apical and environmental factors and a "Spring dormancy" which was temporary and not fully understood (Newton *et al.,* 1990), but thought to be associated with fragmentation of plants (Hay *et al.,* 1988), a sharp decline in stem starch content (Hay *et al.,* 1989) and vigorous root growth (Brock *et al.,* 1988).

Generally there were few differences in morphological characteristics between Astred and Gualdo when grown as spaced plants with no cutting or grazing. However, cultivars should also be sown in pure swards before conclusive comparisons can be made (McWilliam *et al.,* 1970). This similarity in morphological characteristics could be seen as encouraging from the success point of view of this plant type (vegetatively reproductive red clover populations have been maintained over time in different agricultural systems), as Astred and Gualdo originated from wild accessions from opposite sides of the European continent (Portugal and Italy).

An understanding of vegetatively reproductive red clover parent plant growth as a complete unit is required before this plant type can be fully understood. It is clear from this research that vegetatively reproductive red clover plant types are distinctly different from crown type red clover selections, not just in the formation of plantlets and rooted plantlets, but also in primary stem production, primary stem tissue turnover and flowering, when compared to literature descriptions of crown type red clover plants (Fergus *et al.,*1960; Taylor *et al.,*1979; Bowley *et al.,*1984; Smith *et al.,*1985; Taylor *et al.,*1996; Frame *et al.,*1998). Due to the majority of rooted plantlets being found to form on Spring grown stems (5 to 6 months old) in the following Autumn, grazing management will have to be lax over the Spring and Summer periods. This should ensure some stems remain alive to provide possible sites for plantlets to form if vegetatively reproductive red clovers are to maintain a population through clonal propagation.

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5. EFFECTS OF MOISTURE AND FLOWERING ON ROOTED PLANTLET FORMATION IN VEGETATIVELY REPRODUCTIVE RED CLOVER (*Trifolium pratense* L.)

Plate 5.1 Establishing Astred plants in the rainout shelter area. Note buried steel tubes that plants are growing in.

5.1 ABSTRACT

It is not clear what triggers and encourages plantlets to be produced by vegetatively reproductive red clover under different physical and environmental conditions. Two ungrazed field experiments were conducted using transplanted Astred red clover seedlings from September 1997 to May 1998. One experiment investigated what effect dry (dry/wet) or wet (wet/wet) surface soil moisture levels had on rooted plantlet formation over time under a rainout shelter with irrigation, in a completely randomised design with 30 parent plants per treatment. Significantly more ($P<0.001$) rooted plantlets were produced per parent plant under the wet/wet irrigation treatment (22.2% gravimetric surface soil water content) compared to the dry/wet (3.7% gravimetric surface soil water content) treatment imposed from 25/2/98 to 26/3/98. There was no difference in the total number of rooted plantlets produced when the irrigation treatments had been the same for two months. The other experiment examined the effect of removal and non-removal of early developing flower heads on rooted plantlet formation in a completely randomised design with 25 parent plants per treatment at 2 x 2m spacings. There was no significant difference ($P<0.958$) in the number or frequency ($P<0.185$) of rooted plantlets produced per parent plant between treatments. Surface soil moisture was critical for rooted plantlet formation to take place, whereas flowering did not affect the number of rooted plantlets produced.

Key words: flowering, gravimetric surface soil water content, rooted plantlet formation.

5.2 INTRODUCTION

It is clear (see Chapters 3 and 4, Smith 1993; Smith *et al.,* 1993;) that rooted plantlets are produced in varying numbers in vegetatively reproductive red clovers, with differences between selections under the same environmental conditions (see Chapter 3), but there are no literature reports of the number of rooted plantlets produced when different environmental conditions exist. It is not clear what triggers vegetative rooting, whether it is climatic conditions or a combination of physiological and morphological aspects.

For efficient use and production of vegetatively reproductive red clovers, there is a need to understand detailed plantlet and rooted plantlet formation (Hyslop *et al.,* 1998) so that timely and correct grazing/environmental management strategies can be implemented to ensure long term sward persistency. Many selections of vegetatively reproductive red clover have been observed to produce plantlets that do not form roots (see Chapters 3 and 4), but whether this is due to management requires further clarification.

Past observations (see Chapters 3 and 4) have found that large numbers of rooted plantlets form on stems that are in a dense sward environment, and hence have a moist micro climate. Soil surface moisture levels have been reported to be important in the growth of white clover (*Trifolium repens*) nodal roots (Ueno, 1982; Stevenson *et al.,* 1985; Thomas, 1987a), and in the germination of oversown seed and seedling establishment (Campbell, 1968; Campbell *et al.,* 1973; Barley, 1976; Chapman *et al.,* 1985). The reliance on adequate surface soil moisture for rooted plantlet production may contribute to the highly seasonal production of rooted plantlets which has been reported in white clover (Chapman, 1983).

Smith *et al.,* (1993) reported that Astred grew strong stems in late Summer which rooted plantlets later formed on after flowering. This observation suggested that plantlet production may be determined by the relative degree of flowering or connected to flowering in some way by resource allocation within the plant. The removal of seed heads to eliminate apical dominance in Spring sown, crown type red clover promotes the development of strong rosettes and vegetative growth before the onset of Winter through the redistribution of assimilate. Failure to remove heads may result in loss of stands (Smith *et al.,* 1985). As plantlets are formed from lateral stem buds (see Chapter 4), the removal of newly developing flower heads may affect the initiation and development of plantlets on these stems. The removal of white clover flower heads or stem apices stimulated the development of lateral axillary buds and nodal roots (Curll, 1980; Thomas 1987b).

The objectives of this research were to examine the effect of the removal of early developing flower heads on the number of rooted plantlets formed in Astred, a vegetatively reproductive red clover, and to examine whether surface soil moisture levels affect the number and rate of rooted plantlet formation.

5.3 MATERIALS AND METHODS

5.3.1 Experimental sites

Two field experiments were conducted using Astred red clover seedlings from September 1997 to May 1998. Experiment 1 was conducted at the Pasture and Crop Research Unit, Massey University, Palmerston North, New Zealand (latitude 40°23'S). The soil type was an Ohakea silt loam, with pH 6.1 and Olsen P 16.7µg/g soil. Experiment 2 was conducted under a rainout shelter at AgResearch, Palmerston North, New Zealand (latitude 40°23'S). This structure automatically covered the complete experimental area when rainfall started, allowing soil moisture levels to be manipulated (see Plates 5.1 and 5.2). The soil type was a Manawatu silt loam, with pH 6.2 and Olsen P 50µg/g soil. The area has an annual rainfall of 995 mm (10 year average) and mean soil temperature of 12.8°C at 100 mm (10 year average).

5.3.2 Design and management

5.3.2.1 Experiment 1

Seeds were sown in root trainers in the glasshouse on 3/4/97 and transplanted to the field on 9/7/97, with 25 plants randomly placed at 2 x 2 metre spacings for each treatment in a completely randomised design. The planting area was prepared by spraying with 4l/ha of Gallant® (100g/l haloxyfop as an emulsifiable concentrate) and 15 g/ha of Granstar™ (750 g/kg tribenuron methyl as a water dispersible grain) in 200l/ha of water by boom sprayer on 25/6/97 to control grass and broad leaf weeds respectively. 150 kg/ha of diammonium phosphate (D.A.P) was broadcast at planting with 10 kg/ha of Mesurol® (20 g/kg methiocarb) slug and snail bait. Weeds between plants were removed by push hoe but no hoeing took place under the spreading stems of each plant. All plants were irrigated to field capacity using vari-flow drippers, once weekly from 1/1/98 for the remainder of the experiment. No cutting or grazing took place in this experiment.

5.3.2.2 Experiment 2

The area was sprayed with 6 l/ha Roundup (36% glyphosate) in 200l/ha of water on 25/8/97 before rotary hoe cultivation on 9/9/97. Plants were raised as for Experiment 1, and transplanted on 10/9/97 into 250mm diameter x 350mm deep, bottomless, galvanised steel tubes sunk into the soil with 40mm protruding (see Plate 5.1), in a completely randomised design. This ensured the taproots of parent plants could be watered while still maintaining a dry soil surface surrounding the parent plant. 150kg/ha of diammonium phosphate (D.A.P) was broadcast at planting. 30 plants from each treatment (dry/wet or wet/wet) were randomly placed under the rainout shelter at 1.5 x 1.5m spacings, and plants in the wet/wet treatment were surrounded by an impermeable membrane (see Plate 5.2), 1m in diameter, to a depth of 300mm. Water could then be applied to the soil in a 1m diameter circle surrounding the wet/wet treatment parent plants while the dry/wet parent plant surroundings remained dry. The automated rainout cover was turned on from 1/10/97 so the soil around the parent plants had time to dry out before the irrigation treatments began in February 1998.

All parent plant taproots in both treatments were irrigated by drip tubes (0.5mm diameter) with 450ml of water per parent plant, once every day from planting. Alternative watering treatments for areas surrounding the parent plant started on 25/2/98. Micro sprinklers were used to apply 600ml of water per parent plant in the wet/wet treatment surroundings (1m diameter), morning and night, every day. The dry/wet treatment had no water applied into its surrounding areas until 26/3/98, from then on both treatments received 600ml of water per parent plant morning and night, every day until the end of the experiment (10/6/98). Plants were cut with hand shears when they reached 400mm in height, to a residual height of 200mm. Weeds were controlled as for Experiment 1.

Plate 5.2 Wet/wet treatment plants being surrounded by an impermeable membrane

5.3.3 Measurements

5.3.3.1 Experiment 1

Treatment 1 involved removing all the flower heads from each plant at weekly intervals from 19/1/98 to 1/5/98. In treatment 2 the flower heads remained on the plants until the end of the experiment. The flower heads were removed before the florets opened, and counted. A destructive harvest of each parent plant took place on 22/6/98 where rooted plantlets were counted. Plantlets were defined as nodal shoots with roots firmly embedded in the soil.

5.3.3.2 Experiment 2

Rooted plantlets were counted on each parent plant in both treatments every two weeks from 25/2/98 to 10/6/98. A destructive harvest at which each parent plant was dug up was conducted on 10/6/98 and the rooted plantlets counted. Rooted plantlets were defined as in Experiment 1. Samples for gravimetric soil water content were taken every four weeks with a 25mm cork borer to a depth of 30mm and then oven dried for 72 hours at 80°C from 3/3/98 to 10/6/98. Herbage was cut on 13/2/98 and samples were oven dried at 80°C for 48 hours for dry matter determination.

5.3.4 Statistical analysis

Means for all treatments were analysed by analysis of variance using the General Linear Model GLM procedure of SAS (SAS Institute Inc. 1990) for a balanced completely randomised design (see Tables 5.1 and 5.2). The relationship between flowers removed and rooted plantlets produced was analysed by using a regression analysis (SAS Institute Inc. 1988). A Chi-square (χ^2) test was performed on rooted plantlets/parent plant frequency data (see Figure 5.2). A repeated measures analysis was used for rooted plantlets produced over time, and differences based on the Wilks Lambda significance test (see Figure 5.3 and 5.4).

5.4 RESULTS

5.4.1.1 Experiment 1

There was no significant difference ($P<0.958$) in the number of rooted plantlets produced on parent plants that had all flower buds removed before the florets had opened, compared to parent plants that went to full reproductive maturity. The coefficient of variation for the numbers of rooted plantlets produced was a high 140% (see Table 5.1). The number of rooted plantlets produced per parent plant varied from 0 to 48 with flowers removed, and 0 to 19 with no flowers removed. No relationship ($P<0.410$) was found by simple linear regression ($r^2 = 0.0344$) between the number of flowers removed and the number of rooted plantlets produced per parent plant (see Figure 5.1). The frequency of the number of rooted plantlets produced per parent plant under the two treatments was not significantly different ($P<0.185$), see Figure 5.2.

Peak flowering occurred from late February to early March. The overall mean number of flowers removed per parent plant was 1123, with a coefficient of variation of 76.1% (range 122-4298) (see Table 5.2). Coefficients of variation for the number of flowers produced per parent plant were high across all harvest dates (see Table 5.1).

Plate 5.3 A parent plant remaining vegetative when flowers have been removed.

Plate 5.4 Senesced reproductive stems when flower heads were not removed and new regrowth from the crown.

Table 5.1 The number of rooted plantlets produced by Astred red clover over 159 days when flowers were removed or retained. (Expt 1).

Treatment	Flowers removed	Flowers not removed
rooted plantlets per parent plant (mean)	5.72	5.84
s.e.m.	1.62	1.62
Coefficient of variation (%)	140	140
Median	2	5
Mode	0	1

Table 5.2 Flowers removed from parent plants of Astred red clover over 109 days. (Expt 1).

Date	Flowers removed (mean)	Maximum	Minimum	s.e.m.
19/1/98	5.9	25	0	2.0
23/1/98	21.7	68	0	4.4
27/1/98	70.9	251	0	16.2
2/2/98	121.5	725	0	30.8
9/2/98	139.6	544	0	24.7
13/2/98	95.9	315	0	13.8
23/2/98	193.6	817	8	32.1
2/3/98	110.3	527	3	22.9
9/3/98	101.8	464	2	21.2
16/3/98	85	288	0	14.9
24/3/98	62.2	188	0	11.2
31/3/98	41	174	0	9.2
6/4/98	19.8	102	0	5.6
15/4/98	24.5	154	0	8.0
24/4/98	18.1	112	0	5.8
1/5/98	11.6	71	0	3.4

Figure 5.1 The effect of flowers removed on plantlet production. (Expt 1).

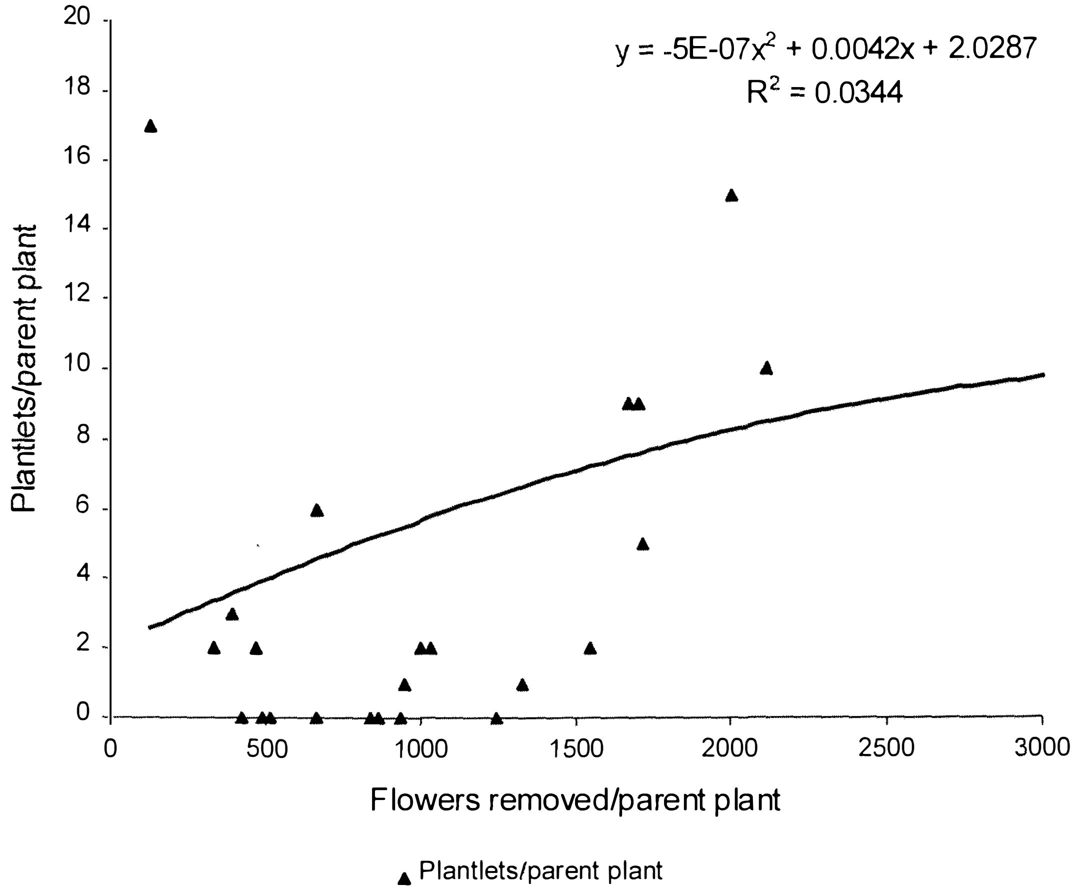
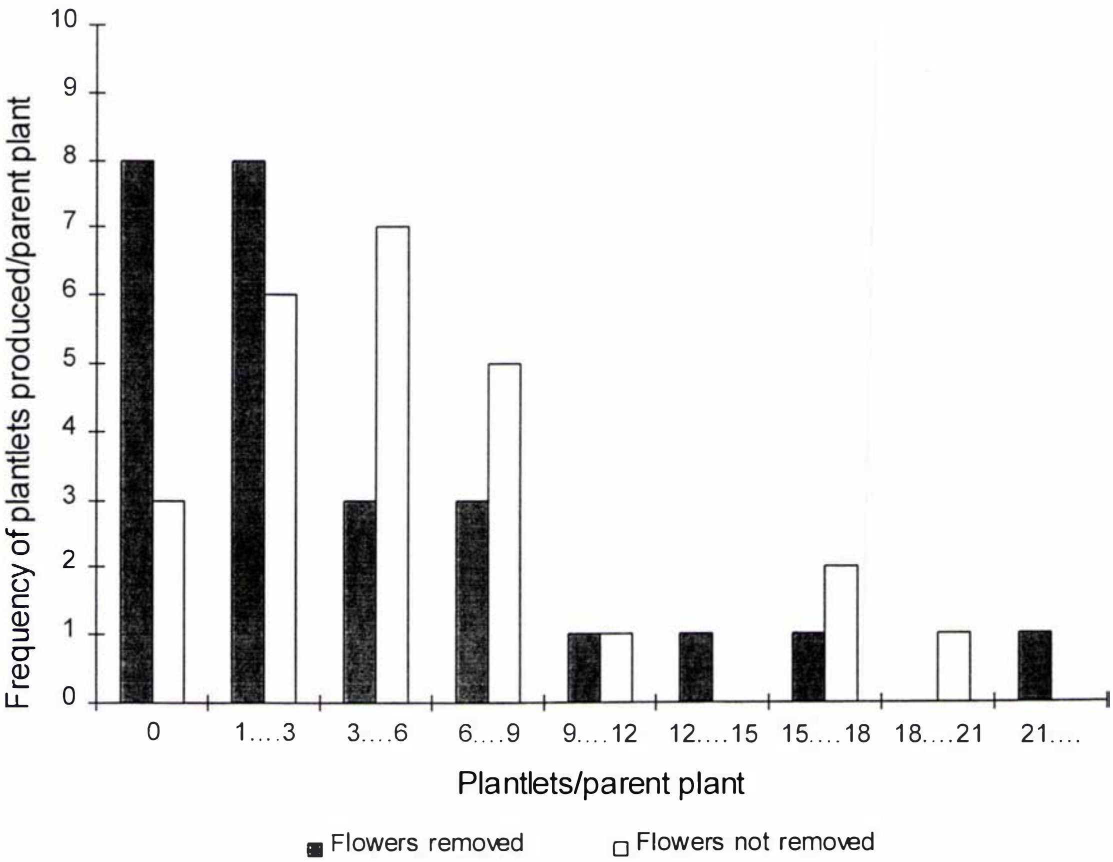


Figure 5.2 Frequency of rooted plantlets produced per parent plant with the removal and non removal of flowers. (Expt 1).



5.4.1.2 Experiment 2

Significantly more (P<0.001) rooted plantlets were produced per parent plant over time in the wet/wet than the dry/wet irrigation treatment (see Figure 5.3). The number of rooted plantlets was significantly lower (P<0.001) in the dry/wet than the wet/wet treatment prior to implementation of surface watering, but there was no difference in the number of rooted plantlets produced when the irrigation treatments were the same after 26/3/98. There was a significant (P<0.001) time and time by irrigation (P<0.008) effect. Rooted plantlets were formed at significantly (P<0.05) greater rates for two weeks in the dry/wet treatment once irrigation in the surrounding areas began on 26/3/98 (see Figure 5.4).

The destructive harvest of parent plants on 10/6/98 showed previous non-destructive rooted plantlet counts per parent plant had under estimated the number of rooted plantlets by 13.5%.

Plate 5.5 Aerially rooted plantlets forming in a moist micro-climate.

Gravimetric soil water contents of the treatments were significantly different (see Figures 5.3 and 5.4) on all dates. The gravimetric soil water content of dry/wet and wet/wet irrigation treatments had a difference of 18.5% (s.e.m. ± 0.53) on 3/3/98. When treatments were irrigated the same (all wet/wet) for one month, there was still a significant difference ($P<0.001$) in gravimetric soil water contents. However the differences were small, (1.2% and 1.9%) for 12/5/98 and 12/6/98, respectively.

Dry matter cut per parent plant within and across treatments was highly variable (coefficient of variation 73.3%) and ranged from 2.7g to 540.5g over the five month period. Dry matter accumulation was significantly different ($P<0.004$) between treatments with 121.6g and 220.0 g (s.e.m. 22.8 g) for dry/wet and wet/wet treatments, respectively.

Figure 5.3 Total rooted plantlets produced over time with dry/wet and wet/wet irrigation treatments. Surface of dry/wet treatment watered from 3/4/98 onwards. Vertical bars represent s.e.m. (Expt 2).

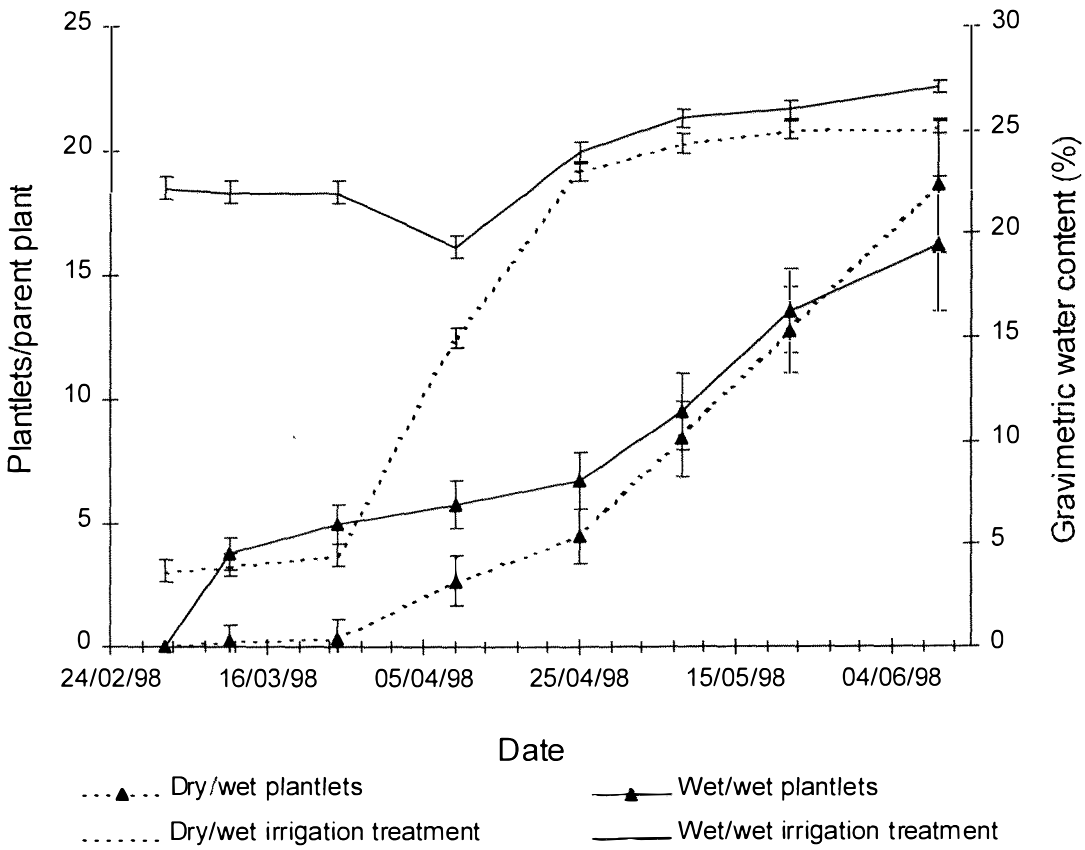
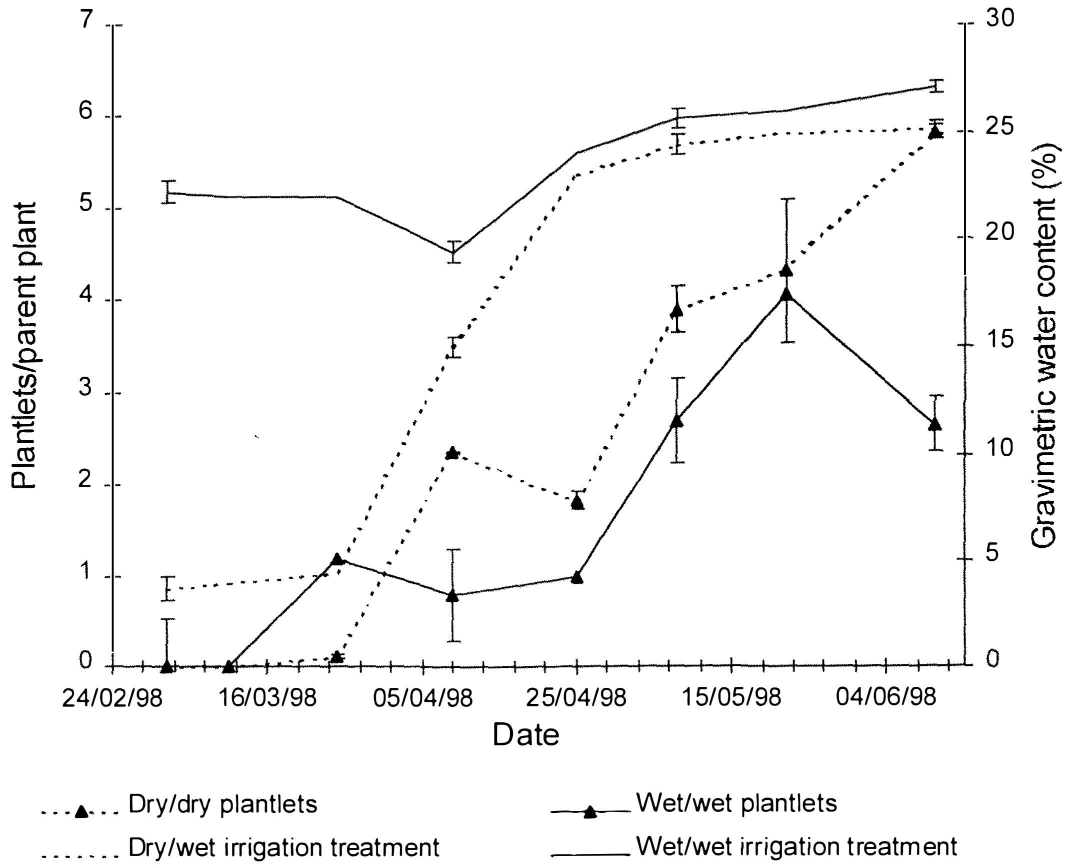


Figure 5.4 Fortnightly change in the number of rooted plantlets produced over time with dry/wet and wet/wet irrigation treatments. Vertical bars represent s.e.m. (Expt 2).



5.5 DISCUSSION

The seasonal production (Autumn/early Winter) of rooted plantlets in vegetatively reproductive red clovers is partly related to morphological aspects of the parent plant (see Chapter 4), but is also influenced by environmental conditions. Surface soil moisture levels are particularly important in the successful formation of a rooted plantlet (see Figure 5.3).

Adequate surface soil moisture was essential for rooted plantlet formation, and under dry conditions there was no nodal rooting (see Figures 5.3 and 5.4). Similar conditions are required for white clover nodal rooting (Ueno, 1982; Thomas, 1984; Stevenson *et al.,* 1985). Plantlets were formed on the lower stems of Astred parent plants growing over dry soil, but did not develop roots until irrigation commenced in the dry/wet treatment. Response to irrigation was immediate, with plantlets producing roots after two days (see Figures 5.3 and 5.4). A high proportion of plantlet roots were initially not in direct contact with the soil surface and had to extend down to contact the soil. A moist micro-climate (Ueno, 1982; Stevenson *et al.,* 1985) within the sward would benefit this process (Hyslop unpub) as plantlet roots grew up to 60mm though the air before reaching the soil (see Plate 5.5).

The degree of flowering played no significant role in the number of rooted plantlets produced per parent plant. There was no relationship between the number of flowers removed and the number of rooted plantlets (see Figure 5.1). Nevertheless, although flower heads were removed before the florets opened, this could have been too late to prevent allocation away from plantlet production. Parent plants may produce more rooted plantlets if always kept vegetative, while still maintaining live mature stems, as found in white clover (Grant *et al.,* 1991; Gooding, 1993). It was not the objective here to maintain completely vegetative red clover parent plants. But the rooted plantlet production by such parent plants would be worthy of investigation.

Some red clover plants senesced all leaf and stem material on stems that terminated in a seed head after seed set (see Plates 5.3 and 5.4), which has also been reported in lucerne (Buxton *et al.*,1985). The lower portion of these stems is where most plantlets are produced (see Chapter 4), hence little or no rooted plantlets were produced on such stems. These parent plants then regrow stems from crown buds (see Plate 5.4) as do crown type red clovers (Fergus *et al.*,1960; Bowley *et al.*, 1984; Frame *et al.*, 1998). Other red clover plants had stems which remained alive even when they went to full seed head maturity, and therefore had a greater chance of producing rooted plantlets (see Figure 5.2).

Even though only one dry/wet period treatment could to be imposed over time in this experiment, it could be concluded from other plantlet counts (see Chapters 3 and 4, and Experiment 1) that the development of plantlet roots can take place at any time within the Autumn or early Winter period, provided adequate surface soil water is available. Plantlets display a compensatory rooting ability once moist conditions exist (see Figures 5.3 and 5.4), but a longer period of wet soil surface would result in more plantlets developing into rooted plantlets before Winter. Early Autumn rainfalls would create this situation.

How long adequate surface soil moisture levels would need to be maintained for plantlets to develop a root system capable of surviving a dry period is unknown. If the first set of roots on a plantlet died from lack of moisture it is not known whether more would be produced when rainfall occurs. Rooted plantlets are still connected to their parent plant for some period, therefore only part of their requirements need to come from the developing nodal root system. A more detailed study is needed to explore this relationship between the rooted, developing plantlet and its parent plant. The formation of the non-rooted plantlet early in the season seems to be more critical to the effectiveness of the vegetative reproduction process, as long as adequate soil moisture is available for plantlets to produce roots sometime in the Autumn and early Winter period.

Part of the variation in the number of rooted plantlets produced in both experiments came from the inherent genetic ability of Astred red clover to vegetatively reproduce. Nodal rooting has a heritability of $h^2 = 0.531$ (Mirzaie-Nodoushan 1993), but the variability within Astred seedlings to express this vegetatively reproductive trait was high (see Figures 5.1 and 5.2). Mirzaie-Nodoushan (1993) reported coefficients of variation of 20.2% in nodal rooting within a selection, which is a function of rooted plantlets produced, as some selections develop nodal roots without a plantlet above.

The under estimation of rooted plantlets by non-destructive counting shown by the destructive harvest of parent plants, illustrated the difficulty in detecting if roots have developed on the base of some plantlets, without risking damage to the young root structures by lifting them from the soil surface. As Autumn progresses, and with moist soil conditions, worm casts can completely bury some of the lower stems, as found by Hay (1983) with white clover, making accurate counting even more difficult. Similar problems were found in counting chicory growing points, with destructive harvesting by digging being the only accurate method (Li 1997).

The dry matter accumulation per parent plant was not an accurate estimation of parent plant size or health. The cut was made 200mm above ground and did not account for variation in the degree of prostrate growth of each parent plant. Cutting height was at 200mm so that the plants were not in contact with the rainout shelter when it moved over the experiment during rain.

Plantlet and rooted plantlet formation are critical to the effective use of vegetatively reproductive red clovers, with moist Autumn/early Winter surface soil conditions being one of the requirements for vegetative reproduction to take place. Flowering status of the parent plant was not one of the factors involved in rooted plantlet production in this study. Other environmental and physiological factors such as temperature (chilling), light levels, non-rooted plantlet initiation, vegetative growth, and the relationship between a rooted plantlet and its parent plant need to be investigated.

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6. HERBAGE PRODUCTION, PERSISTENCE AND MANAGEMENT OF ASTRED RED CLOVER (*Trifolium pratense* L.) COMPARED TO GRASSLANDS PAWERA

6.1 ABSTRACT

Spreading red clovers such as Astred are able to vegetatively reproduce through the production of daughter plantlets at stem nodes as well as though the production of seed. A grazing management experiment compared the herbage production and parent plant persistence of Astred (*Trifolium pratense* L.) to Grasslands Pawera, with the objective of developing grazing management recommendations for swards of Astred red clover. The twelve treatments consisted of two cultivars (Astred, Pawera); two grazing intensities (hard, lax); three grazing frequencies (4, 6 and 8 weeks) over a three year period in a randomised complete block design (48 x 118 m^2 plots) grazed by sheep. There was no difference in the total herbage accumulated between Astred and Pawera over the first growing season, across all grazing treatments, but Astred produced significantly more (extra 2538 kgDM/ha for 8 weeks, hard) herbage at the first Spring grazing. Astred swards had significantly higher ($P<0.001$) percentages of parent plants surviving across all treatments after two seasons grazing. Grazing intensity and frequency affected ($P<0.05$) the number of rooted plantlets produced which ranged from 29 to 66 m^2. The recommended grazing management for pure swards of Astred red clover is to either graze every 4 to 6 weeks, or when pre-grazing height reaches 30cm, whichever occurs latest. A minimum post grazing height of 10cm throughout the whole grazing season is also critical for effective rooted plantlet production.

Key words: Astred, grazing management, rooted plantlets, *Trifolium pratense* L.

6.2 INTRODUCTION

With the release of cultivars Astred in 1992 and Gualdo in 1997, vegetatively reproductive red clovers are becoming commercially available to farmers and will possibly be included in pasture mixtures. These clovers have the ability to vegetatively reproduce through the production of daughter plantlets at stem nodes as well as through the production of seed, (see Chapter 3, Smith, 1992; Smith, 1993; Smith *et al.,* 1993; Baresel *et al.,* 1997), and are aimed at offering better persistence and similar production when compared to red clovers currently available. There has been considerable speculation (Smith *et al.,* 1988) over the means of increasing or maintaining legumes in mixed pastures, and the production of rooted plantlets may be one effective method to do this for red clovers.

Research has mainly concentrated on the persistence of this type of red clover in mixed swards. In New Zealand, Orr *et al.,* (1996) reported Astred was the most prevalent of five introduced legumes (*Adesmia bicolor, Trifolium pratense, Trifolium repens, Trifolium fragiferum* and *Trifolium semipilosum*) on easy slopes, and contributed in excess of 30% to dry matter in Spring. Astred continued to persist under 40 day rotational grazing with sheep over a three year period. In Tasmania, under three years set stocking with sheep grazing down to a 5cm residual, Astred retained 55% ground cover compared to 5%, 2%, 0% for Grasslands Turoa, Grasslands Hamua and Redwest respectively, (Smith *et al.,*1993). Hyslop *et al.,*(1998) concluded the persistency of Astred in mixed swards was directly related to grazing management and its ability to effectively vegetatively reproduce. Astred presented similar persistency problems to Grasslands Pawera under the same management in a mixed sward. Vegetative replacement by Astred mainly occurs during Autumn, (see

Chapter 3), but is related to previous Spring and Summer management (see Chapter 4).

Recommended grazing management for conventional red clover to maintain its productivity and persistence is lenient (5-10cm residual), infrequent grazings of approximately 45 day intervals (Brougham 1959; Hay *et al.,* 1989; Curll *et al.,* 1989; Taylor *et al.,* 1996). When grazing pressure is hard (3-5cm post height) and frequent (every 30 days or less) parent plant density decreases leading to total stand loss by year 2,3 or 4, (Brougham 1959; Brougham 1960; Harris *et al.,* 1980; Cosgrove *et al.,* 1985; Sheath *et al.,* 1989; Frame 1990; Hume *et al.,* 1995).

In a mixed sward, grazing management decisions often relate more to the other sward components than to red clover (Brougham 1959; Curll *et al.,* 1989) compromising known positive grazing management policies for red clover, particularly in the late Autumn and Winter, (Hay *et al.,* 1989). For this reason it would be useful to grow vegetatively reproductive red clover as a pure sward so it could be managed for maximum persistency and yield. It could then be considered a truly perennial, seasonal forage crop, capable of producing high quality dry matter in early Spring and Summer (Hyslop *et al.,* 1998).

There has been insufficient information published to determine the effects of grazing management on vegetatively reproductive red clovers (see Chapter 3). A field experiment was established with the objective of providing information on vegetatively reproductive red clover parent plant persistence, rooted plantlet formation, yield and seasonal growth, when sown and grazed as a pure sward and compared to a crown type red clover, Grasslands Pawera.

6.3 MATERIALS AND METHODS

6.3.1 Experimental site

The experiment was conducted at the Pasture and Crop Research Unit, Massey University, Palmerston North, New Zealand (latitude 40°23'S) from September 1996 to May 1998 (see Plate 6.1). The soil type was a Manawatu silt loam (Hewitt, 1992), with pH 5.7 and Olsen P 17µg/gsoil. The area has an annual rainfall of 995 mm (10 year average) and mean soil temperature of 12.8°C at 100 mm (10 year average).

Seed was sown on 8/3/96 at 5.4kg/ha and 13.3kg/ha for Astred and Pawera (coated) respectively, to ensure 200 seeds were placed per square metre (germination 96% and 97%) using a V ring roller drill into a conventionally cultivated seed bed. 150kg/ha of diammonium phosphate (D.A.P) was broadcast at sowing and harrowed in with 3l/ha of Treflan® (400g/l trifluralin in the form of an emulsifiable concentrate) in 300l/ha of water for pre emergent weed control. 4l/ha of MCPB (385g/l MCPB as the sodium salt in the form of a soluble concentrate) was applied by boom sprayer on 18/4/96 in 200l/ha of water for selective control of broad leaf weeds and 4l/ha of GallantNF® (100g/l haloxyfop as an emulsifiable concentrate) in 200l/ha of water for control of grasses.

6.3.2 Design and Grazing management

All grazing was by mature Romney ewes at a stocking density equivalent to 1300-1700 sheep/ha for 1 or 2 days per plot (118m^2). Two grazing intensities, hard and lax, (post grazing heights of 5cm and 10cm respectively) and three grazing frequencies (4, 6 and 8 week intervals) were applied over both cultivars, Astred and Pawera to a randomised complete block design with four blocks. This equated to nine, 4 week; six, 6 week; and five, 8 week grazings over the 34 week season. Grazing started on 16/9/96 so that all grazing frequencies coincided on 24/2/97 which left "spelling" periods of 4, 6, and 8 weeks for plantlet production from 1/3/97 onwards. All plots were grazed on 5/5/97 to even out sward post grazing heights before Winter. Sheep were removed from each plot as the desired post grazing height was reached.

Plate 6.1 Sheep grazing the experimental site at the Pasture and Crop Research Unit, Massey University.

6.3.3 Measurements

Pre and post grazed herbage masses were measured by cutting two randomly allocated replicates from each plot to ground level using a 0.25m^{-2} quadrat. Botanical composition measurements were obtained by dissecting one of these replicates into red clover and other sward components. All samples were oven dried at 80°C for 48 hours.

Twenty sward height measurements were randomly taken per plot using a sward stick at both pre and post grazings. Height was recorded when the first contact was made with the sward canopy.

Plant establishment counts were taken every two weeks from sowing for six weeks using a $0.5m^2$ quadrat, with two samples taken per plot. Parent plant densities were measured every two months from 5/12/96 to 12/5/98, by counting parent plant crowns in three samples per plot using a $0.25m^2$ quadrat after grazing. Rooted plantlets (which only occur in Astred) were counted as parent plant crowns only when they became detached from their parent. Rooted plantlets were counted before grazing in two fixed $0.25m^2$ quadrats per plot on 23/3/97, 21/4/97 and 10/6/97.

6.3.4 Statistical analysis

All data presented were evaluated by analysis of variance using the General Linear Model GLM procedure of SAS (SAS Institute Inc. 1990) for a balanced randomised complete block design.

6.4 RESULTS

6.4.1 Parent plant persistence

Astred swards had significantly higher ($P<0.001$) percentages of parent plants remaining across all treatments after two seasons grazing compared to Pawera (see Table 6.1). By year 3, there were significant differences in the percentages of parent plants remaining between grazing intensities ($P<0.001$), except for Pawera 8 weeks and Astred 6 weeks. Grazing frequency was also significant ($P<0.05$) by this date across both cultivars. There was a marginal interaction ($P<0.06$) between grazing intensity and frequency (see Table 6.1). The greatest parent plant death was over Winter and early Spring, particularly in the hard grazed, 4 week treatments of both cultivars.

6.4.2 Total herbage accumulation

There was no difference in the total herbage accumulated by Astred and Pawera over the first growing season across all grazing treatments (see Table 6.2). No significant differences resulted between grazing intensities or grazing frequencies, nor were there significant interactions between cultivar, grazing frequency and grazing intensity ($P<0.067$). However, there was a difference of 3361 kgDM/ha between the highest producing treatment (Astred, 6 weeks, lax) (see Plate 6.2) and the lowest producing treatment (Astred, 8 weeks, lax).

Table 6.1 Persistence of parent plants (%) over time under different grazing intensities and frequencies.

Cultivar	Astred						
Grazing frequency	4 weeks		6 weeks		8 weeks		
Grazing intensity	hard	lax	hard	lax	hard	lax	s.e.m.
Date							
5/12/96	100	100	100	100	100	100	
15/6/97	68	78	78	90	69	87	4.55
10/1/98	51	77	69	73	65	76	6.26
12/5/98	41	70	64	65	51	69	4.52
2/11/98	26	59	52	58	47	66	6.32

Cultivar	Pawera						
Grazing frequency	4 weeks		6 weeks		8 weeks		
Grazing intensity	hard	lax	hard	lax	hard	lax	s.e.m.
Date							
5/12/96	100	100	100	100	100	100	
15/6/97	83	86	90	84	86	77	4.55
10/1/98	67	57	74	64	61	53	6.26
12/5/98	52	57	66	59	52	47	4.52
2/11/98	13	48	30	43	39	34	6.32

Table 6.2 Total seasonal herbage accumulation (kg DM/ha) under different grazing intensities and frequencies.

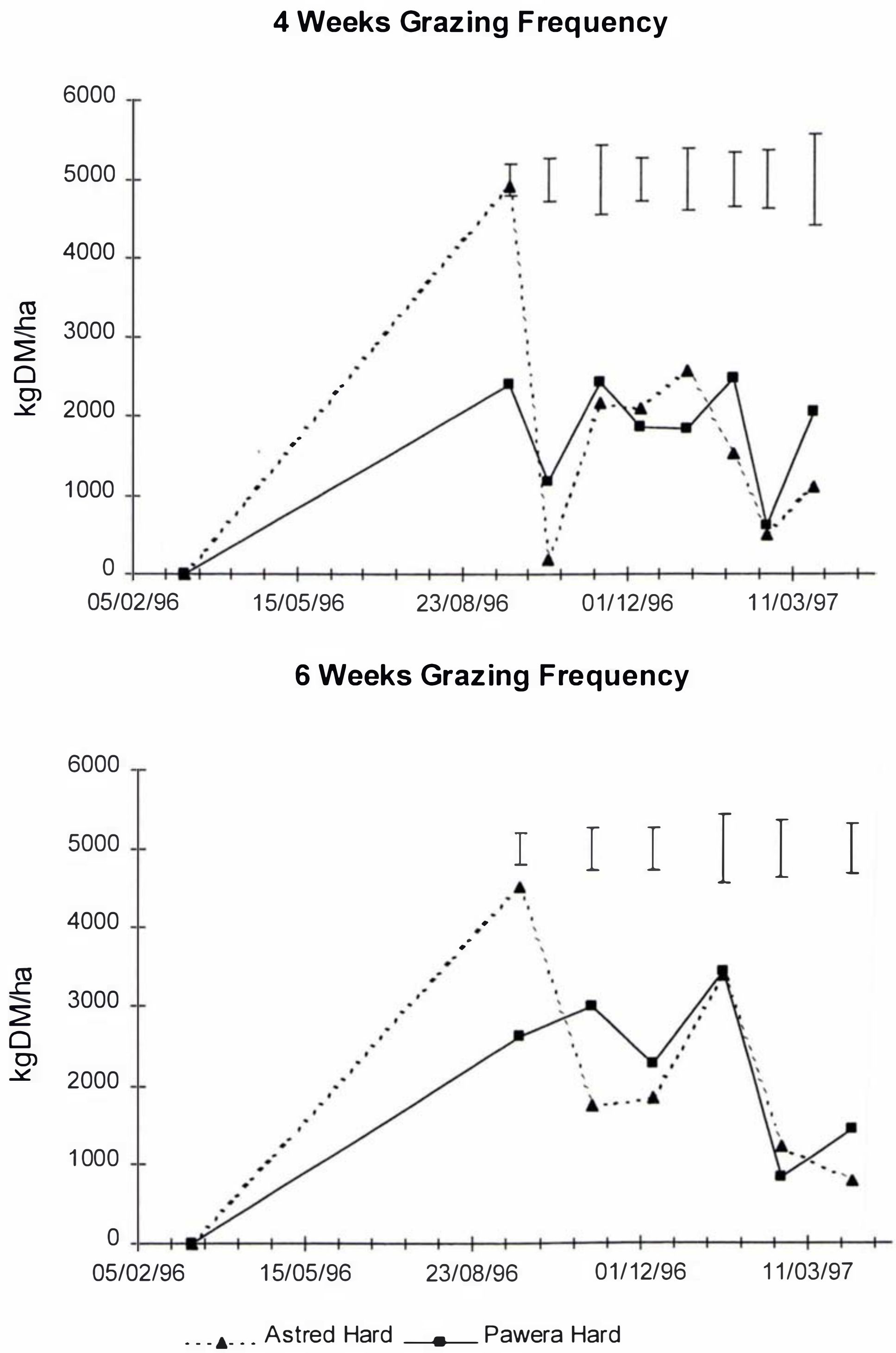
Grazing frequency	4 weeks		6 weeks		8 weeks		
Grazing intensity	hard	lax	hard	lax	hard	lax	s.e.m.
Astred	15027	14038	13592	15358	14782	11997	722.6
Pawera	14847	14482	13624	13214	14383	14353	722.6

Plate 6.2 Sheep grazing a six week, lax grazed Astred sward.

6.4.3 Seasonal herbage accumulation

All Astred grazing treatments accumulated significantly ($P<0.001$) more herbage from sowing to the first Spring grazing on 20/9/96 when compared to Pawera (see Figure 6.1). The differences ranged from an extra 2538 kgDM/ha for Astred, 8 weeks, hard treatment, to 1914 kgDM/ha for Astred, 6 weeks, hard grazed treatment. There were no other significant differences between species at all other harvests, and in all treatments, except for Pawera yielding more than Astred on 14/10/96 with 4 week hard and lax grazing, on 3/11/96 with 6 week hard and lax grazing, and on 14/11/96 with 8 weeks hard and lax grazing. These differences were partly due to Pawera 4, 6 and 8 week, lax grazed plots not being grazed on 20/9/96 because they were under the minimum lax grazing height of 10cm.

Figure 6.1 Seasonal herbage accumulation of Astred and Pawera under 4 week and 6 week grazing frequencies to 5cm residual. Vertical bars represent s.e.m.



6.4.4 Pre and Post grazing heights

Pre grazing sward heights varied over the season due to changes in plant growth rates between grazings, and peaked for both cultivars in January 1997 (see Figure 6.2). Significant differences ($P<0.001$) in height resulted from hard (5cm residual) and lax (10cm residual) grazing intensities across all treatments on all harvest dates. Pawera grew significantly ($P<0.001$) taller than Astred at each harvest from 3/11/96 to 20/1/97. However, for the first harvest on 20/9/96, Astred was on average three times the height ($P<0.001$) of Pawera. All post grazing harvest heights had significant differences in grazing intensity ($P<0.001$) at all harvest dates, across all treatments. No other effects were significant.

6.4.5 Pre grazing weed botanicals

In Spring (grazings from 20/9/96 to 9/12/96) there were differences in the amount of weeds present in Astred ($P<0.001$) and Pawera ($P<0.05$) swards, but thereafter weed content was similar in both (see Figure 6.3). Grazing frequency and grazing intensity had a marked effect on the amount of weeds that established and grew in each treatment. Eight weeks grazing frequency had significantly ($P<0.001$) less weeds than the 4 week grazing rotation at all harvests that coincided. Lax grazing intensity also decreased weed content significantly ($P<0.05$) in all treatments after 14/11/96 harvest, except the 4 week grazing frequency.

Figure 6.2 Pre grazing heights of Astred and Pawera swards under 4 week, 6 week and 8 week grazing frequencies and lax (10cm residual) and hard (5cm residual) grazing intensities. Vertical bars represent s.e.m.

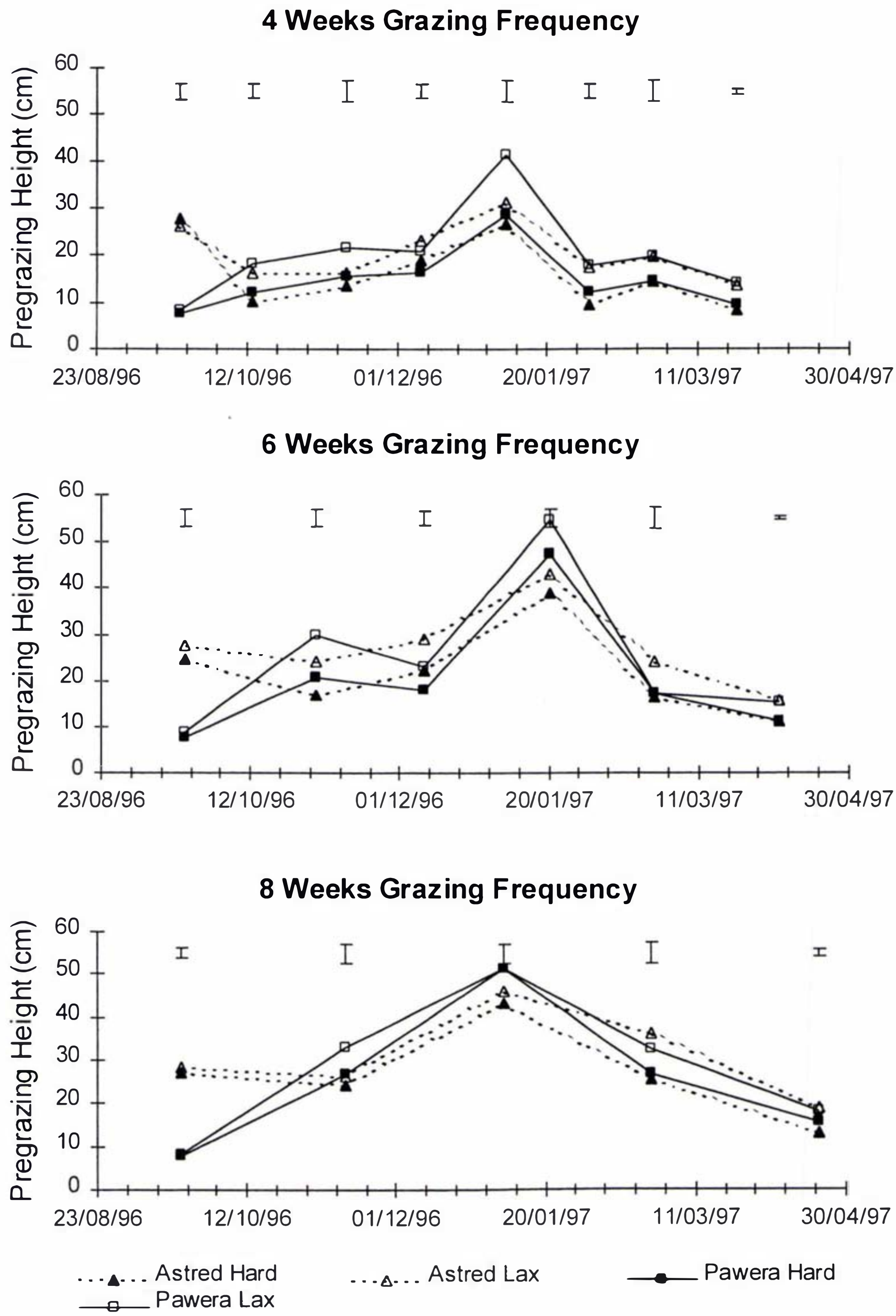
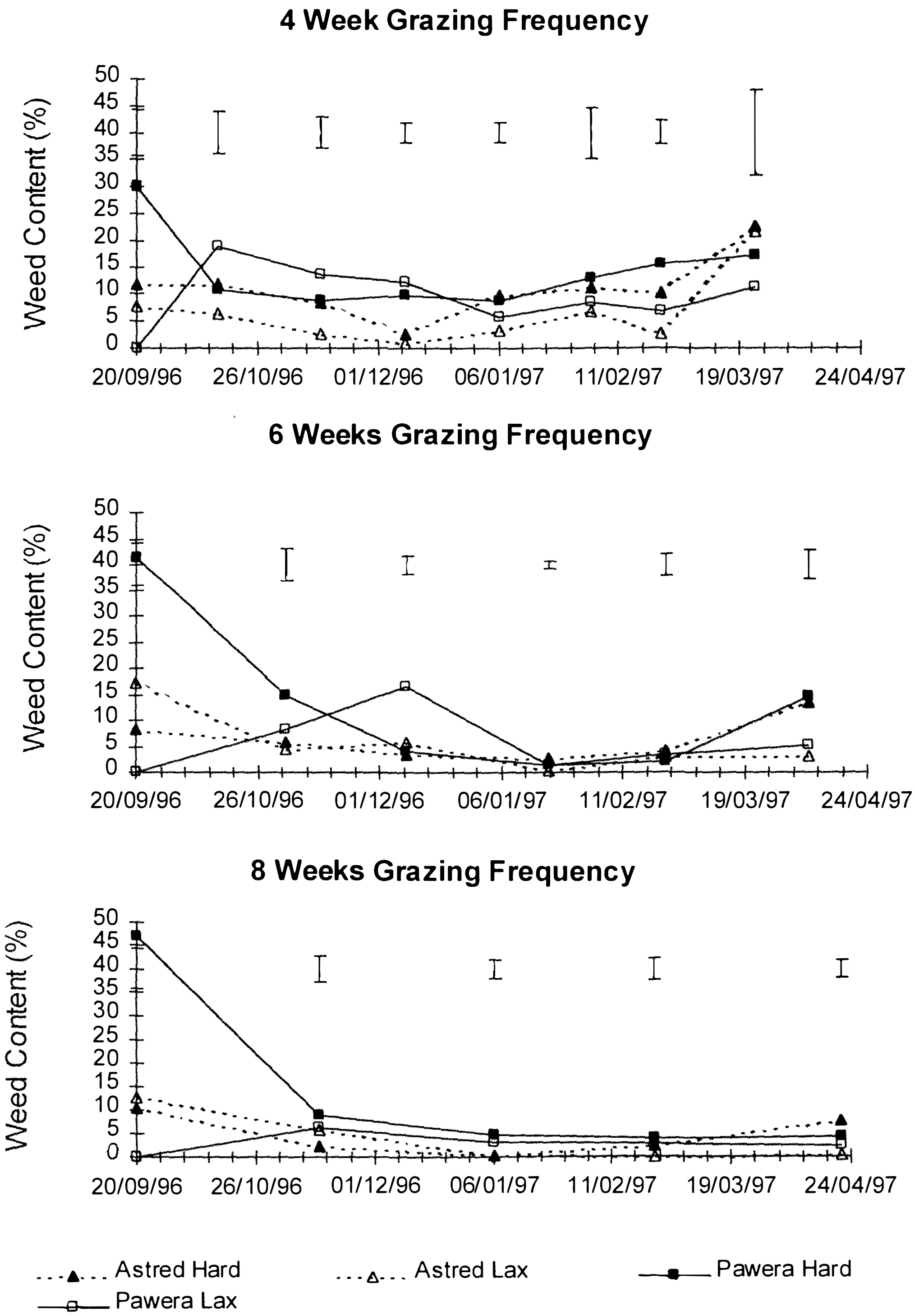


Figure 6.3 Pre grazing weed content (%) in Astred and Pawera swards under 4 week, 6 week and 8 week grazing frequencies and lax (10cm residual) and hard (5cm residual) grazing intensities. Vertical bars represent s.e.m.



6.4.6 Rooted plantlet counts

There was a significant ($P<0.05$) interaction between grazing frequency and grazing intensity for the number of rooted plantlets formed (see Table 6.3). The Astred 6 weeks, hard produced the least rooted plantlets, with 44% of the plantlets produced by Astred, 8 weeks lax and 81% of Astred 8 weeks hard. Grazing intensity at 4 weeks, hard did not affect the number of rooted plantlets produced relative to 4 weeks, lax grazed plots. However, this was not the case for 6 and 8 week grazing frequencies, where more rooted plantlets were produced when the grazing intensity was lax. The least number of rooted plantlets were produced from grazing hard (5cm post grazing height) every 6 weeks. Note, Pawera had no plantlets.

Table 6.3 Rooted plantlets (number m^{-2}) produced by Astred under different grazing intensities and frequencies.

Cultivar	Astred						Prob>F
Grazing frequency	4 weeks		6 weeks		8 weeks		0.0641
Grazing intensity	hard	lax	Hard	lax	hard	lax	0.0025
Date							s.e.m..
10/06/97	43	42	29	46	36	66	5.1

6.5 DISCUSSION

Regardless of grazing management, there is an inevitable decline in parent plant density over time in red clover stands (Fergus *et al.,* 1960; Taylor *et al.,* 1977; Bowley *et al.,* 1988), therefore it is important to promote the formation and survival of rooted plantlets to counteract this in vegetatively reproductive red clover cultivars (Hyslop *et al.,* 1998). All grazing management conclusions are based on maximising parent plant and rooted plantlet numbers, not herbage accumulation, however total annual herbage accumulation between treatments was not found to be different (see Table 6.2).

It is clear from other studies with taprooted forage plants that sward persistence is decreased by intensive grazing pressure and increased grazing frequency (Brougham 1959; Brougham 1960; Harris *et al.,* 1980; Cosgrove *et al.,* 1985; Sheath *et al.,* 1989; Frame 1990; Hume *et al.,* 1995). Parent plants per square metre declined over time in all Astred and Pawera grazing management treatments (see Table 6.1), but the rate of decline was less for Astred swards, particularly in the third Winter (1998). This greater plant density of Astred could have been due to the addition of rooted plantlets to the Astred swards each Autumn. It may take time for parent plant populations to reach an equilibrium under grazing conditions from the time of sowing. This lag time until equilibrium parent plant numbers are reached, occurs in lucerne (Tesar 1979), crown type red clovers (Westoby 1984) and many other grazed plant species. Further research is being conducted on the population dynamics of Astred under grazing.

The grazing management treatments imposed were based on set time periods and intensities, rather than morphological changes of the parent plants, as plantlets were formed and became rooted plantlets. The rotations were calculated back from 1/3/97 when rooted plantlets were known to start to form (see Chapter 3), so that "spelling" periods from grazing of 4, 6 and 8 weeks were set up to allow rooted plantlets establishment. These periods of "spelling" may be better imposed when the right environmental conditions exist rather than by a date basis (see Chapter 5). Improved management decisions for the increased production of rooted plantlets may also be better based on parent plant morphology, as found with lucerne management (Bibbey, 1960; Leach, 1970; Singh *et al.,* 1974; Johnson, 1984), now that knowledge of where and what affects rooted plantlet formation has improved (see Chapters 3,4 and 5).

The percentages of parent plants remaining in the Astred swards after three years of grazing are not only a function of the grazing treatments imposed, but also a function of rooted plantlet production and survival. Because rooted plantlets were not counted as parent plants until the first Spring grazing in the next season (one year from when they were first formed), parent plant numbers recorded in the Autumn and Winter did not reflect any new vegetative replacement that had taken place.

Rooted plantlets are predominately produced on stems that grow in the preceding Spring (see Chapter 4), which supports the finding of less rooted plantlets being produced under the hard grazing (5cm residual) treatments (see Table 6.3). However, even under hard grazing when only 5-10cm of stem remained, rooted plantlets were still able to be produced (29-43m^{-2}), but they were very close to the parent crown, smaller in size, and less likely to reach maturity (see Chapter 3) than rooted plantlets produced at a greater distance from the parent plant in open gaps in the sward (see Chapter 4). It should be emphasised that significant numbers of rooted plantlets can form under grazing conditions (see Table 6.3).

Even though total herbage accumulation between cultivars was not significantly different across all grazing treatments (see Table 6.2), it was only measured over the first grazing season (1996-1997) when parent plant numbers had not significantly declined in either cultivar. By 1998-1999, when Astred averaged 38 plants m^2 (52% of original population) and Pawera 15 plants m^2 (28% of original population) over all treatments, yield differences may have become apparent between cultivars. Herbage accumulation measurements were not conducted in years 2 and 3 because of confounding with other measurements. It is possible that a plant size, density compensation could reduce this yield difference in the short term (Sheaffer *et al.*, 1988), but Smith *et al.,* (1993) found Astred produced significantly higher yields by year 3 when compared to Grasslands Hamua and Redwest under sheep grazing.

Astred, when grown as a spaced plant, is classified as semi-prostrate in growth habit (Smith, 1992; Smith, 1993), but when sown as a pure sward, it can reach over 50cm in height under an 8 week grazing rotation (see Figure 6.2). A distinct loss of herbage quality was noted at the bottom of these tall swards as lower leaves senesced and died. A loss in yield was also apparent (see Table 6.2). The best weed suppression was achieved under the 8 week rotation, but it was similar to the 6 weeks, lax grazing treatment (see Figure 6.3).

Astred had a clear seasonal yield advantage in the late Winter/early Spring over the later flowering Pawera (Hyslop *et al.,* 1998), (see Figure 6.1). Unfortunately, at the time of sowing this Winter activity was not reported in the literature as it would have been useful to compare Astred with a similarly Winter active cultivar such as Grasslands Colenso. Areas in New Zealand to take advantage of this late Winter/early Spring production need to be Mediterranean in climate, and have free draining, fertile soil with adequate soil moisture levels.

In conclusion, the recommended grazing management for pure swards of Astred, vegetatively reproductive red clover, would be to graze every 4 to 6 weeks, or when pre grazing height reaches 30cm, whichever is later. A minimum post grazing height of 10cm over the whole grazing season is also critical for effective rooted plantlet production. Swards should be spelled for 6 to 8 weeks in the Autumn after plantlets begin to take root, to increase plantlet survival. This would not significantly affect annual herbage accumulation or farm management grazing options because this is near the end of the seasonal growth phase for red clover swards. Animals should be removed if soil conditions become wet under foot. Such a management strategy did not cause a significant loss in total herbage accumulation while producing high numbers of rooted plantlets and suppressing weeds. The only difference between these management recommendations and those for crown type red clovers is the greater post grazing or post cutting residual of 10cm instead of 5cm. These increased levels of residual may decrease annual herbage accumulation and utilisation in some situations, but this was not the case in this experiment. A detailed morphological study of when vegetatively reproductive red clovers should be grazed in relation to plantlet formation is required to "fine tune" grazing management recommendations.

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7. BIOMASS ALLOCATION AND PERSISTENCE OF ASTRED, A VEGETATIVELY REPRODUCTIVE RED CLOVER, (*Trifolium pratense* L.) UNDER DIFFERENT GRAZING INTENSITIES AND FREQUENCIES

7.1 ABSTRACT

The biomass allocation and the dynamics of vegetative reproduction in Astred (*Trifloium pratense* L.) are important in the understanding of sward persistency. Experiment 1 consisted of 12 treatments (two cultivars (Astred, Pawera); two grazing intensities (hard, lax); three grazing frequencies (4,6 and 8 weeks)), and compared aspects of stem to leaf ratio, taproot mass, regrowth after grazing and parent plant-life span of Astred to Grasslands Pawera under rotational grazing by sheep. Experiment 2 studied plantlet dynamics under two grazing intensities, hard and lax, and 2 grazing frequencies, 4 and 8 weeks in pure Astred swards in a randomised split plot design for six months under rotational grazing by sheep. In Experiment 1, Pawera and Astred had contrasting ($P<0.001$) components of leaf and stem from 20/9/96 to 6/1/97. Pawera had significantly ($P<0.001$) thicker stems than Astred in all corresponding grazing treatments. The percentage of regrowth for Astred or Pawera was not significantly different at two of the three harvest dates, for either crown leaf/stem regrowth or for leaf/stem regrowth from stem nodes. Over all grazing treatments, Astred plots had higher taproot populations than Pawera (72 vs 44, s.e.m. ±4.81, roots m^{-2}, $P<0.001$), but Pawera had heavier ($P<0.001$) individual taproots in all corresponding grazing treatments. The percentages of tagged parent plants remaining in 6 weekly hard grazed plots decreased at similar rates, except on the last measurement date ($P<0.001$). Grazing intensity and frequency affected ($P<0.001$) the number of all plantlets (plantlets with no roots, aerially rooted or rooted) produced, which ranged from 0 to $11m^{-2}$ for rooted plantlets at monthly and bi-monthly post grazing measurements. Morphologically, Astred and Pawera parent plants are not distinctly different in many ways, but grazing management needs to be lax (10cm post grazing height) and infrequent (every 4 to 6 weeks) for Astred's vegetative reproductive mechanism to function effectively.

Key words: Astred, biomass allocation, plantlet dynamics, regrowth, stem to leaf ratio, taproots, *Trifloium pratense* L.

7.2 INTRODUCTION

An understanding of the growth and development of parent plants and the plantlet dynamics of Astred (a vegetatively reproductive red clover) in a grazed, pure sward is required if sward persistency and productivity are to be maximised (see Chapter 3, Hyslop *et al.,* 1998). Currently, there is no published literature on the effect of grazing intensity and frequency on the growth and development of Astred in pure swards and how this extrapolates to the number of plantlets produced, and hence long term persistency of Astred swards. It is also unclear whether there is a significant "cost" to vegetative reproduction in Astred red clover to the detriment of annual production or sward quality (higher proportion of stem/leaf) when compared with crown type red clovers.

There has been a lack of grazing experiments aimed at devising the best grazing regimes based on growth characteristics, such as the seasonal pattern of development of crown buds for crown type red clovers (Frame *et al.,* 1998). Infrequent and lax grazing has been widely reported (Brougham 1959; Hay *et al.,* 1989; Curll *et al.,* 1989; Taylor *et al.,* 1996) as the best method of management for maximum production and persistence of crown type red clovers, but these management recommendations have all been based on rotation lengths and post grazing heights, not parent plant growth and development state. Crown type red clovers do not produce the numbers of distinct crown bud "flushes" (normally two with double cut cultivars) (Bowley, 1984) produced by lucerne (Leach, 1969; Leach, 1970; Singh *et al.,* 1974), which have been found to be the best indication of the correct time to graze (Marble *et al.,* 1985). Such understanding would be useful to the management of both crown type and prostrate red clover dominant swards used for cutting and grazing Frame *et al.,* (1998).

The morphology of Astred as a single spaced parent plant has been described in detail by Smith (1992) under Tasmanian conditions where leaf size was both shorter and narrower by 4-6mm and 2mm respectively, and stems were thinner compared to Grasslands Hamua, Grasslands Colenso, Redquin and Redwest. However, Astred has larger leaves and thicker stems than cv. Gualdo (see Chapter 4). The morphology of plantlets and the dynamics of their production were not presented by Smith (1992). In Chapter 3 it was found that spaced parent plants of Astred produced a total plantlet mass per parent plant of 15.9g in May, increasing to 348g by December, compared to Grasslands Turoa's 1.6-26.2g respectively, over the same period. Chapter 3 also reported greater survival rates of Astred rooted plantlets than F2419 and Turoa rooted plantlets when managed under hard and lax (5cm and 10cm post grazing heights respectively) rotational grazing by sheep over a 48 week period. This preliminary investigation concluded that the total mass of single parent plants was not significantly different between Astred, F2419 and Turoa and that the allocation of mass to stem tissue was also similar, suggesting that the growth potential of Astred was not disadvantaged by its obviously greater production of rooted plantlets on stems.

An objective of this research was to determine the biomass allocation of parent plants in a pure sward of a vegetatively reproductive red clover (Astred) and Grasslands Pawera under different grazing intensities and frequencies. A second objective was to gain an understanding of the population dynamics of plantlet production within an Astred red clover sward under different grazing intensities and frequencies that will assist in the development of appropriate grazing management decisions to maintain sward persistency.

7.3 MATERIALS AND METHODS

7.3.1 Experimental site

Both Experiments 1 and 2 were conducted at the Pasture and Crop Research Unit, Massey University, Palmerston North, New Zealand. The information on the site, establishment and agronomic management relevant to both experiments are in Chapter 6. The red clover swards were in their second year of growth in Experiment 2.

7.3.2 Design and Grazing management

7.3.2.1 Experiment 1

Design and grazing management are detailed in 6.3.2 as this experiment was overlaid on the grazing frequency by intensity experiment (see Chapter 6).

7.3.2.2 Experiment 2

Two year old plots of Astred which had been hard grazed (post grazing height of 5cm) at grazing frequencies of 4 or 8 weeks by mature Romney ewes at a stocking density equivalent to 1300-1700 sheep/ha for 1 or 2 days in the previous season (1996/97). These plots were divided into half (split plot design) by an electric fence to give 16, 59m^2 plots in a randomised complete block design with four blocks as replicates. Each split plot was grazed to a post grazing height of either 5cm (hard) or 10cm (lax) at the same grazing frequency as the previous 34 as the previous 34 week season (either every 4 or 8 weeks, see Chapter 6). Sheep were removed from each plot when the desired post grazing height was reached (see Plate 7.1).

Plate 7.1 Lax (10cm) and hard (5cm) post grazing heights in a split plot replicate.

7.3.3 Measurements

7.3.3.1 Experiment 1

Leaf and stem ratios were obtained by dissecting one randomly selected $0.25m^{-2}$ quadrat cut to ground level at pre and post grazing from each plot of Astred and Pawera for the complete 34 week grazing season. The quadrat samples were divided into red clover (leaf and stem) and other (weeds) and oven dried at 80°C for 48 hours. One set of stem diameter measurements were taken on 26/2/98 by randomly selecting 10 stems per plot (48 plots) and

The allocation of regrowth after grazing between remaining stem buds and crown buds was measured for Astred and Pawera grazed hard (see Plate 7.2) and lax (see Plate 7.3) with 5cm and 10cm post grazing heights respectively, every 8 weeks. Successive dry matter cuts (two randomly chosen quadrats ($0.25m^2$) per plot) were made to ground level 1, 2 and 3 weeks after the grazing on 5/1/98. Cuts were dissected into red clover parent plant stem, regrowth from the crown, and regrowth from stem nodes, and oven dried at 80°C for 48 hours.

Root measurements were taken in all six grazing treatments for both Astred and Pawera on 25/3/98, two years from sowing, by digging two randomly chosen, $0.25m^2$ quadrats per plot (48 plots), and separating the roots from the soil so the complete root was extracted. Above ground plant parts were cut off in the cotyledonal area. The roots were washed by hand and oven dried at 80°C for 72 hours. The number of taproots per quadrat was counted. If there was no obvious taproot, the bunch of adventitious roots were counted as one taproot per parent plant. Individual root mass was calculated by dividing the taproot mass per m^2 by the number of taproots per m^2.

Two hundred parent plants were tagged using wire stakes pushed into the soil near the plant one year after sowing (3/11/96) in the Astred and Pawera, 6 week, hard grazed treatment (4 replicates). Each plant was recorded as alive or dead, post grazing until 27/10/98. These tagged plants enabled the parent plant life span of a selection of individual parent plants under standard recommended red clover grazing management (every 6 weeks to 5cm post grazing height) to be monitored.

7.3.3.2 Experiment 2

Pre and post grazing plantlet counts were taken from 8/1/98 to 1/5/98 at 4 and 8 weekly intervals (depending on grazing frequency) by counting plantlets with no roots, plantlets with roots not embedded in the soil (aerially rooted), and plantlets with roots firmly fixed in the soil (rooted plantlet) inside three fixed 0.25m^2 quadrats within each split plot. Plantlets that had roots firmly fixed in the soil were tagged pre grazing with a twist of coloured wire so they could be distinguished from previously rooted plantlets.

Two randomly selected 0.25m^2 quadrats were cut to ground level after each grazing in each split plot and dissected into red clover (leaf and stem) and other (weeds), then oven dried at 80°C for 48 hours to obtain kgDM/ha of each post grazing component.

Sward height was measured post grazing with twenty randomly taken heights per split plot with a sward stick, with the height recorded at the first contact with the sward canopy.

7.3.4 Statistical analysis

The model for repeated measures was used to analyse the parent plant measurements conducted in Experiment 1 within the SAS GLM procedure (SAS Institute Inc. 1990)

Data sets in Experiment 2 were evaluated by analysis of variance using the General Linear Model GLM procedure of SAS (SAS Institute Inc. 1990) for a balanced split plot design.

All other data presented were evaluated by analysis of variance using the General Linear Model GLM procedure of SAS (SAS Institute Inc. 1990) for a balanced randomised complete block design.

7.4 RESULTS

7.4.1 Experiment 1

7.4.1.1 Tissue allocation of stem to leaf

Astred swards had less leaf and stem dry matter ($P<0.001$) than Pawera swards, 57% and 72% respectively, from 20/9/96 to 6/1/97 (Tables 7.2 and 7.3, Figures 7.1 and 7.2). There were also significant differences ($P<0.05$) between Astred and Pawera at two later harvest dates (24/2/97 and 21/4/97) (see Figures 7.1 and 7.2). Grazing intensity significantly ($P<0.001$) affected the percentages of leaf and stem at all harvests, except for 3/11/96, 6/1/96 and 24/3/96. The percentage of leaf was greater in hard than lax grazed swards, but grazing frequency had no effect on the percentage of leaf or stem at any harvest date (Figures 7.1 and 7.2).

Stem diameter increased ($P<0.001$) with lengthening grazing frequency, and as grazing intensity decreased from 5cm to 10cm post grazing height. Pawera had significantly ($P<0.001$) thicker stems compared with Astred in all corresponding grazing treatments (see Table 7.1).

Plate 7.2 Post grazing residual in Astred, 8 weeks, hard.

Plate 7.3 Post grazing residual in Astred, 8 weeks, lax.

7.4.1.2 Regrowth after grazing

There was no significant difference between Astred and Pawera for either leaf plus stem regrowth from the crown, or for leaf regrowth from stem nodes, except on 12/1/98 when leaf plus stem regrowth from the crown was significantly higher ($P<0.05$) for Astred (see Tables 7.2 and 7.3).

Grazing intensity had the most effect on regrowth. There were significant differences ($P<0.05$ to $P<0.001$) between hard and lax grazing for the percentage of regrowth from the crown and the stem nodes at all harvests. Proportionally less ($P<0.001$) regrowth came from the crown than the stem nodes under lax grazing (10cm post grazing height), but this was compensated for by proportionally less regrowth from stem nodes (see Tables 7.2 and 7.3). Original parent plant stem percentage decreased at each harvest due to senescence.

7.4.1.3 Taproot measurements

Over all grazing treatments, Astred plots had higher taproot populations than Pawera (72 vs 44, s.e.m. ±4.81, roots m^{-2}, $P<0.001$) (see Table 7.4). There was no significant difference ($P<0.407$) in the number of roots m^{-2} at the different grazing intensities, except between Astred 4 weeks hard and 4 weeks lax.

Pawera had heavier ($P<0.001$) individual taproots than Astred in all corresponding grazing treatments (see Figure 7.3) with an average root mass of 2.40 gDM/root to Astred's 1.60 gDM/root. As grazing intensity and frequency decreased, taproot mass increased ($P<0.001$) in both cultivars (see Figure 7.4).

There was a significant ($P<0.001$) interaction between grazing intensity, frequency and cultivar on root mass m^{-2} (see Figure 7.3). Increased grazing intensity (from 10cm to 5cm post grazing sward height) decreased root mass m^{-2} (gDM m^{-2}) in both Astred and Pawera with the largest differences being between Astred 4 weeks hard and 4 weeks lax (60.8 and 136.2 gDM m^{-2} respectively), and Pawera 4 weeks hard and 4 weeks lax (60.8 and 125.4 gDM m^{-2} respectively). Astred had a significantly ($P<0.001$) greater root mass m^{-2} in the 6 week lax grazing treatment than Pawera, but not in any other grazing treatment.

7.4.1.4 Parent plant life span

The percentage of tagged parent plants that survived in the Astred and Pawera, 6 week hard grazed treatment differed only on the last measurement date (27/10/98) at 31 months of age, when 35% of tagged Pawera plants were surviving compared with Astred's 55% ($P<0.039$) (see Figure 7.5).

Figure 7.1 Pre grazing percentage leaf in Astred and Pawera swards under 4, 6 and 8 week grazing frequencies and lax (10cm residual) and hard (5cm residual) grazing intensities. Vertical bars represent s.e.m. (Expt 1).

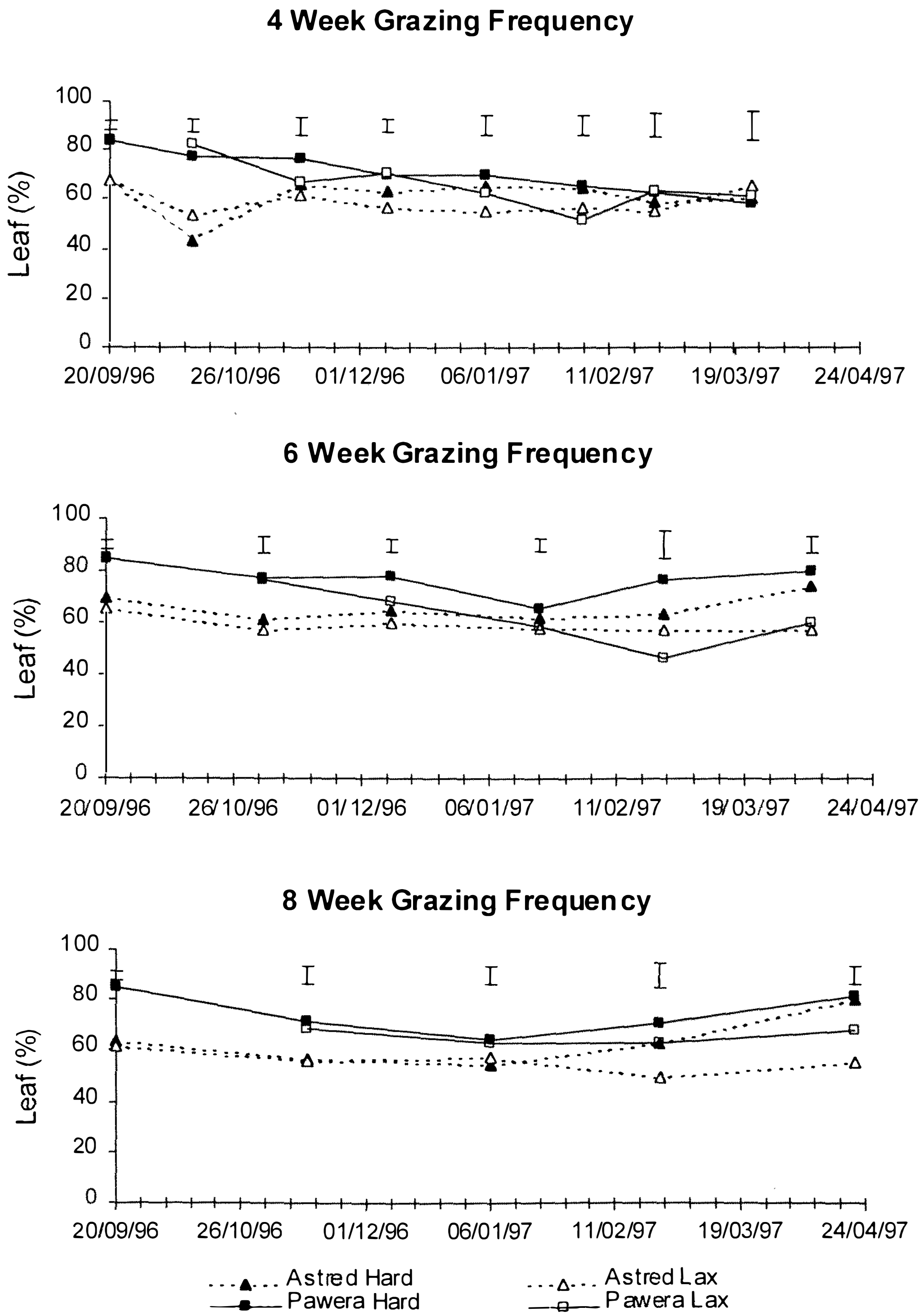


Figure 7.2 Pre grazing percentage stem in Astred and Pawera swards under 4, 6 and 8 week grazing frequencies and lax (10cm residual) and hard (5cm residual) grazing intensities. Vertical bars represent s.e.m. (Expt 1).

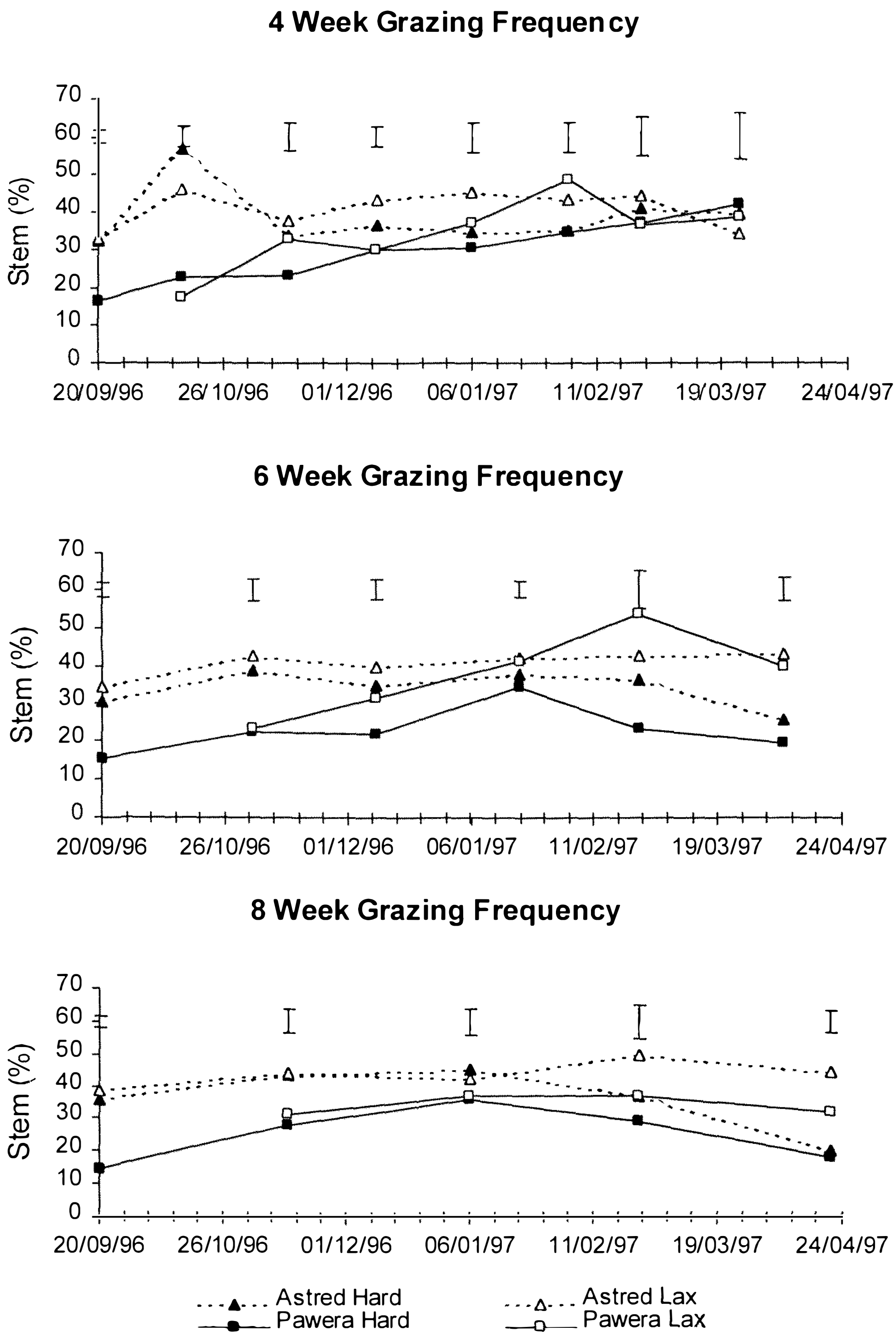


Table 7.1 Stem diameters (mm) of Astred and Pawera swards under 4 week, 6 week and 8 week grazing frequencies and lax (10cm residual) and hard (5cm residual) grazing intensities. (Expt 1).

Grazing frequency	4 weeks		6 weeks		8 weeks		
Grazing intensity	hard	lax	hard	lax	hard	lax	s.e.m.
Astred	3.04	3.97	3.52	3.96	3.77	4.10	0.16
Pawera	4.11	5.16	4.55	5.46	4.87	5.49	0.16

Table 7.2 Percentage regrowth of shoot components after grazing in 2 year old pure swards of Astred and Pawera grazed every 8 weeks to 5cm post grazing height. (Expt 1).

Grazing intensity	Hard (post grazing height of 5cm)								
Component	Stem from crown			Leaf from crown			Leaf from node		
Cultivar	Astred	Pawera	s.e.m.	Astred	Pawera	s.e.m.	Astred	Pawera	s.e.m.
Date									
12/1/98	75.8	76.5	3.39	12.3	7.0	1.29	11.8	16.3	2.87
19/1/98	61.3	63.0	2.86	25.6	21.3	3.33	13.0	15.5	2.08
26/1/98	46.6	40.7	2.4	41.6	44.8	2.29	11.6	14.3	2.26

Table 7.3 Percentage regrowth of shoot components after grazing in 2 year old pure swards of Astred and Pawera grazed every 8 weeks to 10cm post grazing height. (Expt 1).

Grazing intensity	Lax (post grazing height of 10cm)								
Component	Stem from crown			Leaf from crown			Leaf from node		
Cultivar	Astred	Pawera	s.e.m.	Astred	Pawera	s.e.m.	Astred	Pawera	s.e.m.
Date									
12/1/98	77.8	73.7	3.39	3.6	3.7	1.29	18.5	22.5	2.87
19/1/98	74.1	69.1	2.86	9.0	6.1	3.33	16.8	24.6	2.08
26/1/98	61.1	58.3	2.4	13.7	17.1	2.29	25.0	24.5	2.26

Table 7.4 The number of individual taproots m^{-2} harvested by digging on 25/3/98. (Expt 1).

Grazing frequency	4 weeks		6 weeks		8 weeks		
Grazing intensity	hard	lax	hard	lax	hard	lax	s.e.m.
Astred	62	88	80	78	59	66	4.81
Pawera	38	51	54	37	50	37	4.81

Figure 7.3 Taproot mass per plant (gDM/plant) of Astred and Pawera parent plants 2 years from sowing under 4, 6 or 8 week grazing frequency and hard or lax grazing intensity (5cm and 10cm post grazing height, respectively). Vertical bars represent s.e.m. (Expt 1).

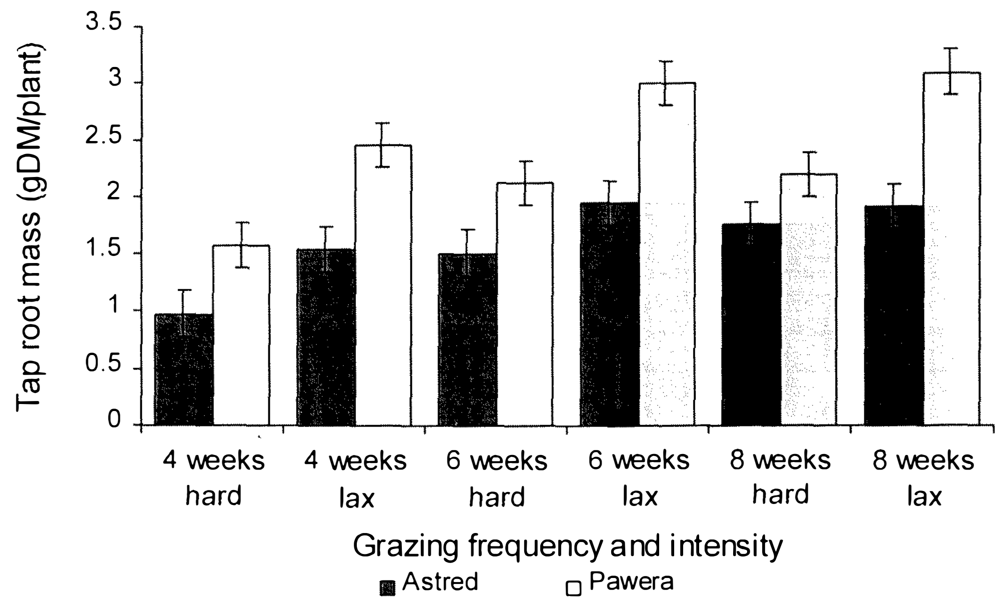


Figure 7.4 Taproot mass per unit area (gDM/m^2) of Astred and Pawera parent plants 2 years from sowing under 4, 6 or 8 week grazing frequency and hard or lax grazing intensity (5cm and 10cm post grazing height, respectively). Vertical bars represent s.e.m. (Expt 1).

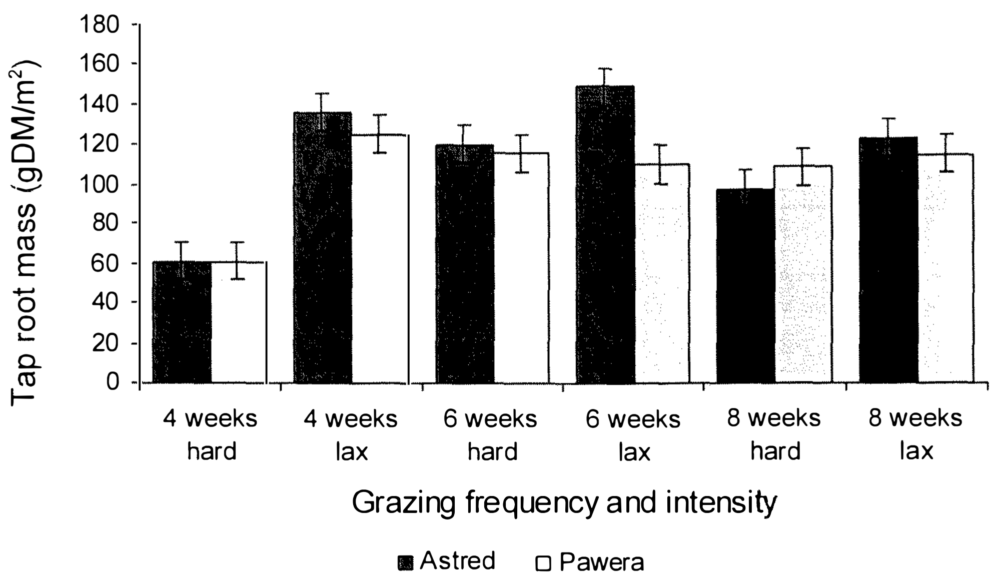
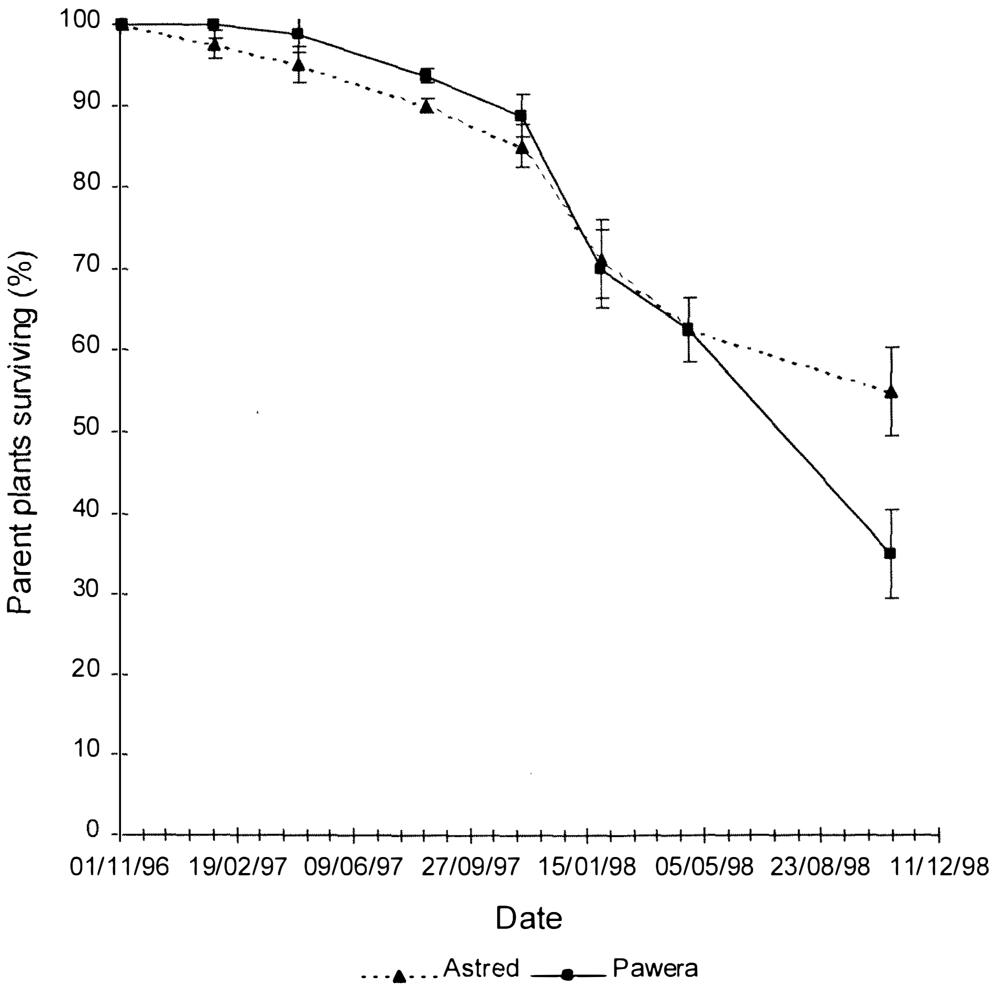


Figure 7.5 Percentage of tagged parent plants surviving in Astred and Pawera pure swards grazed every six weeks between September and May each year to 5cm residual sward height by sheep. Vertical bars represent s.e.m. (Expt 1).



7.4.2 Experiment 2

7.4.2.1 Pre grazing plantlet counts

The percentage of plantlets with no roots peaked in early March (ranging from 90-130 plantlets m^{-2}) for all grazing treatments, except for the 4 week, hard grazed (5cm post grazing height) treatment in which the percentage of unrooted plantlets declined from the first measurement (see Figures 7.6 and 7.7). There were significant ($P<0.001$) differences in all treatments in the percentage of plantlets with no roots at different measurement dates and grazing intensities, declining to an average of 30 plantlets m^{-2} across all treatments by June.

Aerially rooted plantlet percentages were significantly different ($P<0.001$) between grazing frequencies and intensities and at different measurement dates, but were relatively constant within each grazing treatment until 30/4/98, and thereafter declined in every treatment (see Figures 7.6 and 7.7).

A smaller proportion of rooted plantlets compared to plantlets with no roots or with aerial roots existed at any one time in all grazing treatments, except after 30/4/98 when rooted plantlets increased ($P<0.001$) in number over all grazing treatments, but especially ($P<0.001$) in 4 and 8 weeks lax (10cm post grazing height). However, the effect of grazing frequency was also significant ($P<0.001$), with a greater number of rooted plantlets in the 8 week grazing treatment than the 4 week grazing treatment.

7.4.2.2 Post grazing plantlet counts

Grazing intensity and measurement date had significant (P<0.001) effects on the percentage of plantlets with no roots after grazing, but all treatments had more than 80% of the total population of plantlets consisting of plantlets with no roots at any one time. Grazing frequency (either 4 or 8 weeks) made no difference (P<0.457) to the number of surviving plantlets with no roots.

The proportion of aerially rooted plantlets increased (P<0.001) over time in all grazing treatments and ranged from 0%-17% of the total population. The 4 and 8 weeks lax (10cm post grazing height) treatments had the highest percentage of aerially rooted plantlets, but as with plantlets with no roots, grazing frequency did not have a significant effect (P<0.600) (see Figures 7.8 and 7.9).

The overall trend for the percentage of rooted plantlets remaining after grazing was that the lax grazed (10cm post grazing height) treatments grazed either every 4 or 8 weeks had significantly (P<0.001) more rooted plantlets than the hard grazed (5cm post grazing height) treatments grazed either every 4 or 8 weeks. There was a marginal effect (P<0.07) of grazing frequency, with more rooted plantlets surviving when grazed every 8 weeks than every 4 weeks. Treatments grazed every 8 weeks to 5cm post grazing height (hard) had the highest percentage of surviving rooted plantlets at any one time. Lax grazing every 4 weeks had the highest apparent, accumulated rooted plantlets (see Figure 7.10).

7.4.2.3 Post grazing morphological components

Morphological components varied significantly ($P<0.001$) between grazing frequencies and intensities (see Tables 7.5 and 7.6). As grazing frequency decreased from 4 to 8 weeks, percentages of post grazing leaf and stem increased. The weed component was particularly high (average of 65% over all harvests) in the 4 week grazed treatment, but decreased with decreased grazing frequency (8 weeks).

7.4.2.4 Pre and post grazing sward heights

Grazing intensity affected ($P<0.001$) pre grazing sward heights at all harvests and in both grazing frequencies, except for the 4 weeks hard and lax on 2/2/98 and the 8 weeks hard and lax grazing treatments on 8/1/98 and 30/4/98. All pre grazing heights declined as the season progressed (see Figure 7.11). There was a significant ($P<0.001$) interaction between harvest date, grazing frequency and grazing intensity.

All post grazing sward heights were significantly different ($P<0.001$) between hard and lax (5cm and 10cm post grazing sward heights) grazing treatments at all harvest dates.

Figure 7.6 The pre grazing percentage of plantlets with no roots, plantlets with aerial roots, and rooted plantlets under 4 weeks grazing frequency, with hard and lax grazing intensities (5cm and 10cm) (s.e.m. 2.90, 1.54, 2.12, respectively). (Expt 2).

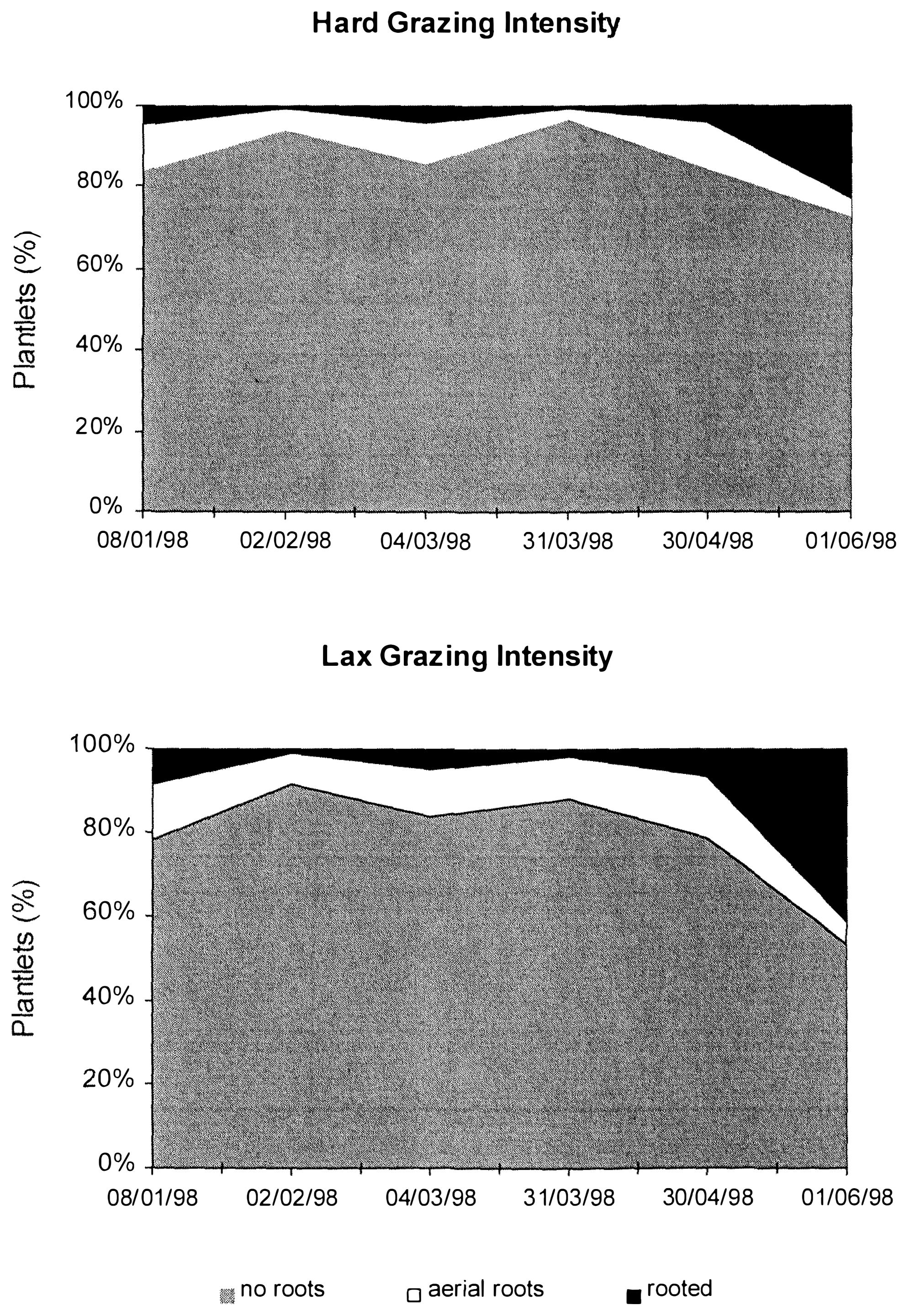


Figure 7.7 The pre grazing percentage of plantlets with no roots, plantlets with aerial roots, and rooted plantlets under 8 weeks grazing frequency, with hard and lax grazing intensities (5cm and 10cm) (s.e.m. 2.90, 1.54, 2.12, respectively). (Expt 2).

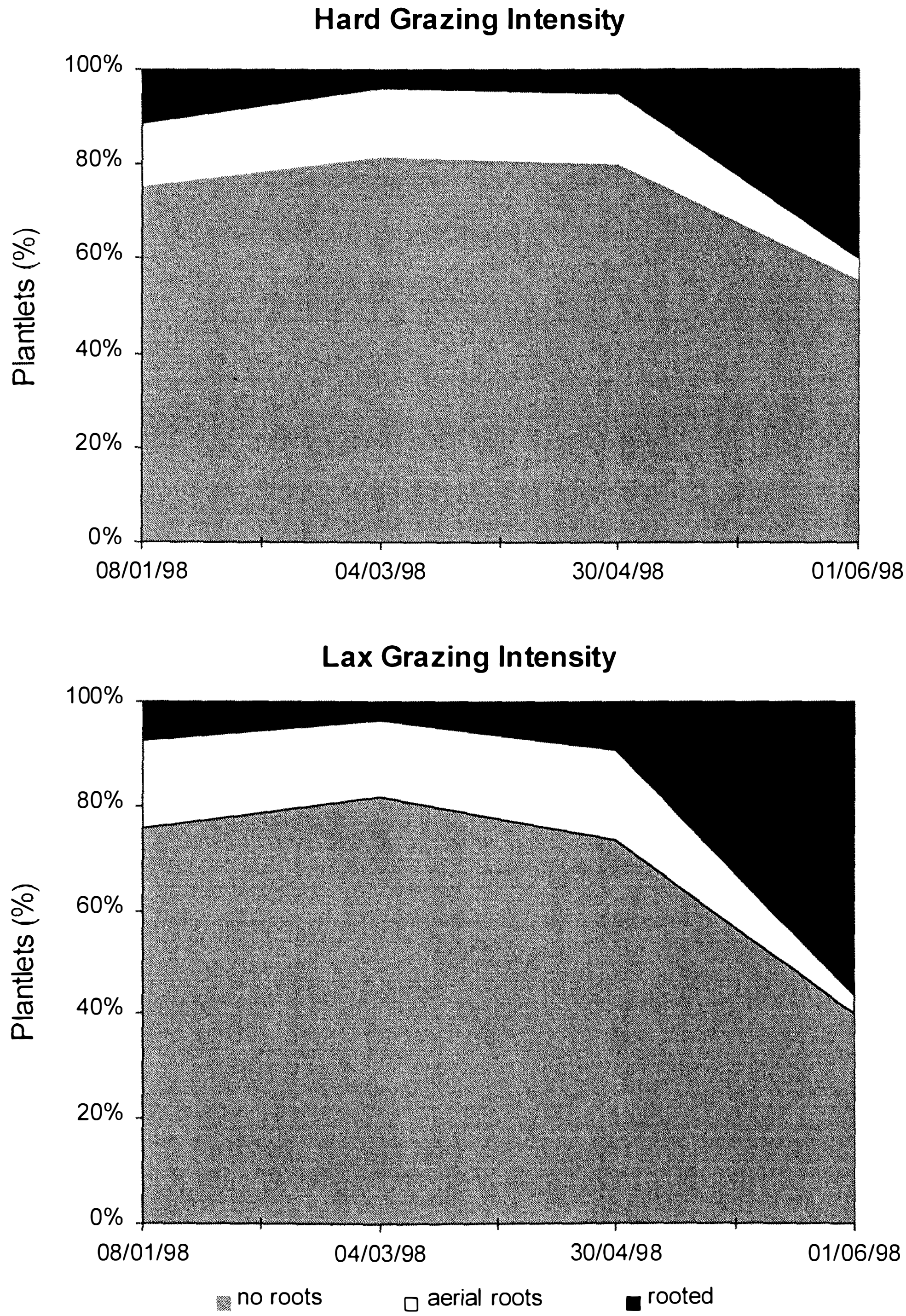


Figure 7.8 The post grazing percentage of plantlets with no roots, plantlets with aerial roots, and rooted plantlets under 4 weeks grazing frequency, with hard and lax grazing intensities (5cm and 10cm) (s.e.m. 4.91, 1.92, 4.81, respectively). (Expt 2).

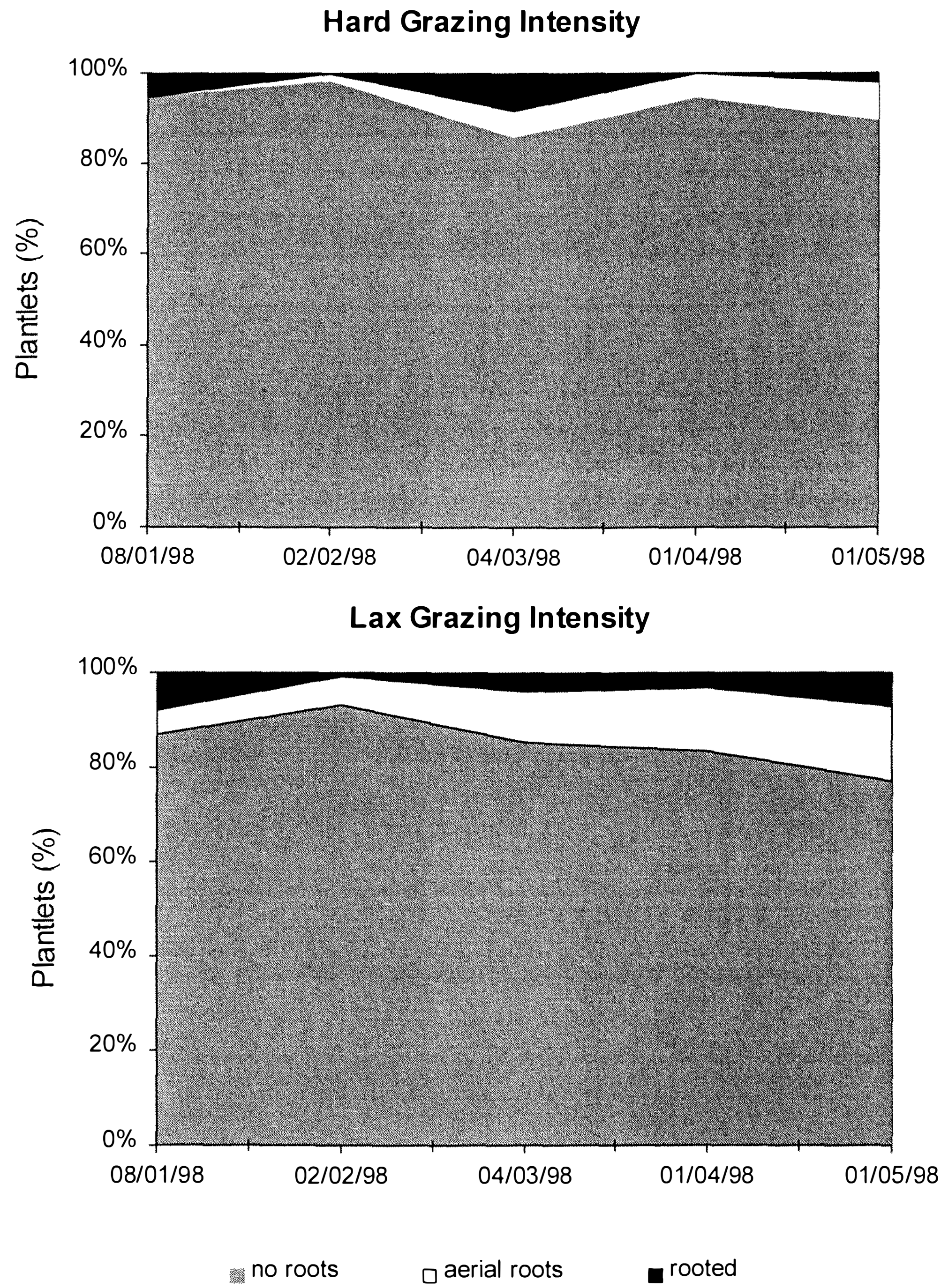


Figure 7.9 The post grazing percentage of plantlets with no roots, plantlets with aerial roots, and rooted plantlets under 8 weeks grazing frequency, with hard and lax grazing intensities (5cm and 10cm) (s.e.m. 4.91, 1.92, 4.81, respectively). (Expt 2).

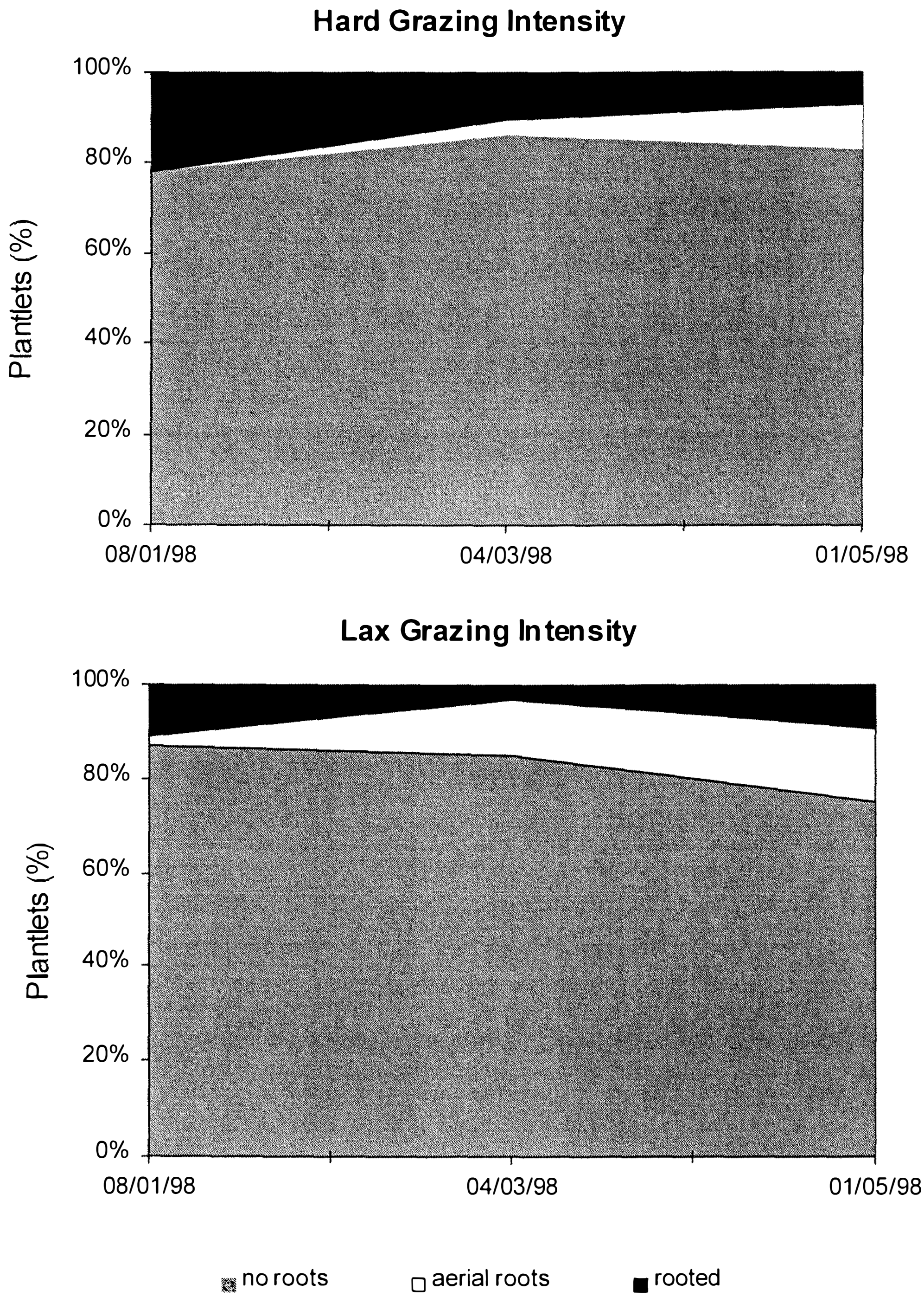


Table 7.5 Composition (DM) of Astred grazed hard or lax every 4 weeks. (Expt 2).

Grazing intensity	Hard (5cm post grazing height)			Lax (10cm post grazing height)		
Component	Leaf	Stem	Weeds	Leaf	Stem	Weeds
Date						
13/1/98	4.9	28.6	66.4	17.5	31.1	51.2
5/2/98	9.1	25.6	65.2	21.5	33.2	45.1
9/3/98	11.8	26.5	61.5	27.8	34.9	37.2
3/4/98	13.0	16.1	70.8	26.4	29.7	43.8
5/5/98	13.8	21.4	64.6	29.8	37.5	32.7
s.e.m.	3.62	3.43	5.53	3.62	3.43	5.53

Table 7.6 Composition (DM) of Astred grazed hard or lax every 8 weeks. (Expt 2).

Grazing intensity	Hard (5cm post grazing height)			Lax (10cm post grazing height)		
Component	Leaf	Stem	Weeds	Leaf	Stem	Weeds
Date						
13/1/98	8.0	64.7	27.2	27.4	37.1	35.4
9/3/98	19.1	52.3	28.5	33.3	52.5	14.0
5/5/98	29.0	39.1	31.8	42.2	49.8	7.8
s.e.m.	3.62	3.43	5.53	3.62	3.43	5.53

Figure 7.10 Apparent accumulated number of rooted plantlets of Astred under 4 week and 8 week grazing frequencies, with hard and lax grazing intensities (5cm and 10cm post grazing heights respectively). Vertical bars represent s.e.m. (Expt 2).

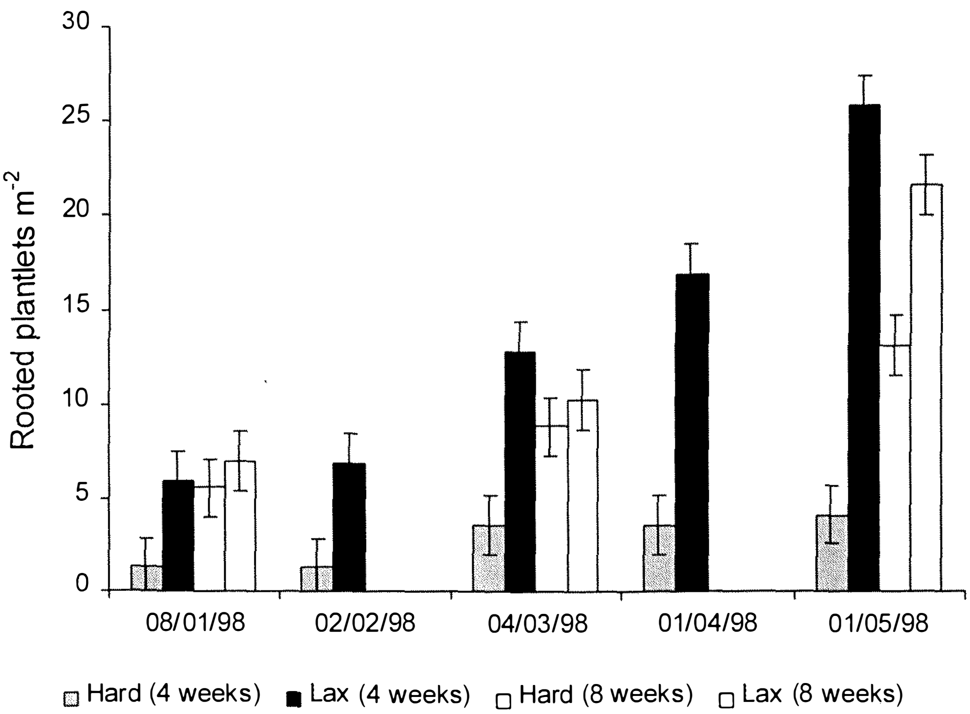
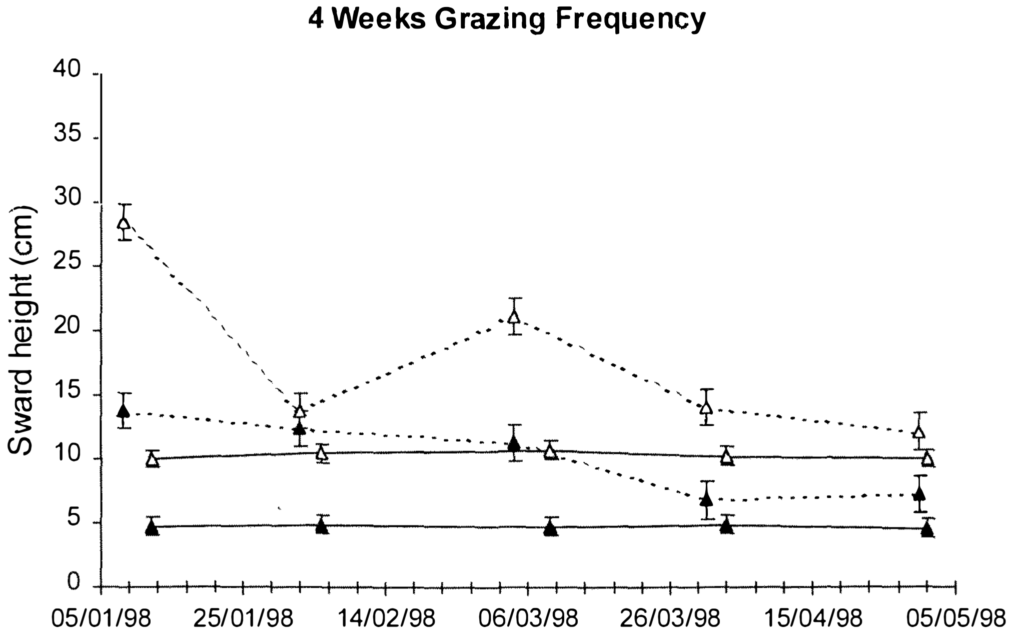
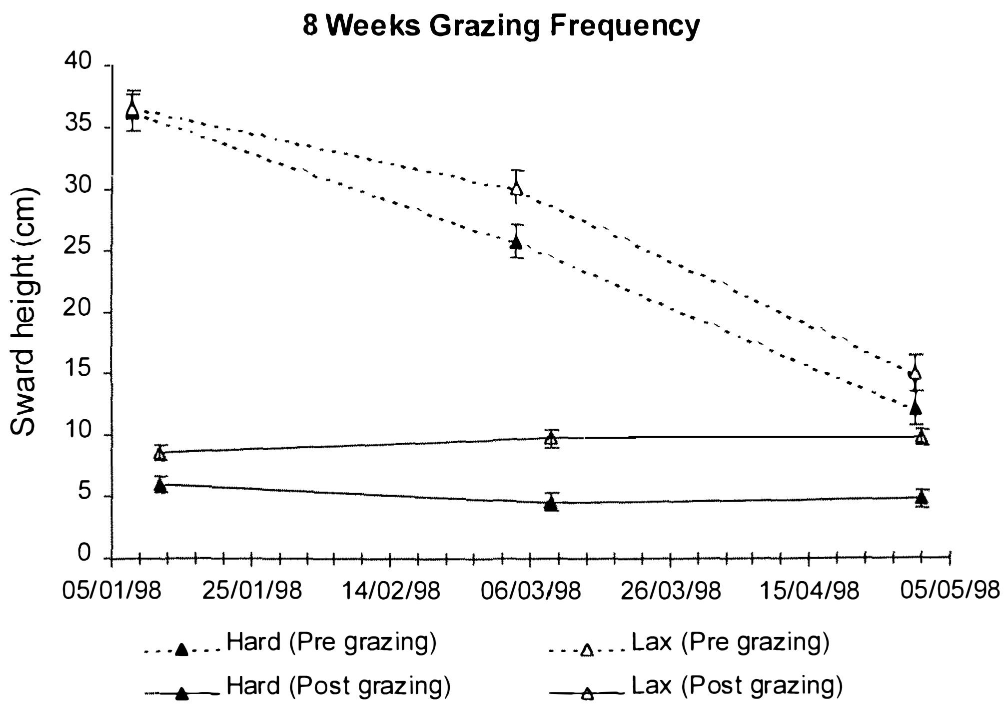


Figure 7.11 Pre and post grazing sward heights (cm) of Astred with hard and lax grazing intensities every 4 or 8 weeks





7.5 DISCUSSION

The biomass allocation of mature plants of Astred did not differ significantly from that of Pawera in a grazed pure sward. Astred and Pawera had similar responses to grazing intensity and frequency with respect to leaf and stem ratios over the growing season. The contrasting production of leaf and stem from 20/9/96 to 6/1/97 between Astred and Pawera were due to the different seasonal growth pattern of each cultivar (see Figures 7.1 and 7.2, Chapter 6). Pawera remained as a rosette later into the Spring than Astred and consequently stem elongation occurred later. Not until 6/1/97 were there no significant differences in the morphological allocation of the two cultivars under comparable grazing treatments. From February onwards, Astred swards under lax grazing (10cm post grazing sward height) and 8 week grazing frequency produced significantly more stem and less leaf than Pawera, possibly as a consequence of developing plantlets on the stems or from leaf senescence in the lower canopy of the sward (Collins *et al.,* 1984; Sheehan *et al.,* 1985). This difference was not present in any of the other grazing treatments. If Astred swards are managed for maximum rooted plantlet production, increased ratios of stem to leaf will result as the Spring and Summer progress, because plantlets are mainly produced on Spring grown stems (see Chapter 4) which will accumulate in the bottom of the sward.

More assimilate is possibly partitioned to stems and developing plantlets in Astred than Pawera, instead of going to new crown buds because less are initiated, which would have an effect on yield (Taylor *et al.,* 1996) when left until seed set. Plantlets, like seedlings and red clover parent plants, do not undergo stem elongation until the increasing photoperiod in the following Spring (Bowley *et al.,* 1987), therefore they add to the high quality, leaf fraction of the sward.

Despite Astred having significantly ($P<0.001$) thinner stems than Pawera in all corresponding grazing treatments, it was still able to reach a pre grazing height of

40-50cm. Red clover stem diameter appears not to be correlated with lignin content and *in vitro* digestible dry matter (Aman *et al.,* 1983), possibly because as stem diameter increases, so does the diameter of the hollow centre. However, increased lignin content is highly correlated with stem maturity (Taylor *et al.,* 1996).

Astred parent plants grown from the sown seed persisted for as long, and longer under 6 weekly hard grazing, than did sown Pawera parent plants (see Figure 7.5). This contrasts with the ecological theory (Franco *et al.,* 1996) that clonal parent plants would be expected to have reduced life spans due to the high cost of reproducing by vegetative means. Astred production levels (kgDM/ha) in the first year were comparable to Pawera (see Chapter 6) which suggested there was similar partitioning of assimilate to above ground parts by the two different red clover plant types. It is possible that Astred is less susceptible to one or more of the causes of poor plant persistence in red clovers, for example increased tolerance to grazing, insect or disease resistance (Smith *et al.,* 1973), and a decreased susceptibility to internal break down (Cressman, 1967) due to having multiple taproots (Montpetit *et al.,* 1991). However controlled studies of Astred parent plant longevity need to be conducted over a longer time to fully determine longevity.

Another reason for the better persistence of Astred under grazing (see Figure 7.5, Chapters 3, 6, 7, and 8, Smith, 1993; Smith, *et al.,* 1993; Hyslop *et al.,* 1998) is that it tends to have higher plant densities than Pawera (see Table 7.4). Parent plant size-density compensation would play a lesser role in the annual production of Astred swards than in Pawera swards, because the parent plant population remains at a high density due to new plantlets being produced each Autumn. These plantlets successfully occupy vacant spaces within the sward, similar to *Ranunculus repens* (Sarukhan 1974; Sarukhan *et al.,* 1973).

Individual taproot mass (see Figure 7.3), taproots m^{-2} after two years grazing (see Figure 7.5), and parent plant density (see Chapter 6), at either 4 weeks, 6 weeks or 8 weeks grazing frequency and under either lax (10cm post grazing sward height) or hard grazing (5cm post grazing sward height), confirm a higher population of smaller taproots for Astred compared to Pawera. However, taproot mass m^{-2} was similar in all comparable grazing treatments and both cultivars demonstrated a similar trend with decreased individual taproot mass as grazing intensity and frequency increased. This indicated that the smaller taproot mass of Astred is an inherent character and related to plant density rather than an indicator of sward ill health.

Fewer rooted plantlets were produced that survived to post grazing compared to the number of aerially rooted plantlets produced per square metre with the potential to become rooted plantlets (see Figures 7.6, 7.7, 7.8 and 7.9). Even under lax grazing (10cm post grazing sward height) every 8 weeks, the number of rooted plantlets post grazing were only 10.5% (or 11 plantlets m^{-2}) of total plantlet numbers (i.e. plantlets with no roots, plantlets with roots but not in the soil and rooted plantlets) in the period (May) with greatest plantlet numbers.

As rooted plantlet measurements were not cumulative over measurement dates, the seasonal total of surviving rooted plantlets was not recorded. However, the apparent accumulated, rooted plantlet number post grazing gave some indication of the possible number of surviving rooted plantlets and supported the finding that lax grazing is important if established, rooted plantlets are to be produced (see Figure 7.10). High (up to 70 plantlets m^{-2}) numbers of rooted plantlets can be produced in mixed swards (Hyslop *et al.,* 1998), in pure swards (see Figure 7.10, Chapter 6), and from single plants (Chapters 3 to 6) under suitable environmental and management conditions.

The dynamics of plantlet production in pure swards with grazing frequencies of 4 and 8 weeks and hard and lax grazing intensities (5cm and 10cm post grazing heights) suggested that grazing removes (see Figures 7.6, 7.7, 7.8, and 7.9) plantlets that have yet to produce roots, damages plantlets with roots which are not yet in the soil, and also damages and removes some already rooted plantlets. Part of this damage is caused by the desiccation of roots (see Chapter 5) as the moist micro climate within the sward is destroyed by the removal of herbage by the grazing animal. Only a small percentage (0-21%) of plantlets had roots that were not anchored in the soil at any one time, which would indicate that individual plantlet rooting takes place over a short period of time (less than 4 weeks), but plantlets remain vulnerable to animal damage (pulling) for a longer period of time after they have roots in the soil. This period of vulnerability requires further study so the optimum grazing management for rooted plantlets (spelling periods) can be defined. In Chapter 3, 83% of tagged plantlets produced by spaced parent plants were surviving after 48 weeks when rotationally grazed by mature ewes.

Long term studies are required to trace the survival of plantlets in pure and mixed swards under different grazing managements. Experiment 2 indicated that an Astred sward of lower density can be partly restored by the successful production of rooted plantlets through lax, infrequent grazing (at least 10cm post grazing height and 4 to 8 week grazing frequency) for one season (see Figure 7.10).

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8. PRELIMINARY INVESTIGATIONS INTO THE PERSISTENCE OF A VEGETATIVELY REPRODUCTIVE RED CLOVER CV. ASTRED IN MIXED SWARDS AT HAWKES BAY AND MANAWATU♣

♣ A modified version of this chapter has been published in *Proceedings of the Agronomy Society of New Zealand* **28**, 117-122 (Hyslop *et al.*, 1998).

8.1 ABSTRACT

All red clover (*Trifolium pratense* L.) cultivars currently used in New Zealand grow from crowns and predominantly reproduce from seed, but lack persistence in temperate pastures, particularly where grazing pressure is high. Vegetatively reproductive red clover selections that have prostrate stems that produce clonal replacement plants at nodes (plantlets) are available. A vegetatively reproductive red clover, cv. Astred was sown in mixed swards on three farm trials at Hastings, Dannevirke, and Palmerston North in 1996. Each experiment had contrasting grazing pressure over time with all grazing management decisions made by each farmer. Astred parent plant persistence decreased from 30 plants m^{-2} to 4 plants m^{-2} at Palmerston North; however significantly more ($P<0.001$) survived at Dannevirke after two seasons than for cv. Grasslands Pawera, a crown type red clover, used as a comparison. Astred out yielded Pawera from September to November by 997kgDM/ha (s.e.m. ± 296) at the Dannevirke site and contributed 55% of pasture yield in the first summer at Palmerston North. The Hastings trial did not persist due to a dry Winter after sowing, followed by a dry Summer, which showed that the drought tolerance of Astred and Pawera was similar. Vegetatively reproductive red clovers appear to have the potential in mixed swards to maintain a stable population through plant replacement by vegetative reproduction, but only under suitable environmental and management conditions.

Additional key words: Astred, persistence, plantlet, *Trifolium pratense* L., vegetatively reproductive

8.2 INTRODUCTION

Red clover (*Trifolium pratense* L.) can provide high quality summer feed in New Zealand's temperate grazing systems (Hay *et al.,* 1978; Cosgrove *et al.,* 1985; Ussher 1986; Hay *et al.,* 1989). However, its use by farmers in pasture mixtures and for pure specialist swards has declined in recent years, due mainly to poor persistency.

Lancashire (1985) reported that red clover is one of the least persistent clovers in temperate pastures, particularly where grazing pressure is high (Cosgrove *et al.,* 1985). All red clover cultivars currently available in New Zealand grow from crowns and predominantly reproduce from seed. The crowns are susceptible to treading damage and fungal infection, particularly in Winter, resulting in poor persistence (Hay *et al.,* 1989). Pure stands of red clover usually only persist three to four years with no vegetative replacement.

Red clover selections exist that have prostrate stems producing clonal plants at stem nodes. The only cultivar with seed available in commercial quantities in New Zealand at present is Astred, which has shown improved persistency relative to crown type red clovers in grazed pastures in Tasmania (Smith, *et al.,* 1993). Astred red clover has a prostrate growth habit and has the ability to vegetatively reproduce (see Chapter 3, Smith, 1992; Smith, 1993; Smith, *et al.,* 1993; Orr *et al.,* 1996). Astred produced both the largest and the greatest number of rooted plantlets per parent plant when compared with other vegetatively reproductive selections in spaced plant trials (see Chapter 3).

A grazing trial with sheep in Tasmania which compared cultivars Astred, Grasslands Turoa, Grasslands Hamua and Redwest, showed Astred retained 55% ground cover after three years, compared to 5%, 2%, 0% for cv.

Grasslands Turoa, Grasslands Hamua and Redwest respectively (Smith *et al.*, 1993). The perennation and productivity of vegetatively reproductive red clovers requires evaluation to determine their potential in New Zealand farming systems, and to develop appropriate management strategies to ensure clonal, rooted plantlets develop and establish.

The objective of this research was, therefore, to evaluate the plant survival and productivity of Astred red clover when sown in pasture mixtures on farms with contrasting grazing management and environmental conditions.

8.3 MATERIALS AND METHODS

Three field experiments were conducted using the red clover cultivars, Astred and Grasslands Pawera within pasture mixtures at three distinct meteorological sites. Pawera was used as a comparison because it is widely considered the most persistent red clover available at present within New Zealand. At each site the cultivars were sown in 0.2ha strips at a rate of 5.4kg/ha for Astred and 13.3kg/ha for Pawera which was coated. These sowing rates ensured that for both cultivars 250 viable seeds were planted per square metre. Red clover seed was sown in addition to the other herbage species used at each site (see individual experiments). Each 0.2ha strip was divided into four strata for non biased sampling and measurement. Parent plants were counted every three months over all experiments using a $0.5m^2$ quadrat. Rooted plantlets were only counted in Autumn when they were identifiable within the sward. Herbage samples were oven dried at $80^{\circ}C$. All grazing management decisions were made by each farmer with grazing time and the number and type of animal recorded. Experiment 1 was grazed with young replacement dairy stock, Experiment 2 with a milking herd, and Experiment 3 with beef steers, lambs, hoggets and ewes.

8.3.1 Experiment 1

Experiment 1 was conducted at Kiritaki Road, Dannevirke, New Zealand (latitude $40^{o}15$'S) from September 1996 to June 1998 (see Plate 8.1). Annual rainfall is 1329mm (10yr avg.). The soil type is a Kopua silt loam, with pH 5.5 and Olsen P 21µg/g soil. The paddock was ploughed, harrowed and rolled after being in Winter green feed oats, then sown with the red clovers in a mix with 12kg/ha perennial ryegrass (*Lolium perenne* L.), Yatsyn 1, 4kg/ha white clover

(*Trifolium repens* L.), cv. Aran and 4kg/ha cocksfoot (*Dactylis glomerata* L.), cv. Sorborto on 27/9/96 with a V ring roller drill. Potassic 15% superphosphate 320kg/ha was applied in Spring and 75kg/ha of urea was applied in Autumn of both years. Establishment counts were taken on 4/11/96 and 3/12/96 using a 0.5m^2 quadrat, and one set of rooted plantlet counts was taken on 27/5/98 using a 0.25m^2 quadrat with 12 replicates.

8.3.2 Experiment 2

Experiment 2 was conducted on No.1 Dairy Farm, Massey University, Palmerston North, New Zealand (latitude 40°23'S) from February 1995 to June 1998. Annual rainfall is 1031mm (10yr avg.). The soil type is a Manawatu silt loam, with pH 6.2 and Olsen P 50 µg/g soil. The paddock was sprayed with 6 l/ha Roundup (36% glyphosate) and 200gm/ha Lorsban (9.9% chloropicrin) in 300 l water ha^{-1} eight days before drilling on 28/2/95. Astred was sown in a 0.2ha strip with 12kg/ha perennial ryegrass cv. Embassy, 3kg/ha white clover cv. Grasslands Kopu and 2kg/ha of white clover cv. Grasslands Tahora. Pawera was not sown at this site. Diammonium phosphate (D.A.P, 150kg/ha) was drilled with the seed, 300kg/ha of 50% potassic superphosphate, 200kg/ha D.A.P + selenium and 120kg/ha urea were applied during 1996 and 1997. Establishment counts were taken on 5/3/96, 11/3/96, 18/3/96, 26/3/96 and 4/4/96 by measuring 20 randomly selected, 1m row sections. Herbage accumulation was measured from 8 pre- and post-grazing dry matter cuts using 0.1 m^2 quadrats, and botanical composition was determined from eight subsamples dissected into red clover and other species at each grazing. Rooted plantlets were counted on 6/6/97 using a 0.5 m^2 quadrat and 12 replicates.

8.3.3 Experiment 3

Experiment 3 was sited at "Turamoe", Hawkes Bay, New Zealand (latitude 39°42'S) from May 1996 to June 1998. Annual rainfall is 710mm (10yr avg.), (see Appendix II). The soil type is a Noi II sandy loam on clay pan, with pH 5.4 and Olsen P 15 µg/g soil. The paddock was sprayed with 6 l/ha Roundup (36% glyphosate) in 300 l water/ha, two weeks before sowing, then grazed down to 400kgDMha residual at drilling. The red clovers were sown on 5/5/96 in a mixture of 25kg/ha grazing brome (*Bromus inermis* cv. Grasslands Gala) and 5kg/ha white clover cv. Grasslands Prop using a Bioblade cross slot direct drill with 120kg/ha of Cropmaster 20 (20N - 10P - 0K) drilled with the seed. Establishment counts were made on 12/6/96 and 20/7/96 by measuring 20, 1m row sections. 125kg/ha of Cropmaster 20 was applied in late Autumn of 1997 and 1998.

8.3.4 Statistical analysis

All data presented from Experiments 1, 2 and 3 were evaluated by analysis of variance using the General Linear Model GLM procedure of SAS (SAS Institute Inc. 1990) for a balanced completely randomised design.

8.4 RESULTS

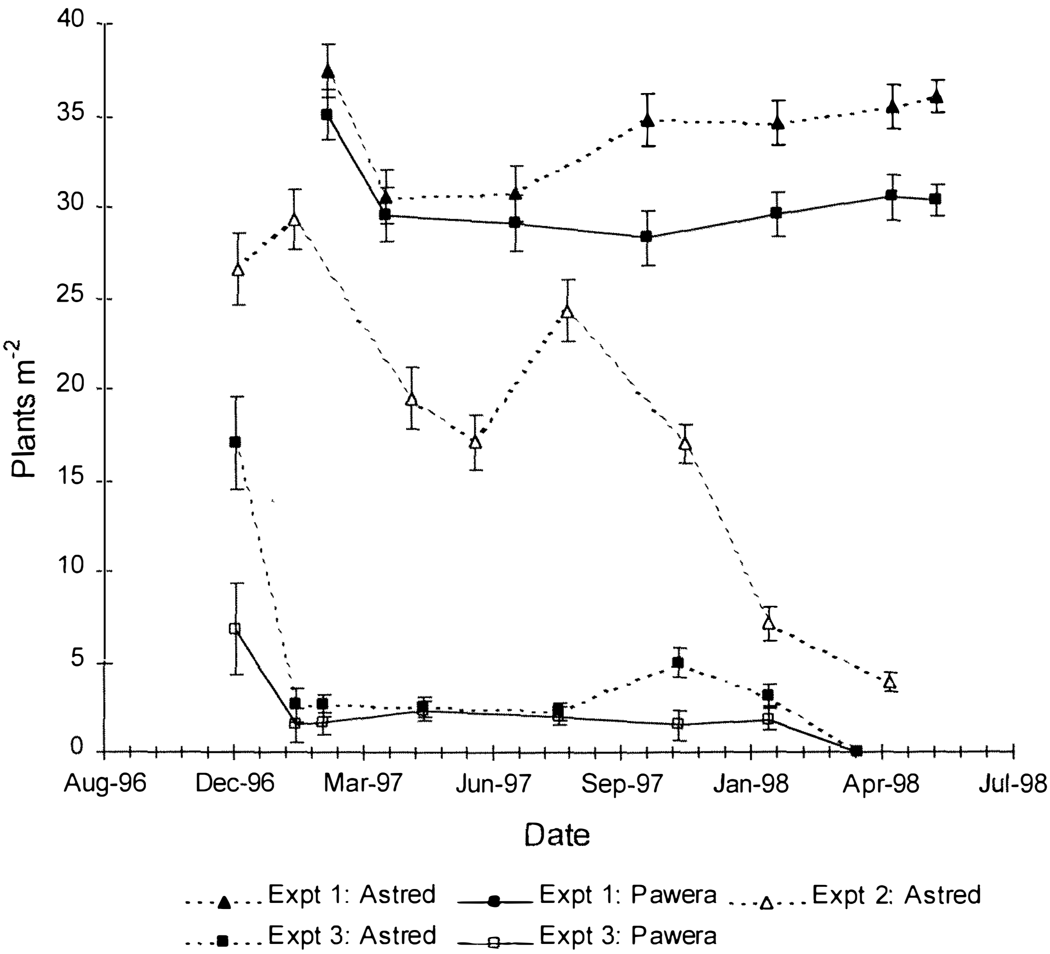
8.4.1 Plant population

Established plant populations varied substantially between sites (from 7-37 plants m^{-2}), even though sowing rates of viable seed did not differ (see Figure 8.1). Significant differences ($P<0.05$) between Astred and Pawera populations did not start to become apparent until the Spring of 1997 for Experiment 1. The last measurement of plant population in Experiment 1 on 27/5/98 showed a significant ($P<0.001$) difference between cultivars with Astred and Pawera having 36 and 30 plants m^{-2} (s.e.m. ± 0.87), respectively. Rooted plantlet density in Astred on 27/5/98 was 64 rooted plantlets m^{-2} (s.e.m. ± 1.7). Pawera did not produce plantlets.

In Experiment 2, parent plant numbers of Astred steadily declined from 30 plants m^{-2} (1996) to 4 plants m^{-2} (1998), except for an increase in Winter/Spring 1997 when rooted plantlets that were formed in Autumn (14 rooted plantlets m^{-2} s.e.m. ± 2.4) were counted as independent parent plants (see Figure 8.1).

Late sowing in Autumn (May) led to a poor establishment (7-17 plants m^{-2}) in Experiment 3 (see Figure 8.1) and a very dry Spring in 1996 meant that plant numbers did not recover. Rainfalls of 29mm (92mm), 15mm (83mm) and 15mm (79mm) were recorded for August, September and October respectively at this site (normal monthly average in brackets), (see Appendix II). Complete parent plant population death occurred in the Summer of 1998 due to lack of soil moisture (see Figure 8.1).

Figure 8.1 Astred and Pawera parent plant populations in mixed swards over time. Vertical bars represent s.e.m.



8.4.2 Grazing pressure

Grazing pressure was calculated as average stock units per hectare (SU/ha) with grazing days for each season recorded for each experimental paddock. Average SU/ha were used because of the variety of stock across experiments and the different frequencies of grazing events. Total stock unit/grazing days per hectare (SUgd/ha) indicates the overall total grazing pressure received by each experiment from sowing (see Table 8.1). Experiment 2 had three times more grazing pressure than Experiment 3 and 3.5 times the grazing pressure of Experiment 1, after allowing for the different sowing dates.

Plate 8.1 Winter break grazing of Experiment 1 at Dannevirke.

Table 8.1 Grazing pressure over time for the three experiments.

Year	Season	Experiment 1	Experiment 2	Experiment 3
1996	Autumn	-	904(2)	-
	Winter	-	904(1)	53(2)
	Spring	-	904(4)	113(5)
	Summer	110(13)	904(2)	14(22)
1997	Autumn	110(6)	904(3)	57(18)
	Winter	78(25)	294(4)	61(17)
	Spring	cut		135(9)
	Summer	78(25)	294(8)	132(10)
1998	Autumn	78(15)	294(6)	-
Total SU grazing days/ha		7160	16735	5577

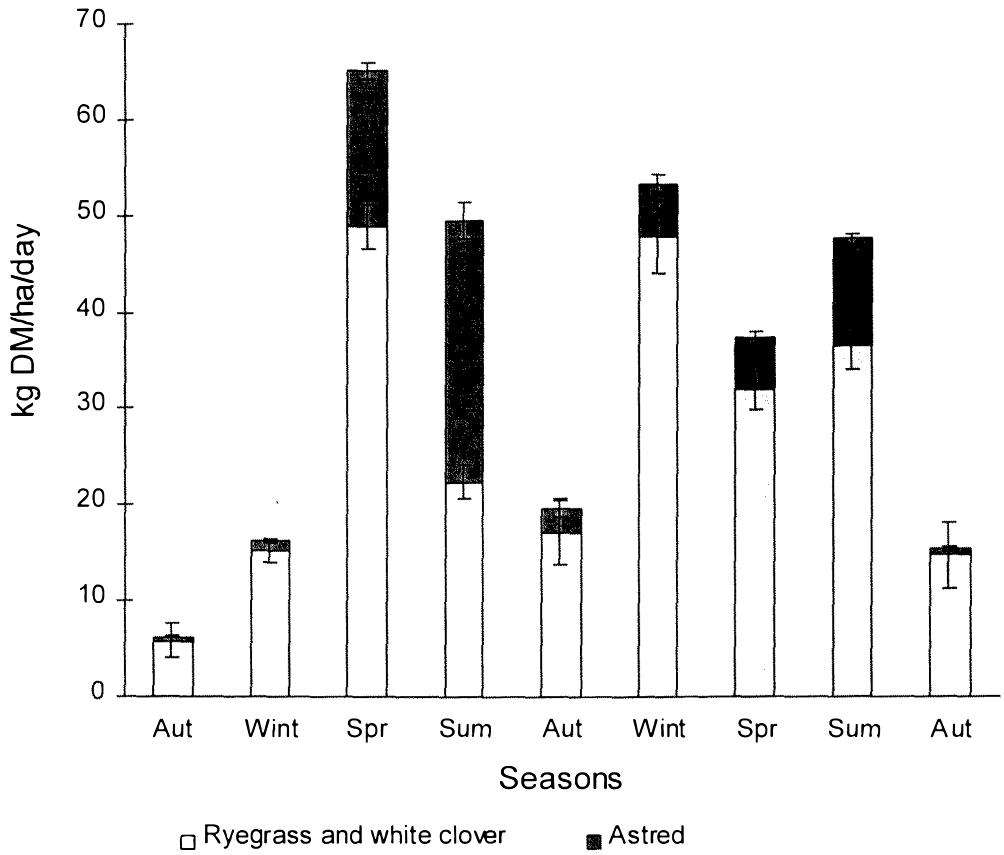
Units: stock units/ha(days grazed) Stock units based on; ewe 1.0, hogget 0.8, lamb 0.6, beef steer 4.7, cow 7.5, dairy yearling 3.5, dairy weaner 2.5

Plate 8.2 Post grazing residual after Winter break grazing of Experiment 1.

8.4.3 Herbage accumulation

In Experiment 2 the Astred red clover component produced 1483 (s.e.m. ± 46.4) and 2410 (s.e.m. ± 25.0) kgDM/ha in Spring and Summer of 1996/1997, compared to 4446 (s.e.m. ± 239.1) and 1995 (s.e.m. ± 159.1) kgDM/ha for the rest of the sward components (see Figure 8.2). This equated to 55% of the production coming from the red clover for that summer period. Total production over the experimental period of 26 months comprised 21917kgDM/ha ryegrass/white clover (s.e.m. ± 2232.4) and 6338kgDM/ha of red clover (s.e.m. ± 607.5), with 4038kgDM/ha of red clover being produced in the first year.

Figure 8.2 Herbage dry matter accumulation of Astred in a mixed sward at Palmerston North. Vertical bars represent s.e.m. (Expt 2.)



8.4.4 Silage production

In Experiment 1 with a conservation period of 102 days, Astred produced 997kgDM/ha more than Pawera and contributed 43% of the total yield compared to 34% for Pawera (see Table 8.2). This cut was taken on 7/11/97, one year from sowing and with respective parent plant densities of 35m^2 and 30m^2 for Astred and Pawera. In contrast, Astred contributed only 7.7% of the silage yield in Experiment 2 when cut 1.75 years from sowing with a conservation period of 53 days (see Table 8.2). Parent plant density had declined to 15m^2. The mean dry matter production per plant per day for Astred was 0.96kg in Experiment 1 and 0.55kg in Experiment 2.

Table 8.2 Silage produced (kgDM/ha) over 102 days to 7/11/97 in Experiment 1 and over 53 days to 21/11/97 (Expt 2).

Experiment	Cultivar	Plants m^{-2}	Rye/white clover	Red clover	Total	s.e.m.
1.	Astred	35	4477	3425	7902	296
1.	Pawera	30	4525	2380	6905	296
2.	Astred	15	5192	435	5627	240

8.5 DISCUSSION

Astred red clover appears to have similar susceptibility to drought (Experiment 3) and intensive grazing pressure (Experiment 2) as Pawera. However, the increase in plant density in Astred in its second growing season in Experiment 1, where the grazing pressure was less than in Experiment 2, and the increase in plant density during Spring in Experiment 2, suggested that Astred has the potential for greater persistence than Pawera when conditions are suitable for rooted plantlet production and survival (see Figure 8.1, Table 8.1). Similar results were reported by Smith *et al.,* (1993) in Tasmania. Therefore, like Pawera the persistency of Astred will be dependent on the intensity, frequency and timing of grazing (Cosgrove *et al.,* 1985).

When grazing pressure was high, and sometimes very high in the case of Winter break grazing (see Plates 8.1 and 8.2), parent plant density of Astred declined over time, despite evidence that some rooted plantlets were produced in Autumn and survived into Spring (see Figure 8.1). This decline in plant density demonstrated that management based on what a mixed pasture can withstand, but not taking into account the requirements of red clover, is as detrimental to Astred as it is to crown type red clovers (Cosgrove *et al.,* 1985; Hay *et al.,* 1989). Although both Astred and Pawera maintained an effective plant population of greater than 30 plants m^{-2} (Hay *et al.,* 1989) under the grazing management in Experiment 1, the increase in plant density of Astred in the second growing season showed that this cultivar can produce new vegetative plants in a grazed pasture. Further monitoring of the Experiment will be required before the capacity of Astred to maintain an effective plant density in a pasture beyond the 2 to 3 years usually achieved by Pawera can be determined. Grazing management that ensures Astred produces rooted plantlets which persist will need to be developed.

When grown as spaced plants, Astred has produced up to 150 rooted plantlets per plant so it has the potential to develop a self-sustaining plant population (see Chapter 3). The dynamics of plantlet and rooted plantlet initiation and development are poorly understood at present, but plantlets and rooted plantlets mainly develop in Autumn on branches of old stems (see Chapter 4).

Astred is an early season, early flowering type of red clover (see Chapter 5) of Mediterranean origins, and has a comparable growth pattern to cv. Grasslands Colenso and cv. Grasslands Hamua, whereas cv. Grasslands Pawera peaks and flowers later in the growing season (Rumball, 1983). The higher Winter activity of Astred could be useful in a mixed forage for calving cows or for adding quality and bulk to the first silage cut of the season (see Table 8.2). The making of silage had no serious adverse effect on the number of rooted plantlets produced.

8.6 REFERENCES

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9. DISCUSSION♣

♣ A modified version of this chapter has been published in *Proceedings of the New Zealand Grassland Association* **61**, 121-126 (Hyslop *et al.*, 1999). (See Appendix I).

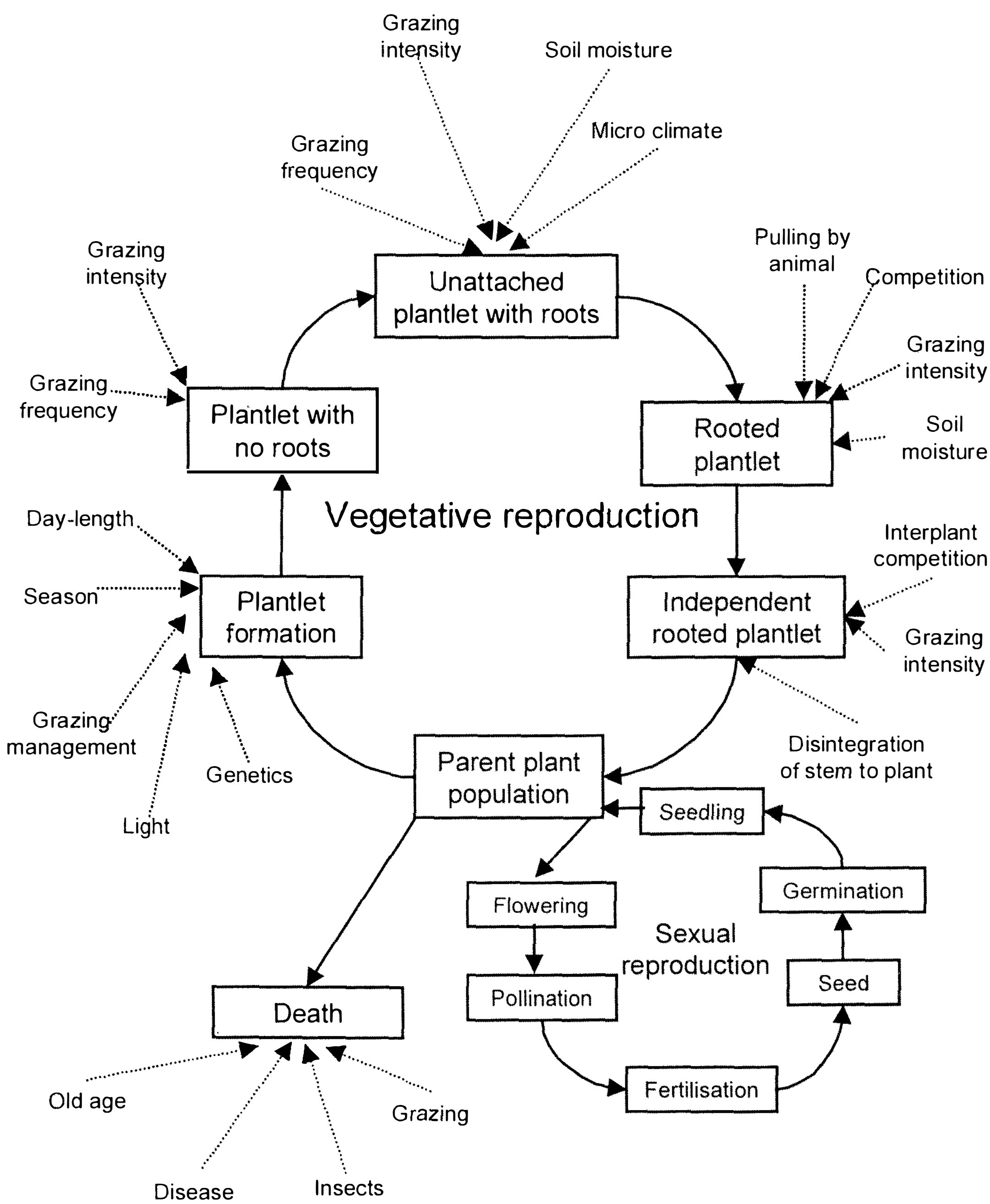
9.1 INTRODUCTION

Red clover has proven to be an excellent forage species in terms of yield and forage quality (see Chapter 2), but long term persistency, particularly in grazed swards has always been a problem with crown forming cultivars (see Chapters 2, 6 and 8). Selections exist that have the ability to vegetatively reproduce (Smith, 1992; Smith, 1993; Smith *et al.,* 1993) and these could improve the persistency problem (see Plate 1.1). However, there is little information on vegetative reproduction in *Trifolium pratense* and the mechanisms involved have not been previously studied.

A series of nine field and spaced plant trials were conducted to study the growth, productivity, perennation, and vegetative reproduction of Astred, Gualdo and F2419, vegetatively reproductive red clover cultivars and selection. Results are discussed in detail in Chapters 3 to 8. This Chapter contains an integrated general discussion of the results from those Chapters.

The ability of vegetatively reproductive red clover to produce rooted plantlets will result in the re-consideration of the use of red clover in New Zealand pastoral systems if suitable cultivars are released. The main reason that has limited the use of crown type red clovers has been poor persistence, which vegetatively reproductive red clovers overcome. This discussion begins with a diagrammatic representation (see Figure 9.1) of the main factors involved with vegetatively reproductive red clover population dynamics and plantlet formation, which provides a framework for the overall discussion. Proceeding sections focus on some of the aspects that influence the reproductive ability of these red clover types and how their reproductive mechanism works. The possible uses of vegetatively reproductive red clover types in New Zealand and practical management recommendations will also be discussed, followed by the main conclusions from this evaluation.

Figure 9.1 Vegetatively reproductive red clover parent plant population dynamics with particular reference to the vegetative reproductive cycle.



9.2 PLANTLET FORMATION, AND FACTORS AFFECTING PLANTLET FORMATION AND SURVIVAL

There was a large morphological variation within and between the selections of the red clovers studied, which affected the capability of each selection to vegetatively reproduce (see Chapters 3, 4 and 5). However, the same general mechanisms of plantlet production were found to apply to all selections and will be discussed as such, except where specific differences exist.

Astred and Gualdo plant types produced aerial stems from crown buds in the Spring that grew up to 500mm in height when sown as a pure sward, and in Autumn bent to touch the soil surface. Plantlets were initiated from buds on these stems. Plantlets then produced roots and grew into small independent plants as the stem connection to the parent plant disintegrated. F2419 stems crept along the ground and produced nodal roots as they grew, provided the surface soil was moist. Plantlets mainly formed at branch points above an already existing nodal root, then the connection to the parent plant decayed.

The year to year production of rooted plantlets was highly variable between and within all experiments and was greatly influenced by a combination of secondary factors such as grazing history and environmental conditions. Despite this, there is clear evidence that rooted plantlet production is highly seasonal and occurs mainly in the Autumn/early Winter (see Chapters 5 and 7). The reasons for this seasonality are likely to be a combination of complex chemical switches within the plant (possibly related to maturity and daylength), and environmental influences such as soil moisture and temperature.

More rooted plantlets were produced per parent plant when the surface soil moisture content was high (22.2% gravimetric surface soil water content). The evidence suggests that not only is a moist soil surface required for effective rooted plantlet production but, more importantly, a humid micro climate in the lower strata of the sward is also needed, as a high proportion of plantlets form roots out of contact with the soil (aerially rooted) (see Plate 5.5) which leaves the new roots vulnerable to desiccation. Similar conditions are required for nodal rooting in white clover (Ueno, 1982; Thomas, 1984; Stevenson *et al.,* 1985). Very few plantlets formed roots when the soil moisture content was low (0.3 rooted plantlets/parent plant at 3.71% gravimetric surface soil water content).

Spaced plants of Astred produced the same number of rooted plantlets by early Winter after half had been subjected to a dry Autumn and then irrigated, compared to the remainder which had been always been under moist conditions (see Chapter 5). There was a strong compensatory increase in rooted plantlets once moist soil surface conditions were present. Plantlets with no roots rapidly produced roots to anchor the new plantlet to the soil surface in two weeks or less once the soil was moist (see Chapter 5). Provided moist soil conditions exist for a long enough duration at some stage during the Autumn/early Winter period rooted plantlet production can occur. If a dry period follows a wet period, only rooted plantlets with well developed root systems are likely to survive. Even though plantlets are able to produce roots in less than two weeks under ideal environmental conditions (high moisture, no cutting or grazing), often in a grazed sward the process takes longer (4 to 8 weeks) because a new plantlet has to be grown from a stem bud due to the removal of plantlets without roots by the grazing animal.

Smith *et al.,* (1993) concluded that Astred grew strong stems in late Summer and rooted plantlets formed on these stems after flowering. This observation suggested that the relative degree of flowering and plantlet production might be determined by resource allocation within the plant. However, when Astred parent plants were left to mature to either full flower or had their flowers removed before the florets opened, there was no difference in the number of rooted plantlets produced, but the number of rooted plantlets per parent plant was highly variable (see Chapter 5). Sexual reproduction (flowering) appeared not to be connected to rooted plantlet production, but it is also possible that the flower heads were removed too late to affect resource allocation and, therefore, little or no treatment difference existed. It is probable that some interaction exists between plantlets forming at the stem nodes (as found with white clover branch initiation, Sackville Hamilton, 1982) and the developing flower head at the stem apex, such as assimilate partitioning (see Plates 5.3 and 5.4) or auxin distribution or concentration. Where the plantlets form along stems, or on branches off them, may also be affected by the number and position of individual flower heads.

Under conditions without grazing or cutting, Astred produced most of its rooted plantlets on primary stems, or branches off these stems (mainly quaternary branches), that grew from the parent plant crown in September. In contrast, F2419 produced rooted plantlets equally on stems originating from July through to September (see Chapter 4). Morphological factors such as stem length, stems per plant and viable nodes per metre of stem may also be important, but significant differences in rooted plantlet numbers were measured between Astred and Gualdo (see Chapter 4) when these factors were similar. It is unclear why at a given time some nodes remain dormant, some produce plantlets and some produce growth that elongates into a branch, all from similar parent material. Some factors found with white clover nodal development (Newton *et al*., 1990) may apply.

Plantlets are formed closer (within 100mm) to the parent plant under grazing than when ungrazed (Chapter 4, Smith *et al.,* 1993). Except in lax grazed swards, it is unlikely that they are formed on quaternary or tertiary stems, because very few of these stem types are present in hard grazed swards. With no grazing, plantlets form anywhere between 50-600mm from the parent plant crown (mean distance of 300mm), possibly in response to increased light levels (as found with white clover, Beinhart, 1963; Sanderson, 1966) at the extremities of the parent plant, activating otherwise dormant nodal buds, and limiting competition between the parent plant and offspring (rooted plantlets).

The seasonal nature of rooted plantlet production and the location of rooted plantlets on mainly stems of a specific age, or branches off them, means the grazing management of these vegetatively reproductive red clovers will have a direct effect on the formation and production of plantlets and rooted plantlets (see Figure 9.1).

Grazing intensity had more effect on the number of plantlets and rooted plantlets produced than did grazing frequency, with interactions between these variables at some grazing events (see Chapters 6 and 7). Intense (down to 5cm post grazing height, see Plate 7.2) grazing removed a high proportion of the Spring grown stems that would have produced branches where the majority of plantlets are formed in the following Autumn. Even if these Spring grown stems are left in the lower strata of the sward, they must remain alive and undamaged (e.g. by treading) to be able to effectively produce plantlets in the Autumn/early Winter. Grazing, particularly intense grazing, either removed or damaged and pulled out plantlets and rooted plantlets, or destroyed, by removal of herbage, the moist micro-climate that plantlets require for effective rooting to take place (see Chapter 7). Rooted plantlets were produced closer to the parent plant on short lengths of primary or secondary stem as grazing intensity increased, and these had less likelihood of being damaged or removed by the grazing animal. However, larger rooted plantlets survived grazing better than small rooted plantlets (see Chapter 3). As with many plants (Curll, 1980; Thomas 1987), the removal of apical dominance of these primary or secondary stems may increase the number of nodes that produce a plantlet, but if this is done by grazing, a net loss of potential plantlet sites will result from excessive stem removal by the animal, even under lax grazing (see Chapter 7).

Increased light levels in the lower strata of the sward from herbage removal by grazing could also increase the number of stem nodes that initiate plantlets.

Grazing frequency was most important in Autumn/early Winter when plantlets were producing nodal roots to anchor the newly rooted plantlet into the soil surface. Spelling from 1/3/97 for 8 weeks may have been partly responsible for the higher number of rooted plantlets established at the 8 week frequency (66 rooted plantlets m^{-2}) than when the spelling period was 4 or 6 weeks (see Chapter 6), as similar numbers of potential plantlets (either with no roots or aerially rooted) were present in the 4 and 6 week grazing frequencies (see Chapter 7). Production of rooted plantlets in grazed swards mainly took place over distinct periods of time. For example, 31% of the total rooted plantlets produced from January to June, rooted in May under the 8 week grazing frequency and lax grazing. Large numbers of plantlets with no roots, aerially rooted plantlets, and rooted plantlets were produced but did not survive grazing (see Chapter 7).

In mixed swards, rooted plantlet numbers decreased (from 64 to 14 rooted plantlets m^{-2}) with increasing grazing pressure (intensity and duration), and were affected by untimely, intensive grazings, particularly during Winter and Spring (see Plates 8.1 and 8.2) when the management of the companion species compromised the ideal management of the red clover (see Chapter 8). Rooted plantlets still formed in very competitive sward environments (i.e. with *Lolium perenne* and *Trifolium repens*) and some selections (F2419 types) would be more suited to this type of environment because of their ability to vegetatively reproduce at lower post-grazing residual heights than Astred (see Chapters 3 and 4, Plate 3.1).

A greater understanding of which factors are involved in the formation of plantlets is required. For example, the effects of temperature, photoperiod, light intensity, assimilate partitioning and biochemical growth regulators need to be established. A complete demographic study of a vegetatively reproductive red clover population needs to be conducted under grazing to trace the vegetative reproductive mechanism into at least its second generation.

9.3 PARENT PLANT PERSISTENCE AND POPULATION DYNAMICS

Astred parent plant populations tended to have more parent plants per square metre than Pawera, and these individual parent plants were significantly smaller in above ground mass and taproot mass after two seasons of grazing (see Chapter 7). On an area basis, Astred had similar taproot mass to Pawera under comparable grazing managements (see Chapter 7). With the possible addition each year of new parent plants of red clover to the overall plant population via the introduction of mature (one year old) rooted plantlets, presumably over time, even with the cyclic nature of replacement (Autumn) and death (mainly in the Winter), parent plant populations in Astred swards would reach an equilibrium (see Figure 9.1). New rooted plantlets would only become established where competition for resources with existing parent plants allowed.

Astred swards had consistently greater persistence than Pawera after 2 or 3 years in terms of the number of parent plants per square metre when independent rooted plantlets were counted as parent plants after one year of age (see Chapters 6 and 7). Astred and Pawera showed a similar decrease in parent plant density in response to grazing intensity and frequency for two years, but by year three there was a significantly greater percentage of Astred parent plants surviving in the grazing managements that encouraged rooted plantlet formation. This demonstrated that rooted plantlet production was able to maintain the parent plant population of Astred though the application of favourable grazing management methods. The greatest rate of parent plant death was over Winter and early Spring, particularly in the hard and 4 weekly grazed treatments of both cultivars.

Astred sward persistence relative to Pawera was also found to be partly associated with the longevity of individual parent plants, but this only became apparent after 2.6 years of age (see Chapter 7). It is unclear whether Astred parent plants possess a greater tolerance to grazing, or are less susceptible to internal breakdown, disease or insect attack than Pawera.

9.4 PRODUCTION

When Astred was sown in either a mixed or pure sward, annual production was similar to that of Pawera for the first year (see Chapter 6), suggesting that it is possible for a vegetatively reproductive red clover to have at least a similar annual production to that of currently used crown type red clovers. Production decrease was directly associated with a declining parent plant density and parent plant health where grazing pressure was intense and frequent (see Chapter 8), which was also found for lucerne (Smith, 1970; Brownlee, 1973; FitzGerald, 1974; Southwood *et al.*, 1975; Leach, 1979) and crown type red clovers (Brougham 1959; Brougham 1960; Cosgrove *et al.,* 1985; Sheath *et al.,* 1989; Frame 1990; Hume *et al.,* 1995). Where rooted plantlets were not produced due to high grazing pressure, shoot production from the resident parent plants also decreased (see Chapter 7). Production levels could be maintained under careful management with effective annual replacement of dead parent plants by rooted plantlets

Because of Astred's Mediterranean origins, it has a high level of Winter activity similar to that of Grasslands Colenso and Grasslands Hamua. Astred always accumulated significantly more herbage (up to 2540 kgDM/ha) from Autumn to the first Spring grazing than did Pawera which is a later flowering, and more Summer active type (see Chapters 6 and 8). Vegetative reproduction would also be of advantage in Summer active plant types such as Pawera.

9.5 APPLICATION TO AGRICULTURAL SYSTEMS

The perennial nature of these vegetatively reproductive red clover plant types make them an attractive option as a persistent, high quality feed compared to crown type red clovers. Every extra year of grazing increases the economic viability of sowing vegetatively reproductive red clover as opposed to crown type red clover. Pure swards of more erect types (e.g. Astred) could be used as truly perennial, high quality forage crops for finishing young stock over the late Winter, Spring and Summer periods. The high Winter growth activity of Astred could also be useful in mixed species swards for calving cows or for adding quality and bulk to the first silage cut of the season in dairy systems. Prostrate selections, and particularly creeping types (e.g. F2419) (see Chapter 3), could be extensively sown in perennial pasture mixtures in hill country sheep and beef systems for year round production (see Plate 3.1). The Summer growth of these red clovers could be used for flushing ewes in Summer dry areas if selections of low oestrogen content were used.

The experiments in this thesis suggest the following will provide the best management of Astred swards. Pure swards should be grazed every 4 to 6 weeks to a post grazing height of 10cm (lax grazing, see Plate 7.3) over Spring and Summer to ensure sufficient stems survive for the production of plantlets and ultimately rooted plantlets. When rooted plantlets are formed in Autumn/early Winter (when soil moisture levels increase), grazing frequency should be decreased to 6 or 8 weeks, and only continue until Winter if sown on free draining soil. Swards should not be grazed over the Winter period except in areas where sufficient Winter growth occurs with free draining soil, and never below 10cm post grazing height. Grazing in Spring can commence when sufficient herbage has accumulated and soil conditions allow (see Figure 9.1 for the sequence of plantlet formation).

Similar management recommendations apply to Astred in mixed swards but this would be complicated and compromised by the management of the companion specie or species which would often over-ride the above management, particularly in Spring and Autumn. Nevertheless, for plantlet production it is critical that sufficient stem survives from Spring to Summer on Astred parent plants, so lax grazing management is the most important factor.

Conclusive evidence found in this study (see Chapters 3, 6, 7 and 8) and by Smith *et al.,* (1993) of the production, persistence and true perenniality of vegetatively reproductive red clovers make the plant type worthy of further investigation. It was previously suggested that plants of this habit would have poor production (Rumball, 1983) due to the high cost of allocating assimilate to vegetative reproduction. This could still be possible in some selections (e.g. F2419, see Chapter 3) but was not the case in Astred or Gualdo, possibly due to the highly seasonal production of new plantlets.

Large morphological differences exist between plant types that carry the vegetatively reproductive trait, from very prostrate to 50cm in height when grown as a pure sward (e.g. Astred). Breeding and selection programs need to concentrate on splitting these plant types into useful groupings under the environmental and management conditions for which they are to be used, for example: high DM production; cutting and conservation; competitive mixed swards on high soil fertility; low growing, low fertility swards with high grazing pressure; and Winter active, high fertility swards with low grazing pressure. The competitive mixed sward with high fertility, and the Winter active, high fertility, low grazing pressure groupings have the greatest potential in New Zealand agricultural systems. Possible selection traits could be: increased vegetative reproductive ability, disease resistance, insect tolerance, increased production, Winter activity, formononetin content and plant height.

From this research there is no doubt that the persistency, productivity and diverse range of vegetatively reproductive red clover selections could make a significant contribution to New Zealand agriculture as truly perennial, taprooted forage legumes in many different farming systems.

9.6 CONCLUSIONS

The most important conclusions drawn from this evaluation of some selections of vegetatively reproductive red clovers are:

i) The general persistence of vegetatively reproductive red clovers as a population was superior to "crown" type red clovers when management and environmental conditions allowed the formation of rooted plantlets.

ii) Plantlets were initiated in Autumn from nodes of above ground stems, or branches off those stems, that grew in Spring and then had contact with the soil.

iii) Lax (10cm post grazing height) grazing management of vegetatively reproductive red clovers is critical in Spring (to maintain stems) and Autumn (to allow plantlets to root) for rooted plantlet production.

iv) Plantlets with no roots form in Autumn and only produce roots when moist soil surface conditions exist.

v) Rooted plantlets can be produced on parent plants in mixed or pure swards.

vi) The general productive performance of the vegetatively reproductive red clover selections was similar to crown type red clovers and warrants a breeding and selection programme.

9.7 REFERENCES

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APPENDIX I

1. VEGETATIVELY REPRODUCTIVE RED CLOVERS (*Trifolium pratense* L.): AN OVERVIEW♣

♣ The chapter has been published in *Proceedings of the New Zealand Grassland Association* **61**, 121-126 (Hyslop et al., 1999).

1.1 ABSTRACT

Although the persistency of red clover (*Trifolium pratense* L.) can be a problem when grazed in mixed and pure stands, it is still regarded as a valuable source of high quality Summer feed in some farming systems. Vegetatively reproductive red clover selections offer promise to solve this persistency problem but their growth, perennation, reproductive ability and productivity have not been investigated in New Zealand farming systems. This paper is an overview of a four year research programme involving nine experiments investigating these areas. The vegetatively reproductive red clovers evaluated were Astred, F2419 and Gualdo. There was no difference between Astred and Grasslands Pawera, a crown type red clover, in the total herbage accumulated over the first growing season under 4, 6 and 8 week grazing frequencies and lax and hard grazing intensities. After three years of grazing, significantly more Astred parent plants were alive compared to Pawera when rooted plantlets were counted as parent plants after one year. Varying numbers (0 - 66 plantlets m^{-2} per yr) of rooted plantlets were produced by Astred in mixed and pure swards. More rooted plantlets were established per parent plant under wet surface soil conditions. Astred produced 57% of its Autumn rooted plantlets on primary stems developed in September, or branches off these stems. Comparisons of the growth and morphology of Astred, F2419 and Gualdo are presented. Red clover selections that are vegetatively reproductive offer benefits to New Zealand farming systems and could solve some of the persistency problems currently experienced with red clover.

Keywords: Astred, F2419, Gualdo, rooted plantlet, vegetatively reproductive, *Trifolium pratense*

1.2 INTRODUCTION

Red clover (*Trifolium pratense* L.) has been widely reported to have poor persistency when grazed in mixed and pure stands, with total loss of plants after 2 to 3 years (Brougham 1959, 1960; Harris *et al.* 1980; Cosgrove *et al.* 1985; Sheath *et al.* 1989; Frame 1990; Hume *et al.* 1995). However, red clover is a valuable source of high quality Summer feed in some farming systems. All red clover cultivars used in New Zealand grow from crowns and predominantly reproduce from seed, although some reproduce vegetatively to a small extent (Hyslop *et al.*, 1996). The crowns are susceptible to treading damage and fungal infection, particularly in Winter, resulting in poor persistence (Hay *et al.* 1989). Pure stands of red clover usually only persist 3 to 4 years.

Red clover selections exist that have prostrate stems that produce clonal plants at nodes which may improve persistency in grazed pastures. Smith *et al.* (1993) reported the chance discovery of vegetatively reproductive red clover plants by the Department of Agriculture, Tasmania in 1975, in seed accessions gathered from Crato, Portugal. These plants produced strong, prostrate stolons (here termed stems) in late Summer, and clonally formed daughter plants (here termed rooted plantlets). These formed from unelongated growth at lateral stem buds, below which nodal roots formed after flowering. Whether all are capable of producing nodal roots is unknown. The rooted plantlets usually appeared within 100mm of the parent plant, and as they developed roots, the connection to the parent plant degenerated. The cultivar Astred was developed from this material (Smith *et al.* 1993).

Astred was more persistent than conventional cultivars when grazed with sheep over a three year period in Tasmania, retaining 55% ground cover after 3 years compared to 5%, 2% and 0% for Grasslands Turoa, Grasslands Hamua and

Redwest, respectively (Smith *et al*. 1993). In New Zealand, Astred was the most prevalent of the introduced legumes in experiments on easy hill slopes and continued to persist under grazing (Orr *et al*. 1996).

Because of the relatively recent commercial release of the first two vegetatively reproductive red clovers, cv. Astred in 1992 and cv. Gualdo in 1998, published results and information on these cultivars and the plant type in general are limited. Gualdo was bred out of accessions collected in Italy by J.P. Baresel, Strada di Calledro, Italy. F2419 is an accession from material gathered in Portugal and provided by W. Rumball, AgResearch, Palmerston North. Hyslop *et al*,. (1996) concluded that the perennation and productivity of such red clovers required further evaluation to determine their potential in New Zealand farming systems, and the vegetative reproduction mechanism needed to be understood clearly before appropriate management strategies could be developed. The objective of this paper is to present an overview of the research programme that followed. Most research was based at Massey University, Palmerston North, and focused on the production, persistence and demography of the vegetatively reproductive red clover selections, Astred, F2419 and Gualdo. Pawera was used as a comparison as it is widely considered the most persistent red clover cultivar under New Zealand conditions.

1.3 MATERIALS AND METHODS

All experiments are fully described in Hyslop (1999). An overview of the objectives, designs and methods of the nine experiments follows.

The persistence of individual parent plants and the annual and seasonal herbage production of Astred were measured in three field experiments to examine the productiveness of Astred under practical farming conditions:

Experiment 1 (Massey University) compared the herbage production and parent plant persistence of pure swards of Astred and Grasslands Pawera when grazed by sheep. There were 12 treatments (two cultivars, Astred, Pawera; two grazing intensities, hard (5cm), lax (10cm); three grazing frequencies, 4, 6 and 8 weeks) over a three year period in a randomized complete block design (48 x $118m^{-2}$ plots). Two hundred parent plants were tagged one year after sowing in the Astred and Pawera 6 week hard grazed treatments and these were monitored over time (see Figure 1.1). Experiment 2 was sown (27/9/96) as a mixed sward at Dannevirke in a completely randomised design with Grasslands Pawera as a comparison cultivar, and grazed by replacement dairy heifers (Hyslop *et al.*, 1998). Parent plant persistence and rooted plantlet production were measured. Experiment 3 was sown as a mixed sward in a completely randomised design at Massey University, N° 1 Dairy Farm. Parent plant persistence, rooted plantlet production and seasonal production of Astred were measured. Experiment 4 was sown at Hawkes Bay in 1996. However, all plants died in the Summer of 1998 due to lack of soil moisture and the findings will not be reported here (Hyslop *et al.*, 1998).

Experiments 5 to 9 examined morphological, environmental and grazing management effects on the formation of plantlets and rooted plantlets:

Experiment 5 was a split plot design imposed on pure swards of Astred at Massey University that had previously had one season of hard grazing (5cm residual height) by sheep, with grazing frequencies of 4 and 8 weeks. The treatments were hard and lax grazing (residual heights of 5 and 10cm respectively) at 4 and 8 week grazing intervals. The plantlets and rooted plantlets that developed on parent plants over six months (January-June) were counted.

Experiment 6 (Massey campus block) consisted of 60 spaced Astred seedlings in a completely randomised design with no cutting or grazing. Flower buds were removed from 30 plants as they formed and the remaining plants went to full seed set to determine whether flowering affected rooted plantlet formation.

Experiment 7 investigated what effect dry or wet soil surface moisture levels had on rooted plantlet formation over time, in a completely randomised design with 30 parent plants per treatment, under a rainout shelter with irrigation and no grazing at AgResearch, Palmerston North. The soil surface around the parent plants was initially wet or dry, and then after 29 days, water was also applied to the dry treatments (termed dry/wet). All parent plants were regularly watered separately from the surrounding soil watering treatments by the use of waterproof barriers.

Experiment 8 (Massey University) consisted of 15 plants of Astred and 15 of F2419 planted as spaced plants (3m x 3m) in a completely randomised design with no cutting or grazing. Parent plant stem age and number, and rooted plantlet number and position on the parent plant were assessed to identify where rooted plantlets form on parent plants.

Experiment 9 (Massey University) compared morphological characters of two vegetatively reproductive red clovers, Astred and Gualdo, in a completely randomised design with 30 plants of each cultivar. Plants were destructively harvested at 10 months of age.

1.4 RESULTS AND DISCUSSION

1.4.1 Annual production

Astred and F2419 can both reproduce vegetatively via rooted plantlets (0-80/m^2) in mixed and pure swards under New Zealand farming conditions (Experiments 1, 2, 3 and 5, Hyslop *et al.,* 1996; Hyslop *et al.,* 1998;). When Astred was sown in either a mixed or pure sward, annual production was similar to that of Pawera (Experiments 1 and 3) suggesting its production is not unduly compromised by its vegetative reproductive mechanism. Annual production of Astred (13592 kgDM/ha) was not significantly affected by the grazing treatments in the first year although there was a difference of 3361 kgDM/ha between the highest producing treatment (Astred, 6 weeks, lax) and the lowest producing treatment (Astred, 8 weeks, lax) in Experiment 1. In a mixed sward at N° 1 Dairy Farm, Massey University (Experiment 3) Astred's annual production was 4040 kgDM/ha (s.e.m. ± 272) in 1996/1997 and 2300 kgDM/ha (s.e.m. ± 223) in 1997/1998 (see Table 1.1). However, the hard and frequent grazing management of the sward resulted in poor survival of rooted plantlets so the production decline was related to the decline in parent plants of Astred. Astred declined from 30 plants m^{-2} in 1996 to 4 plants m^{-2} in 1998, except for an increase in Winter/Spring 1997 when rooted plantlets that were formed in Autumn (14 plantlets m^{-2}, s.e.m. ± 2.4) were counted as independent parent plants, but these rooted plantlets only slowed the rate of parent plant decline to 4 plants m^{-2} in 1998.

1.4.2 Seasonal production

Because of Astred's Mediterranean origins, it has a comparable growth pattern to Grasslands Colenso and Grasslands Hamua. All Astred grazing treatments in Experiment 1 accumulated significantly more herbage from sowing to the first Spring grazing on 20/9/96 than did Pawera ($P<0.001$) (see Table 1.2). Differences ranged from an extra 2540 kgDM/ha for the Astred, 8 weeks, hard treatment to 1910 kgDM/ha for the Astred, 6 weeks, hard grazed treatment.

Table 1.1 Seasonal production of pure Astred and Pawera swards grazed every 6 weeks to 5cm post grazing height in the first year from sowing (kgDM/ha). (Expt 1).

Harvest date	Astred	Pawera	s.e.m.
8/3/96	0	0	-
20/9/96	4540	2620	204
3/11/96	1760	2990	276
9/12/96	1850	2270	277
20/1/97	3400	3440	442
24/2/97	1240	850	357
7/4/97	800	1450	227
Total	13590	13620	

The high Winter growth activity of Astred could be useful in mixed forage swards for calving cows or for adding quality and bulk to the first silage cut of the season (see Figure 1.1). Astred out-produced Pawera in a mixed pasture sward by 1000 kgDM/ha (s.e.m. ± 296) when cut for silage in Spring (7/11/97), after a conservation period of 102 days, at Dannevirke (Experiment 2) (Hyslop *et al,.* 1998). In contrast, Astred only produced 7.7% of the silage production in Experiment 3 when cut 1.75 years from sowing with a conservation period of 53 days, but parent plant density was only 15 plants m^{-2} (Hyslop *et. al.*, 1998).

In the first year from sowing (1996) in Experiment 3, Astred produced 1480 kgDM/ha (s.e.m. ± 72), or 25% of total sward production in Spring (September, October, November) and 2410 kgDM/ha (s.e.m. ± 165), or 55% of total sward production in Summer (December, January, February) (see Table 1.1). Seasonal production of Astred decreased in 1997, as parent plant numbers declined, to 1000 kgDM/ha (s.e.m. ± 49) or 32.3% of total production.

Table 1.2 Percentage contribution to seasonal yield by Astred sown in a mixed sward (Expt 3).

	Season	Astred	s.e.m.
1996	Autumn	7.4	2.0
	Winter	6.8	1.7
	Spring	25.0	1.2
	Summer	54.7	3.8
1997	Autumn	12.8	4.7
	Winter	10.2	1.8
	Spring	14.8	1.6
	Summer	32.3	1.1
1998	Autumn	4.6	1.6

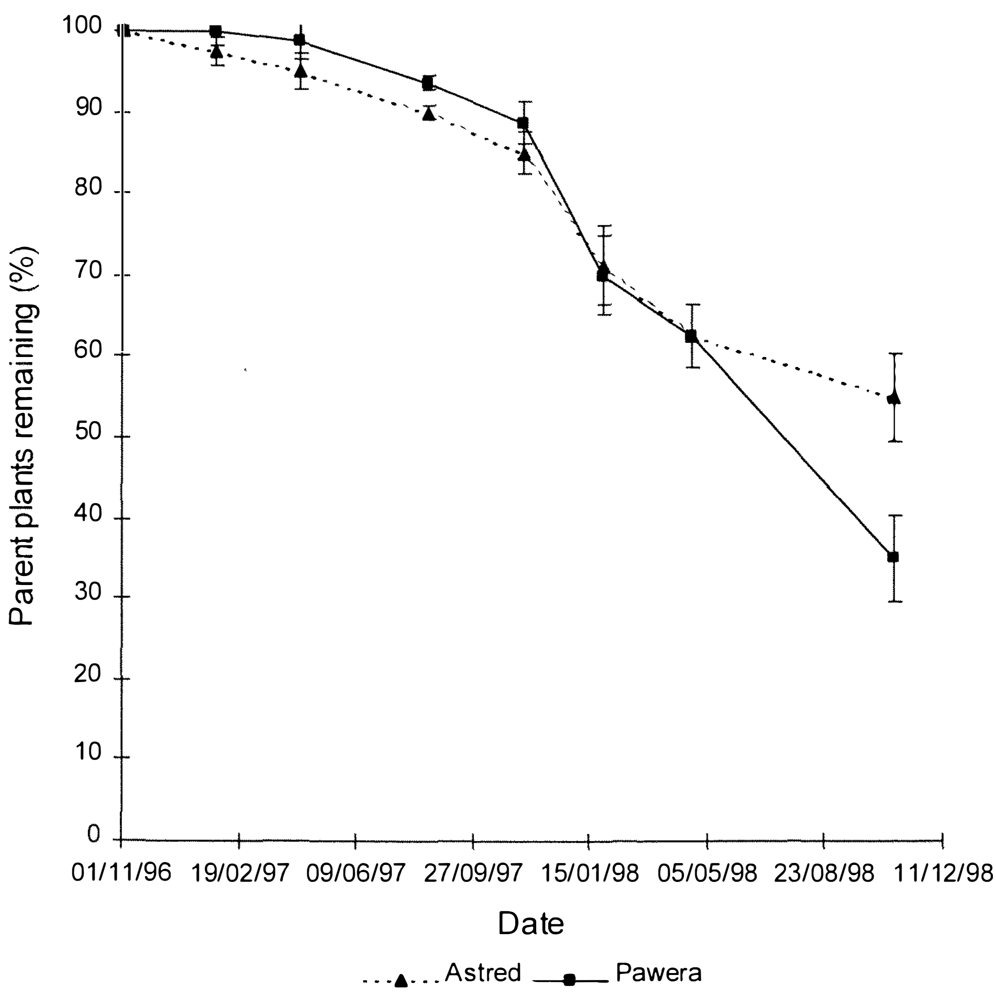
1.4.3 Parent plant persistence in pure swards

Astred and Pawera parent plant persistence were compared in six grazing treatments in Experiment 1. Astred swards had significantly higher ($P<0.001$) percentages of parent plants remaining (rooted plantlets counted as parent plants after one year of age in Astred swards) in all grazing treatments after two growing seasons compared to Pawera (which can only remain the same or decline because of nil parent plant replacement). By year three, there was a significantly greater percentage of parent plants surviving in the lax than the hard grazing intensity ($P<0.001$), and in the 8 week than the 4 week grazing frequency. The greatest rate of parent plant death was over Winter and early Spring, particularly in the hard and 4 weekly grazed treatments of both cultivars.

At 2.6 years of age, after two seasons of grazing, Astred also had significantly more originally sown parent plants than Pawera ($P<0.05$) (see Figure 1.1). At all earlier dates, the rate of original parent plant decline was the same for both cultivars. More Astred than Pawera parent plants were present at Dannevirke (Experiment 2) after two years ($P<0.001$). At the last count on 27/5/98, the parent plant densities of Astred and Pawera, were 36 plants m^{-2} and 30 plants m^{-2} (s.e.m. ± 0.87), respectively (Hyslop *et al.,* 1998).

Astred parent plant populations tended to have more parent plants m^{-2} than Pawera, but these parent plants were significantly smaller in dry mass after two seasons of grazing in comparison to Pawera (Experiment 1). Similarly, Astred (1.6 gDM/root) had lighter individual taproots across all treatments compared to Pawera (2.4 gDM/root, s.e.m. ± 0.197, $P<0.001$). Significantly ($P<0.001$) less taproot mass (0.60 kgDM m^{-2}, s.e.m. ± 0.09) remained in hard (5cm grazing residual height) and frequently grazed (4 week) treatments for both cultivars three years from sowing in Experiment 1 compared to all other treatments (average of 1.26 kgDM m^{-2}, s.e.m. ± 0.09).

Figure 1.1 Percentage of tagged parent plants remaining in Astred and Pawera pure swards that were grazed every six weeks between September and May each year to 5cm residual sward height by sheep). Vertical bars represent s.e.m. (Expt 1).



1.4.4 Plantlet formation

Astred and F2419 rooted plantlet production in Autumn was affected by the number of Spring grown stems on the parent plant that were still present, and by Autumn soil moisture levels (Experiments 6 and 8). Rooted plantlet size is also important for survival in grazed swards (Hyslop *et al.* 1996).

There is large morphological variation in the selections of red clover capable of vegetative reproduction, and some of these selections are able to reproduce vegetatively at lower post-grazing residual heights than Astred (Experiments 8 and 9). In Experiment 8 Astred produced most of its rooted plantlets on primary stems, or branches off these stems, that grew from the parent plant crown in September, whereas F2419 produced rooted plantlets equally on stems originating from July though to September (Table 1.3). In general, a high percentage of rooted plantlets developed on stems originating in Spring, but they also developed to varying extents on stems originating at other times, depending on the red clover selection (Experiment 8). Therefore, because of this seasonal effect, the grazing history of vegetatively reproductive red clover, particularly Astred, will affect plantlet and rooted plantlet formation. Hard grazing of Astred in Spring would appear to greatly diminish the sites available for plantlet formation.

Table 1.3 The frequency of rooted plantlets per 15 parent plants produced by Astred and F2419, on 6/6/97, from primary stems and branches off primary stems produced from 1/7/96 to 1/2/97. (Expt 8).

Month of primary stem growth	Astred	F2419
July	26	330
August	72	353
September	213	392
October	59	154
November	3	9
December	0	0
January	0	0
February	0	0

When Gualdo and Astred were compared morphologically, Astred produced significantly ($P<0.001$) fewer rooted plantlets per parent plant, had longer, wider leaves, thicker stems and was taller at 10 months of age. There were no significant differences in stem length, stems per plant, nodes m^{-2} of stem, total parent plant dry matter and stem, leaf and taproot dry matter.

The seasonal production of rooted plantlets was highly variable between and within all experiments. In the pure swards of Experiment 1 on 10/6/97, there was a significant interaction ($P<0.05$) between grazing frequency and grazing intensity for the number of rooted plantlets produced. Astred, 8 weeks, lax grazed produced 46% (66 plantlets m^{-2}) more rooted plantlets than Astred, 8 weeks, hard grazed (36 plantlets m^{-2}) and 56% (29 plantlets m^{-2}) more than Astred 6 weeks, hard grazed. Grazing intensities at the 4 week grazing frequency did not affect the number of rooted plantlets produced, with 43 and 42 plantlets m^{-2} (s.e.m. ± 5.1) for hard and lax treatments, respectively.

In a mixed sward (Experiment 2), rooted plantlet density of Astred (measured on 27/5/98) after two seasons of grazing was 64 rooted plantlets m^{-2} (s.e.m. ± 1.7) from a population of 32 parent plants m^{-2}. Silage was cut in the second Spring of the experiment. In contrast, when parent plants were under high grazing pressure (Experiment 3), only 14 rooted plantlets m^{-2} (s.e.m. ± 2.4) were counted from a population of 16 (s.e.m. ± 1.4) parent plants m^{-2}.

In Experiment 5, significantly more ($P<0.001$) rooted plantlets formed and survived grazing by sheep under lax grazing intensity at both grazing frequencies. Nine and 11 rooted plantlets m^{-2} (s.e.m. ± 1.5), respectively, were produced and survived grazing in May in the 4 weeks, lax and 8 weeks, lax grazed plots. These represented 31% of the total rooted plantlets produced between January and June. There was no significant difference in the number of rooted plantlets that survived grazing at the two grazing frequencies from January to June. We suggest that Astred swards should be grazed to maintain a post grazing height of 10cm over Spring and Summer to ensure sufficient stems survive for the production of plantlets and ultimately rooted plantlets. When grazing pressure was light, Astred and F2419 rooted plantlets were found up to 900 mm from their parent plant in one season.

Parent plants maturing to full flower did not change the number of rooted plantlets produced relative to parent plants with flowers removed ($P<0.9$) (Experiment 6). The number of rooted plantlets produced per parent plant varied from 0 to 48 with flowers removed and 0 to 19 with no flowers removed, with a high coefficient of variation of 140%. The frequency distributions of the number of rooted plantlets produced per parent plant under the two treatments were not significantly different ($P<0.2$). For example, a similar number of parent plants produced three rooted plantlets in both treatments. No relationship ($P<0.4$) was found by simple linear regression ($r^2 = 0.034$) between the number of flowers removed and the number of rooted plantlets produced per parent plant.

More rooted plantlets ($P<0.001$) were produced per parent plant in the wet irrigation treatment (5 plantlets/parent plant, 22.2% gravimetric surface soil water content) than the dry (0.3 plantlets/parent plant, 3.71% gravimetric surface soil water content) treatment from 25/2/98 to 26/3/98 in Experiment 7. After 26/3/98, surface irrigation was applied to all treatments. There was no difference in the total number of rooted plantlets produced by either wet/wet or dry/wet treatments by the end of the experiment. Rooted plantlets were formed at a significantly greater rate ($P<0.05$) for the first 2 weeks after 26th March in the dry/wet treatment than the wet/wet treatment. The time of onset and the duration of Autumn rain (comparable to the irrigation treatments imposed) would appear likely to affect the number and timing of rooted plantlets formed. There appears to be some capacity for plantlets produced during a dry spell to generate roots when the soil moistens.

1.5 CONCLUSIONS

With some small changes to current grazing management practices and suitable environmental conditions (moist Autumn soil conditions), vegetatively reproductive red clovers appear to be truly perennial forage legumes suitable for use in mixed swards and as forage crops. Like the crown type red clovers currently in use, the persistency of Astred, F2419 and Gualdo will be dependent on the intensity, frequency and timing of grazing, with a post grazing height of 10cm important in Spring to ensure that stems which generate plantlets and ultimately rooted plantlets, fully develop. Red clover selections that are vegetatively reproductive offer benefits to New Zealand farming systems. Their superior persistency over crown type red clovers is worthy of further research and development.

1.6 REFERENCES

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APPENDIX II

1. RAINFALL (mm) FOR HAWKES BAY SITE.

	1996	1997	1998
December	124	13	38
January	62	27	139
February	68	36	51
% of 10yr mean	**141**	**42**	**127**
March	50	102	6
April	59	30	22
May	48	83	39
% of 10yr mean	**157**	**215**	**67**
June	49	150	28
July	166	90	168
August	29	105	50
% of 10yr mean	**110**	**156**	**111**
September	15	76	5
October	15	78	29
November	12	25	37
% of 10yr mean	**27**	**115**	**46**
Total	766	881	485