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Effects of early grazing on the growth and development of red clover (*Trifolium pratense* L.)

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Abstract

Developments in red clover cultivars have shown that persistency issues that have limited the inclusion of red clover in New Zealand pastures in the past are now less of a concern. The seasonality of current New Zealand pastures can lead to poor summer production. Red clover (*Trifolium pratense* L.) offers high drought tolerant pasture along with strong yields and high quality herbage. The objective of this research was to compare recent red clover cultivars with an industry standard red clover cultivar and lucerne (*Medicago sativa* L.), determining the effects of timing of first grazing and grazing frequency.

Two experiments were conducted. The field experiment included two red clover cultivars, Grasslands Relish and Grasslands Sensation, and a lucerne cultivar, Grasslands Torlesse. Treatments consisted of a first grazing 10, 12 and 14 weeks after sowing and a final grazing at 28 weeks after sowing. The species were measured over the experiment for morphological development and production through the establishment period. The second experiment was conducted in a glasshouse which included three red clover cultivars Grasslands Relish, Grasslands Sensation and Grasslands Colenso. Defoliation treatments were applied at 1, 2 and 4 week frequencies while production and development were continuously measured including monthly destructive harvests to further measure total plant biomass content and allocation.

The two recent red clover cultivars showed better production than lucerne and the older red clover cultivar, and especially notable production was seen by Grasslands Relish during the field experiment. Grasslands Relish was able to be grazed earlier than Sensation giving more flexibility, as well as maintaining a high plant population from autumn through to spring. Timing of first grazing showed that it substantially affected yield but had no effect on plant populations. High frequency defoliation resulted in less total herbage mass production and suppression of unique cultivar characteristics, such as growth habit, that was shown in red clover cultivars that were defoliated less frequently.

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CHAPTER 1 - INTRODUCTION

Forage crops have an importance that is seen throughout the world, whether they are annual or perennial, for their ability to produce high quality feed during pasture deficits. Red clover (*Trifolium pratense* L.) has a significant importance as a pasture legume in temperate agricultural systems around the world (Frame, 2005; Sheaffer, Ehlke, Albrecht, & Peterson, 2003). The prevalence of red clover in terms of pasture legumes is only overshadowed by lucerne (*Medicago sativa* L.), also known as 'alfalfa' but will be referred to as lucerne for the entirety of this research, or white clover (*Trifolium repens* L.) depending on which temperate zone is concerned.

The potential for high yields and morphological ability to resist drought along with the nutritional benefits in agricultural systems provide the inclusion of forage crops in pastures valuable for conservation feed or in mixed pastures swards (Frame, 2005). Traditional New Zealand pasture mix of perennial ryegrass (*Lolium perenne* L.) and white clover is often limited in summer production. Red clover's summer yield and quality potential makes it ideal for short term pasture used for fattening livestock or conservation feeds, especially in drought prone areas (Kemp et al., 1999; Cosgrove & Brougham, 1985).

Red clover plays a large role in New Zealand pasture mixes but traditionally red clover does not have the same popularity as white clover which can be a better substitute in mixed swards and lucerne is more persistent as a pure sward in many areas. Limitations such as reproductive problems in ewes from the grazing of red clover along with the susceptibility to cause bloat in cattle, can be an issue particularly when grazing pure swards (Frame, 2005; Kemp et al., 1999). The overwhelming limitation that generated apprehension to the sowing of red clover in New Zealand for many decades was poor persistence past the point of three to four years, and so it was considered a short lived perennial for this reason (Ford & Barrett, 2011). The decline in plant population has been a fundamental focus of breeding efforts and research. Many limitations have been controlled or alleviated through international breeding progress but persistency still remains an issue.

Autumn pasture sowing is a method used throughout New Zealand to establish high quality forages in the form of perennial pastures or perennial forage crops. Due to

perennial tap-rooted forages having semi or complete dormancy during winter, the main management criterion for an autumn sowing is that the plants are established and a grazing is regularly expected before winter.

Recent developments through various international germplasm and breeding efforts have made positive progress in terms of red clover persistence (Ford & Barrett, 2011). Persistence in typical rotational grazing in mixed swards is particularly important in any New Zealand farming systems.

The timing of the first grazing in the important establishment stages of morphological development and the subsequent frequency of grazing are key considerations in terms of productivity of forage legumes.

Therefore the objectives of this research were to:

- i) Compare red clover and lucerne at the timing of first grazing after autumn sowing.
- ii) Compare recent diploid cultivars to older cultivars of red clover and recognise any variation in growth and development under a range of grazing frequencies.
- iii) Understand the role first grazing or grazing frequency play in the survival of traditional and recent cultivars of red clover and lucerne.

CHAPTER 2 - LITERATURE REVIEW

2.1. Introduction

The foundation of pastoral livestock production in New Zealand is a perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.) mix. The limitation of this common pastoral mix is the seasonality of production doesn't allow feed demand to be consistently met, specifically during warm months of the year and areas where drought susceptibility is high.

Forage legumes such as red clover and lucerne also have a significant role in agricultural systems in New Zealand and around the world. The capacity to provide drought tolerant pastures along with high yield potential and nutritive quality combats New Zealand pasture seasonality issues. Red clover and lucerne share many morphological characteristics though their uses and management techniques vary.

Red clover faces the key issue of persisting for no longer than four seasons in either a mixed or pure sward which leads to the wider use of white clover in New Zealand. White clover has the ability to produce numerous stolons and can produce seed in most farming systems in New Zealand under frequent livestock grazing. However, white clover does not possess the ability to tolerate water stress as the absence of a taproot leaves it vulnerable in summer months.

The recent developments in red clover cultivars have shown promising persistence under grazing while maintaining the favourable livestock attributes. Recent red clover cultivars including 'Grasslands Relish' and 'Grassland Sensation' have small amounts of published data, but have demonstrated their successful inclusions in grazing and persistence experiments.

The purpose of this literature review is to provide background information on the abilities of tap-rooted legumes, red clover and lucerne, with particular attention to red clover and the potential of recent cultivars available to the agricultural industry. It will aim to illustrate the morphological growth habits of the legumes through establishment into mature growth under typical management techniques.

2.2. Red Clover Background

2.2.1. Background

Red clover is a short lived perennial legume originating from Asia and Southern Europe. A crown on the soil surface or slightly above produces the plant base from which vegetative growth will begin from the crown buds. Upright hollow stems are produced with distinctive pubescence which continues to the leaves, the trifoliate leaves also have pale crescent shaped mark in the centre of the upper side of the leaf (Frame, 2005). Introduced into Europe in the 1500's then onto New Zealand after European colonisation, red clover was then used in a range of different treatments including over sowing of bush burn and mixtures with various pasture and grain species (Campbell, 1930). More recently, red clover is more typically grown with companion crops or as a monoculture (Abberton & Marshall, 2005).

Globally red clover is regarded as the most important in the clover family as it is the most widely distributed (Frame, 2005; Sheaffer, Ehlke, Albrecht, & Peterson, 2003). It is able to be grown on a range of soil types and requires medium to high fertility, both as a pure sward or in grass mixtures. Red clover is a tap rooted species which can penetrate deep into the soil profile, like lucerne, which gives it an advantage over ryegrass and white clover as water stress can be prevented for further lengths of time (Frame, Charlton, & Laidlaw, 1998). Ideal temperature for growth is 20-25°C though red clover has quite a strong resistance to temperature and can survive 15°C either side of this threshold (Frame et al., 1998; Kemp, Matthew, & Lucas, 1999). Poor persistence has been the major limitation for that of red clover in New Zealand pastoral systems along with marginally low seed yield, susceptibility to fungal diseases and other factors have been the major breeding aims (Abberton & Marshall, 2005).

Red clover population numbers are declining from the day of establishment (Frame, 2005; Kemp et al., 1999), which affects pastoral systems efficiencies as persistence drives profitability (Ford & Barrett, 2011). Two to three seasons of relatively high persistence can be expected for red clover under good management, as grazing or cutting frequency and intensity affect persistence (Frame, 2005; Kemp et al., 1999). Ford & Barrett (2011) provides a further detailed overview of persistence breeding in New Zealand. In the 1930's 'Grasslands Turoa' was one of the first two cultivars released which was specifically selected for persistence under grazing. Breeding efforts

were focused on tetraploid red clovers after demonstrating the best productivity and persistence and disease resistance in the late 1950s. In 1973 ‘Grasslands Pawera’ was the result with improved persistence and resistance to insects and fungi through phytoestrogens (Anderson, 1973). ‘Grasslands Pawera’ was also considered more competitive than any of the other older cultivars from when it was introduced until the early 1990s when other cultivars started to rival or surpass its superiority.

Oestrogenic compounds existing naturally in red clover, however, manifested in reduced conception rates and infertility in pregnant ewes after grazing red clover swards (Anwar, 1994; Kemp et al., 1999). Breeding efforts to produce a more prostrate plant has been the goal in more recent years, as well as reduced phytoestrogens (Ford & Barrett, 2011). ‘Grasslands Sensation’, Plant Variety Right granted in 2002 was one of the first cultivars to be released with these attributes, and more recently (2012) ‘Grasslands Relish’ which are both included in the field and glasshouse experiments of this study (Claydon, Rumball, & Miller, 2003; Ford & Barrett, 2011).

2.2.2. Grasslands Relish

There is limited published literature concerning ‘Grasslands Relish’ as it is such a new cultivar however it has been included in a mixed and pure sward trial performed by Ford and Barrett (2011) as an experimental variety. Relish’s growth habit is semi-erect when compared to the more prostrate growth habit of ‘Grasslands Sensation’.

The single year row trial performed by Ford and Barrett (2011) as part of their 4 year trial found Relish compared favourably against 10 industry standard cultivars and trial cultivars, having both a higher density and top growth score. When Relish was sown in the pure sward trial it was in the top quartile in terms of favourable attributes but not in the top 10% (J.Ford, personal communication, April 15, 2014). The full potential of the Relish was not captured by the single year trial, as strong persistence and yield were seen more clearly in the three and a half year mixed grass and clover sward trial (Ford and Barrett, 2011).

Ford and Barrett (2011) grass and clover mixed trial demonstrated that Relish had usual establishment characteristics in the mixed sward. However, after establishment continuous growth was displayed under grazing when compared to industry standard cultivars and was given the top growth score from year two until the end of the trial in year four which can be seen below in Table 2.1.

Table 2.1: Red clover establishment, growth score and survival under rotational grazing by cattle in replicated mixed sward plot trial at Aorangi Farm near Palmerston North, New Zealand. “Establishment” was scored from 1 (low) to 5 (high). “Growth scores” are the visual red clover yield scored prior to each grazing, from 1 (low) to 10 (high). “Survival” is the percentage of red clover plants surviving after three and a half years, from (Ford & Barrett, 2011)

Entry	Establishment	Growth Score				Mean	Survival (%)
		Year 1	Year 2	Year 3	Year 4		
AberRuby	3.0	4.8	5.2	2.3	1.3	3.6	10
AC Endure	3.7	5.3	5.4	5.9	2.0	5.1	10
Broadway	4.0	8.3	7.5	7.3	5.8	7.4	36
Claret	4.0	4.1	4.5	4.0	1.5	3.9	4
Crossway	3.7	7.0	7.2	5.7	3.5	6.2	21
G27	4.3	6.1	6.0	4.8	3.5	5.3	17
GF148	2.3	4.4	4.7	2.7	2.5	3.7	11
Grasslands Colenso	3.0	7.2	4.9	3.3	0.8	4.3	17
Grasslands Pawera	3.0	6.2	6.8	5.8	4.0	6.0	25
Grasslands Relish	4.0	6.9	7.9	8.8	8.5	8.1	60
Grasslands Sensation	3.7	4.2	4.5	3.3	3.3	3.9	12
Tuscan	3.0	6.3	6.6	6.1	2.5	5.8	21
XCG128	3.3	8.2	8.2	7.7	4.5	7.6	27
XCP89	4.7	9.0	7.1	7.5	5.5	7.4	48
XEC124	2.3	4.4	4.7	2.7	2.5	3.7	5
XLC4	3.3	7.0	6.2	6.4	6.0	6.4	29
XLS5	4.0	7.3	7.7	7.0	6.5	7.2	48
XSP59	4.0	7.7	8.6	7.4	7.7	7.9	48
Mean	3.5	6.5	6.4	5.7	4.2	5.9	22
LSD (5%)	1.3	2.2	1.9	2.2	2.6	1.7	21

Relish also exhibited significantly higher persistence than most other cultivars at the end of the trial as mean survival was 60%. This persistence was seen after medium to long rotational cattle grazing throughout the trial. The formononetin levels (the oestrogen compound that reduces conception rates in ewes) of Relish was lower (0.10%) than all other cultivars in the trial except Crossway (0.06%).

2.2.3. Grasslands Sensation

The full history of Grasslands Sensation was described by Claydon et al. (2003). Hundreds of cultivars were assessed in the late 1970's early 1980's for their agricultural potential, with persistence one of the most sought-after attributes. Four cultivars from Switzerland were some of the most persistent. After the selection process was completed from the various lines, 'Grasslands Sensation' was initiated as a new cultivar. Usual establishment characteristics have been seen for the cultivar (Ford & Barrett, 2011) and a pure sward field experiment by Claydon, Rumball and Miller (2003) which is among the limited published data on 'Grasslands Sensation' concluded that the growth habit of 'Grasslands Sensation' is semi-erect with respect to other red

clover cultivars, but is rather erect with an open structure compared to ‘Grasslands Colenso’ and gives a less dense appearance (Claydon et al., 2003; Ford & Barrett, 2011). The erect growth habit elevates ‘Grasslands Sensation’ higher in the pasture canopy and it is therefore well suited to cattle grazing.

The formononetin levels are similar to that of Colenso and lower than that of ‘Grasslands Pawera’ (Claydon et al., 2003). Ford and Barrett’s (2011) trial too revealed ‘Grasslands Colenso’ and ‘Grasslands Sensation’ were relatively similar although ‘Grasslands Sensation’ also showed a slightly lower level of formononetin. Overall formononetin levels in the Ford and Barrett (2011) trial were much lower than levels seen in Claydon et al. (2003) trial for all cultivars, likely due to differences in environmental conditions varying between trials. ‘Grasslands Colenso’ is an early flowering diploid cultivar, that is included in this research as one of the glasshouse experiment cultivars, it has now been taken off the market but was released as a new cultivar in 1988 (Hickey & Harris, 1989). At the time of release Colenso showed many beneficial attributes that were unsurpassed by other cultivars, its higher persistence in a grazed mixed sward being one of the most notable along with more cool season growth giving a more even production spread (Claydon, E., & Anderson, 1993; Hickey & Harris, 1989). Colenso also has lower formononetin levels than both Hamua and Pawera which had previously been the dominant cultivars used by the industry (Claydon et al., 1993).

Claydon, Rumball and Miller (2003) saw ‘Grasslands Sensation’ out yielded over summer and spring establishment, also late summer and early autumn from the tetraploid cultivars ‘Grasslands Pawera’ and G27 and diploid cultivar Colenso which were controls in the trial. It held a yield advantage over the tetraploid cultivars into the second summer and yielded the best in the third spring. Yield fell off in the fourth summer along with all other cultivars in the trial except for ‘Grasslands Pawera’ which was relatively consistent because of the low yield in previous seasons which can be seen in (Table 2.2). ‘Grasslands Sensation’ also exhibited relatively poor growth scores in the first year of Ford and Barrett’s (2011) mixed sward trial, this is likely attributed to the cultivar not being suited to these type of production conditions.

Table 2.2: Red clover, plot/grazing trial 1990-94. Herbage production (kg/ha DM), from (Claydon et al., 2003)

	Establishment and early 1st summer	Late summer/early autumn	2nd spring	2nd summer	3rd spring	4th summer
'Colenso'	2528	2741	3190	3461	1988	124
'Pawera'	2218	2559	1198	3579	1117	1480
'G27'	2489	2749	2317	3874	2672	1590
'G40' ('Sensation')	3273	3126	3096	5246	2839	1167
LSD 5%	995.2	314.5	566.3	373.7	987.6	1033.0

Sensation has much higher yields than Colenso in total herbage yield and summer yield was up to 50% higher. It was also mentioned that sheep had grazed the trial hard in the third and fourth year and sometimes to ground level (Claydon et al., 2003). Which could be responsible for the lowered yield heading into the final season as Grasslands Sensations growth habit would be less able to accommodate this grazing intensity. Persistence was still high in the Grasslands Sensation plots (78%) while Colenso 28% red clover at the end of the fourth summer, however a high amount of variability was seen by the fourth summer.

'Grasslands Sensation' has an earlier flowering date than the 'early flowering' Colenso or Hamua, and 1-2% of the flowers are white. White clover was an on-going weed in the trial that caused issues while other weeds could be controlled. In terms of pest or disease 'Grasslands Sensation' plots displayed small amounts of leaf rust late into the fourth year. Seed yield potential from 'Grasslands Sensation' demonstrated 13% less than 'Grasslands Colenso' in its first year, however this was under undesirably wet conditions (Claydon et al., 2003).

2.2.4. Conclusion

According to J. Ford (personal communication, April 15, 2014) it was concluded that 'Grasslands Sensation' was bred as a predominately pure sward cultivar and a cut and carry type clover, and Relish was selected more on its ability to compete in mixed grass swards and be directly grazed. Most breeding around the world is focused on pure swards of red clover sown in rows. Pure swards in New Zealand are grown for higher return crops such as maize, chicory, plantain, or sold to farmers as silage and other feed sources. Thus red clover has taken a backwards step in New Zealand, as well as having a high cost seed cost due to relatively low seed production. Additional reasons relate to other species being able to out compete red clover when grown as a conservation crop. This is due to New Zealand farmers being rather traditional in

terms of divergence from regular pasture sward compositions until a certain pasture component is proven in a farming system (Hunter, 1994).

‘Grasslands Sensation’ has the ability to produce high forage yields throughout the season including some cool season growth as well as good persistence until the fourth season. Its growth habit makes it best suited for cattle grazing or conservation feed, provided residuals aren’t too low to inhibit regrowth (Claydon et al., 2003). ‘Grasslands Relish’ has demonstrated high persistence and performance that surpasses almost all of the current cultivars of red clover under grazing. Its ability to fit into a regular rotational grazing frequency and remain persistent into the fourth season of growth is something that has been sort after in red clover cultivars for decades. The potential for creating production efficiencies and increasing animal performance will be a great addition to the New Zealand pastoral industry (Ford & Barrett, 2011).

2.3. Red Clover & Lucerne Establishment

Many of red clover and lucerne establishment characteristics are similar as they have closely related morphology. For this reason they will both be described together and any contrasting characteristics will be noted.

2.3.1. Morphology

After germination of the perennial legume seed, there is an emergence of the (seminal) root followed by the hypocotyl elongating and dragging the two cotyledons through the soil surface (Weber, Borisjuk, & Wobus, 2005). The terminal shoot is then positioned between the two cotyledons. It promptly grows true leaves attached to the primary stem from which each leaf subtends an axillary bud (Thomas, 2003). Initial branches are then developed from both the cotyledonary buds and the bottom axillary buds of the primary stem. These buds allow red clover to elongate their petioles and elevate their leaves above the ground, whereby the primary shoot stops growing after around 10 leaves are produced (Spedding & Diekmahns, 1972; Thomas, 2003). Lucerne uses elongation of the primary stem to elevate its leaves off the ground and has comparatively small petioles (Figure 2.1). Non-flowering stems elongate vertically in both red clover and lucerne but much more elongation is seen in lucerne (Thomas, 2003).

Contractile growth is found in perennial legumes whereby the legume pulls back the first node (cotyledonary buds and low axillary buds) below the soil surface, which continues for the duration of the plants life (Genrich, Sheaffer, & Ehlke, 1998). The amount of contractile growth varies from around 10 mm for red clover and 20 mm for lucerne (Thomas, 2003). For this reason the lucerne crown can be expected to be slightly lower in the soil profile than red clover giving it a marginal advantage from disturbance (Frame et al., 1998; Thomas, 2003), though it is often hard to distinguish.

Tap root forming legumes including red clover and lucerne have the ability to source water further in the soil profile than stoloniferous plants, giving them an advantage in times of water stress (Frame et al., 1998). However, this is not the only advantageous attribute a taproot possesses. The ability to search deeper in the soil profile for water in the early stages of development holds a large advantage for future persistence (Thomas, 2003). The tap root acts as a storage organ for the plant containing water, carbohydrate, starch and protein (Bowley, Taylor, & Dougherty, 1984; Taylor & Quesenberry, 1996). Red clover and lucerne benefit over many other legumes as deep root systems are required early in establishment in dry environments as carbon allocation to rhizomes, stolons or other requirements for plant carbon allocation are either not a priority or not exercised (Thomas, 2003). The tap root becomes the central point from which all vegetative growth will be produced, beginning from the crown (Thomas, 2003). Stems also have the ability to root from the nodes when they are in close contact with moist soil and become a daughter plant (Brock, Hyslop, & Widdup, 2003). The inverse relationship between roots being produced from nodes and tap root production further benefits red clover as longevity is extended with the life of the tap root. Well drained soils limit nodal root production and therefore more carbon allocation to tap root (Thomas, 2003). Adventitious roots are formed which are an important part of the root system, especially as the plant ages and the tap root slowly degrades over time (Frame, 2005). Traditionally, vigour declines over the life of the stand through diseases in the crown and root rots among many other contributing factors; adventitious roots then in some cases provide virtually all rooting functions (Spedding & Diekmahns, 1972). For this reason there has been a positive correlation between presence of adventitious roots and plant persistence (Montpetit & Coulman, 1991).

Growing points are found primarily from the crown buds which are slightly above but often below the soil surface, this allows them to avoid damage from grazing or other climatic conditions such as snow or fire (Humphreys, 1980).

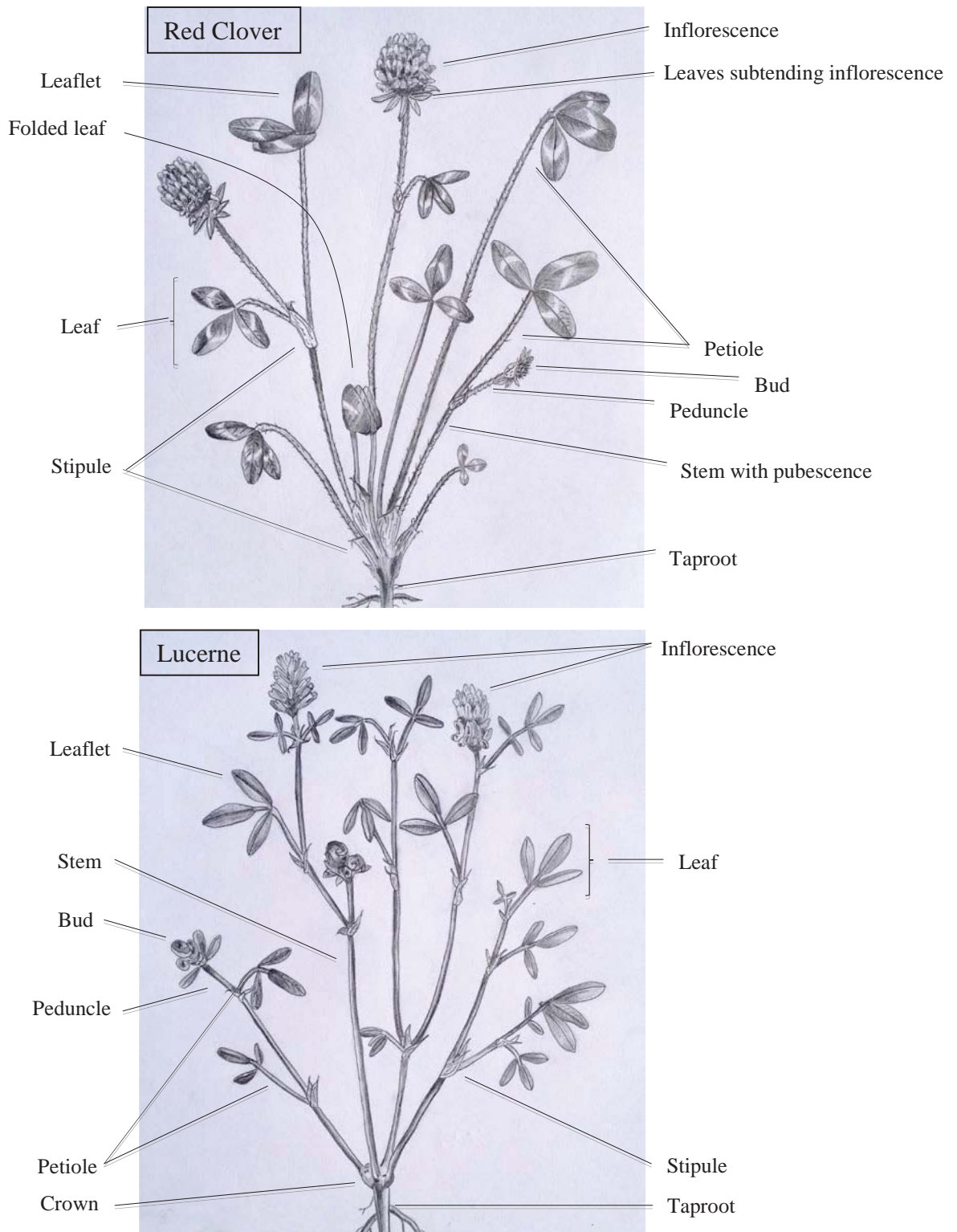


Figure 2.1: Red clover and lucerne morphological diagram (Martin, 2014)

2.3.2. Field Morphology

Red clover is able to establish rapidly and is relatively tolerant, in comparison to lucerne, to adverse soil fertility and drainage conditions (Sheaffer et al., 2003) though excessively wet or acidic soils will not serve as a good environment for either species (Frame, 2005). In the establishment year red clover is not a strong interspecies competitor. Lucerne is also a poor competitor during establishment, though often non-aggressive companion crops are sown to prevent weeds, and once a canopy is developed lucerne becomes much more competitive (Frame, 2005). Vigorous growth can be gained if forceful competitors, in most cases grasses, are avoided in either pure or mixed stands (Frame, 2005; Kemp et al., 1999).

Well cultivated uniform seed bed and a shallow sowing depth of 10-15 mm for both red clover and lucerne are required for good establishment. Rolling soil pre and post sowing maybe required for soil coverage of seeds, along with adequate weather conditions for successful establishment (Frame, 2005). Time of sowing for pasture species should be early autumn or late spring this is because soil temperatures for legumes are closest to the ideal needed for germination. However, clovers and lucerne do not have the same temperature dependence as other legumes, such as lotus and vetch, as clover can be sown later in autumn and lucerne earlier in spring. This can sometimes prove to be difficult for some farming systems as taking farming area out of production early autumn may not be practical (Hampton, Kemp, & White, 1999). Grasses and weeds are the primary reason for poor establishment. Therefore, grasses should be grazed before they reach a height that will interfere with the light interception of the crop, causing a restriction in carbohydrate supply to the root system (Haynes, 1980).

2.3.3. Rhizobium Symbiosis

The rooting bacteria or rhizobium that has a symbiotic relationship with legumes allows for atmospheric nitrogen fixation. Red clover and lucerne are capable of growing without rhizobium but the additional soil nitrogen input is advantageous to the plant. The ability to compete with grasses in a sward is low without the addition of rhizobium in a mixed pasture situation, as ineffective nodulation severely reduces the beneficial characteristics of including a legume (Greenwood & Pankhurst, 1977).

Appropriate rhizobium should be used when planting red clover and lucerne as specific species require compatible strains on rhizobium. This can be in the form of inoculation of seed or if similar crops have been recently grown in the desired establishment area there will likely be enough rhizobium in the soil (Spedding & Diekmahns, 1972). More recently (Lowther & Kerr, 2011) have shown that of New Zealand generally has adequate Rhizobia present in the soil for various clovers and referred to inoculation as an insurance policy against nodulation failure and a greater importance on inoculation is regarded for recently cleared grassland, and land continuously cropped in maize.

Root nodule formation requires an optimal pH 6-6.5, water availability along with appropriate P and K levels are required for seedling development (Frame, 2005). Occurrence of this formation happens as rhizobium bacteria invade the end of the plants root hair. An infection thread is formed and rhizobia enter each cell around the infected area which encourages nodule development (Grove & Carlson, 1972). The removal of P or K, because of plant and bacteria requirements, needs replenishment to benefit the legume. For these reasons it is sometimes advised that grazing or cutting is timed to suit the legume in mixed stands as legumes generally benefit more than a grass companion (Beuselinck et al., 1994).

2.3.4. Temperature

Soil temperature has a strong effect on legume establishment as cool soil temperatures below 10°C or prolonged high temperatures are detrimental to not only establishment but growth and persistency (Spedding & Diekmahns, 1972). Germination will still occur at low temperatures but as temperature moves away from the ideal so does the germination rate. At low temperatures it is low germination rates that are accountable for poor establishment; decreasing soil moisture is the reason for poor establishment at high soil temperatures (Hampton et al., 1999). Red clover has strong winter hardiness but immature plants that have less than 6 leaves tend to be susceptible to sub-zero temperatures (Frame, 2005). Atmospheric temperature is ideally between 20-25 °C for red clover growth for day time temperature (Kemp et al., 1999) and around 18 °C for night time temperature though night temperature doesn't determine yield as much as day time temperature (Spedding & Diekmahns, 1972). Lucerne requires similar or slightly higher temperatures for establishment growth and certain cultivars

can survive temperatures well below -20 °C after they go through a hardening process and become dormant (Frame, 2005). Less nitrogen fixation is facilitated at higher temperatures especially in red clover which could be attributable to greater sensitivity to temperature variation (Spedding & Diekmahns, 1972).

2.4. Red Clover Growth and Development

Red clover has the ability to be grazed in production systems by cattle or sheep, though greater tolerances to grazing pressures have been a focus for breeding progress (Abberton & Marshall, 2005). It is also common, especially in highly arable countries, for red clover to be cut for hay or ensiled (Abberton & Marshall, 2005; Sheaffer et al., 2003).

2.4.1. Competition and Persistence

Red clover growth does decrease after the first season as competition amongst other factors cause it to loose productivity and persistence (Frame, 2005; Kemp et al., 1999). The productivity after 2 to 3 seasons is limited and often stands can be entirely out competed (Ford & Barrett, 2011; Kemp et al., 1999). Both monocultures and mixed stands have the ability to be highly productive when competition isn't high (Frame, 2005). It is important to note that the behaviour of red clover can be dramatically changed by the addition of a grass species relative to a pure sward, for example strong monoculture cultivars have a tendency to perform poorly in a mixed sward for various morphological reasons (Hickey & Harris, 1989). Red clover is often grown with a grass companion, whereby grass species vary from country to country as the main criteria for companionship is avoiding overly competitive grass species (Frame, 2005).

2.4.2. Seasonal Growth

Red clover cultivars have varying amounts of cool season growth (Kemp et al., 1999). Largely, red clover doesn't express an abundance of cool season growth which is amplified in cold areas; plants are essentially dormant in winter (Frame, 2005; Kemp et al., 1999). However, summer production is high due to the red clover being able to source water lower in the soil profile causing a delay in water stress (Kemp et al., 1999).

2.4.3. Creeping vs Spreading growth form

Growth of both reproductive and vegetative shoots from the axillary buds formed on older shoots from the crown. ‘Creeping type’ red clovers were introduced to assist persistence problems which had very prostrate shoots that avoid grazing and grow close to the ground. ‘Spreading type’ red clover which can root from the nodes as the plants are very decumbent and daughter plants can continue as the parent plant dies (Brock et al., 2003).

2.4.4. Growth habit

There are two principal growth habits of a legume: prostrate or erect, and some varieties fall into a category somewhere in between and are often referred to as semi-erect (Spedding & Diekmahns, 1972). This is true for red clover as cultivars come in a range of these growth habits (Kemp et al., 1999). White clover is a prime example of prostrate growth as its stoloniferous growth keeps it close to the ground and tolerant of severe grazing (Frame, 2005). As for lucerne it is a prime example of erect growth especially in the first year as the meristematic apex sits high above ground level (1-3cm), and intensive grazing in the first year can be very destructive to the stand (Spedding & Diekmahns, 1972). Red clover cultivars have predominately prostrate growth habits which mean that they can be susceptible to lodging as the plant matures (Frame, 2005). Growth habits of the red clover cultivars included in both experiments ‘Grasslands Relish’ and ‘Grasslands Sensation’ vary between each other. Grasslands Relish’s semi-erect growth habit was specifically selected to have more erect growth as it was originally rather prostrate; this enabled the cultivar to be marketed as a substitute for ‘Grasslands Colenso’ which is also semi erect. Cultivars such as Broadway and Crossway are more prostrate and have had difficulties with seed production because of this growth habit (J.Ford, personal communication, April 15, 2014).

2.4.5. Defoliation

The grazing management of red clover is confined to rotational grazing, this means that ideally a six week grazing frequency, a 20-25 cm pre grazing height and 8 cm residual should be targeted (Frame et al., 1998; Kemp et al., 1999). Grazing residuals lower than 4-5 cm jeopardizes crown buds, along with overly frequent grazing of 4 weeks or less can also cause crown bud and root starch depletion causing decreased

yield (Kemp et al., 1999). Over-grazing in autumn should be avoided as nitrogen and carbohydrate reserves in the roots are then over used and with the natural depletion during winter, stands can suffer poor persistence (Frame, 2005). High frequency grazing should then be avoided, and well-timed rotational grazing should be considered a priority management objective in order to sustain plant population numbers (Kemp et al., 1999). This is because grazing frequency is inversely related to plant growth and persistence, so as grazing becomes more frequent total dry matter (DM) yield declines along with persistence (Frame, 2005). Under infrequent grazing rotation red clover can produce 12 tonnes of dry matter per hectare through the spring and summer months (Kemp et al., 1999).

A trial conducted by Cosgrove and Brougham (1985) included a red clover and ryegrass mixture. Results after the trial displayed the mixed sward was unaffected by seasonal periods of frequent grazing (summer or winter) compared to yearlong infrequent grazings, though species composition was affected. This can cause future persistency problems and the area that the experiment was conducted allows for frequent grazings (near Palmerston North on Kairanga sandy loam soil) but in different areas of New Zealand this type of treatment may not be able to be facilitated as treading damage among other factors could affect the mixed sward.

2.4.6. Morphology

Red clover is a natural diploid but there are numerous autotetraploid cultivars that have been developed synthetically (Abberton & Marshall, 2005). Red clover varieties are classified by their ploidy level and the flowering date (Kemp et al., 1999). This is in contrast to white clover which is classified by leaf size, whereby smaller leaf size generally allows for a higher capacity of continuous grazing, while greater stolon density also is a large contributor to persistence (Abberton & Marshall, 2005). However, early-flowering red clover cultivars produce more annual herbage in spring and less in summer (Kemp et al., 1999), with two relatively equal cuts followed by lower yielding cuts. Late flowering cultivars such as Grasslands Pawera which produces 50% of their yield in summer, and 30% in spring, the first cut having a higher proportion of the yield and often being more persistent than early flowering varieties (Abberton & Marshall, 2005; Frame, 2005). European breeders use both diploid and tetraploid cultivars, and the US uses predominately diploid cultivars. Neither ploidy

level has been concluded to be dominant for positive grazing or pest resistance traits in New Zealand. Tetraploid cultivar Grasslands Pawera was developed with advanced persistence under grazing and pest resistance and released in 1973 (Anderson, 1973; Ford & Barrett, 2011). However, diploid cultivars such as ‘Grasslands Relish’ and ‘Grasslands Sensation’ are now demonstrating some of the strongest attributes in terms of persistence under grazing, low formononetin levels and pest resistance (Claydon et al., 2003; Ford & Barrett, 2011).

2.4.7. Breeding

Along with most other forage crops red clover has been bred for animal performance, pest resistance, persistence, and high yields. Variation in red clover cultivars are produced as breeding programmes typically consist of phenotypic or recurrent selection which causes a heterogeneous production (Tucak, Popovic, Cupic, Spanic, & Meglic, 2013). Breeding programmes for red clover haven’t had the same concentration as white clover or lucerne which correlates to its prevalence around the world (Frame, 2005). However, genetic gains for red clover have been similar to those seen in lucerne as similar breeding techniques are used (Tucak et al., 2013).

Genetic gains have been relatively slow in terms of yield when compared to other crops such as maize. The focus has been placed on a large set of economically beneficial traits but many are not specifically correlated thus causing a lag or negative genetic yield progress. In addition, slow or non-efficient breeding selection systems being used have contributed to slow genetic gain. Genetically variable and divergent genotypes, in search of preferred traits can be attributed to the success of breeding efforts as variability is essential for predicting genetic advance (Tucak et al., 2013).

2.5. Red Clover Benefits

In terms of clovers, red clover doesn’t receive the same attention when it comes to studies and germplasm improvement compared to white clover. However, the close relationship between model legumes in terms of forthcoming advancements can enable red clover to continue to progress as a forage legume (Abberton & Marshall, 2005).

As mentioned in (the Red Clover & Lucerne Establishment section) the tap root gives many beneficial advantages from deeper water extraction to energy storage (Bowley

et al., 1984; Frame, 2005; Taylor & Quesenberry, 1996). In terms of drought survival the tap root is a critical morphological adaption as the soft mesomorphic leaves and young stems have high water loss, yet are also the high quality feed plant components (Thomas, 2003).

2.5.1. Nitrogen Fixation

Intensive agriculture has a strong relationship with the use of synthetic fertilisers, particularly nitrogen. It is said that synthetic nitrogen can be held accountable for significant human population growth, and it is estimated that close to half of the world is being fed from the use of synthetic nitrogen production (Erisman, Sutton, Galloway, Klimont, & Winiwarter, 2008). Soil nitrogen can be considered as a major limiting factor for global plant production, but system losses are leading to a degradation of the environment, which is a key issue to be addressed. The potential for biological fixation of atmospheric nitrogen has gained more attention as it attempts to reduce the environment issues caused by synthetic fertiliser (Warembourg, Lafont, & Fernandez, 1997). As mentioned forage legumes including red clover and lucerne possesses the ability to fix atmospheric nitrogen through a symbiotic relationship with the bacteria genus *Rhizobium*. The rhizobia bacteria infect root hairs of the legumes and form nodules where atmospheric dinitrogen N_2 is fixed and made available to the legume (Frame, 2005). The symbiotic relationship allows the bacteria to provide nitrogenous compounds to the host plant while accepting a substrate (a place to live) and carbohydrates from the host plant (Spedding & Diekmahns, 1972). This proves to be a large cost reduction for the environment and economic cost reduction for many farming systems as legumes are capable of fixing up to 800kg/ha per year under the most favourable of conditions. However, management of nitrogen fixing crops also is needed to ensure that large amounts of fixed nitrogen aren't leached after animal consumption or through other farming exercises. High nitrogen fixation rates are generally associated with low organic matter content as this increases nitrogen fixation (Moot, 2003). More common nitrogen fixation rates would be 150-250 kg/ha, of which it is normal for close to a third of this to be used by companion crops in a mixed stand via the animal (Abberton & Marshall, 2005; Spedding & Diekmahns, 1972). Nitrogen production is a high energy demanding task for both symbiotic plant-bacteria relationships and synthetic production. Biological fixation may still have

efficiencies to gain to reduce underutilised nitrogen as leaching can still occur at notable rates. However, biological fixation is a priceless tool that agriculture producers can use as a supplement synthetic fertilisers; this also provides a much more sustainable agricultural system which cements clovers environmentally conscious perception (Abberton & Marshall, 2005).

2.5.2. Conservation use

Red clover is widely used across the world as conservation feed in the form of hay and silage (Abberton & Marshall, 2005; Sheaffer et al., 2003). The ability to be grown as a monoculture unlike other clovers such as white clover, along with the infrequent defoliation characteristics allow for conservation cropping (Abberton & Marshall, 2005; Frame, 2005). However, red clover is also commonly sown with companion crops which are generally grasses without overly aggressive nature (Frame, 2005).

2.5.3. Animal Performance

Red clover allows high quality feed to be available in summer when other species are water stressed (Kemp et al., 1999). Red clover that is lush and leafy is capable of having 80% dry matter digestibility (Spedding & Diekmahns, 1972).

Higher animal liveweight gains have been attributed to greater summer and autumn growth of red clover compared to common ryegrass and white clover pastures (Cosgrove & Brougham, 1985). A trial performed by (Fraser, Speijers, Theobald, Fychan, & Jones, 2004) illustrated that lambs grazed on red clover had significantly higher liveweight gains than those fed ryegrass or even lucerne. This enables the slaughter time to be earlier, as well as higher dressing out percentage (ratio of liveweight to carcass weight) was found in the lambs that grazed red clover and in turn increasing producer's profit margin.

Livestock grazing red clover have a much higher intake than when grazing grasses (Fraser et al., 2004; Spedding & Diekmahns, 1972), attributable to the lower cell wall content and higher soluble carbohydrates available (Steinshamn, 2010). At the same stage of maturity and digestibility, intakes are found to be higher than what is found for ryegrass (Frame et al., 1998). Forage legumes are able to be broken down rapidly to a small particle size that is capable of passing through the rumen. The rapid rate of passage allows the forage legumes to have higher nutritive value than ryegrass, and

higher intake can occur, thus giving them higher digestibility and reduced methane production (Dewhurst, Delaby, Moloney, Boland, & Lewis, 2009; Waghorn, Shelton, & Thomas, 1989).

Along with growth rates red clover both grazed and ensiled has enhanced milk yield in cows in contrast with grasses. Red clover is also able to beneficially increase levels of polyunsaturated fatty acids in milk and meat (Dewhurst et al., 2009). Many grazing animals such as sheep, cows and deer have a strong preference for clover as a pasture but also in silage or hay. Clovers also display a stronger preference over grasses in mixed pasture swards (Frame et al., 1998; Fraser et al., 2004; Van Dorland, Wettstein, Aeschlimann, Leuenberger, & Kreuzer, 2007).

2.5.4. Nutritive Value

Red clover is comparatively nutritious forage high in protein, minerals and soluble carbohydrates. Like most pasture species red clover's nutritive value will decline with age as increasing amounts of structural plant material is formed in contrast to more easily digestible material such as leaves and young growth (Frame, 2005). Nutritive value of red clover tends to change depending on stage of growth and maturity, as young leafy herbage can have a dry matter digestibility of 80%, primary growth can reach 70% digestibility and regrowth once established is usually less than 70% digestible. Primary growth and regrowth red clover digestibility is often lower than that of ryegrass at the same harvest date but protein content is superior (Spedding & Diekmahns, 1972). The increase in Neutral Detergent Fibre (NDF)¹ structural material decreases crude protein, although the decrease in digestibility as a result of maturity is less than what is seen in grasses (Thomas, Gibbs, & Tayler, 1981). This means that increasing the frequency of harvests will increase that quality but it will sacrifice yield (Frame, 2005). An enzyme present in red clover is thought to be responsible for the superior protein supply by enhancing the fermentation process (Jones, Muck, & Hatfield, 1995). The high protein component of red clover can be economically supportive to many farming systems as fewer supplements are needed on farm (Abberton & Marshall, 2005). Nitrogen degradability reported for red clover is higher in pasture than in wrapped silage bales (Aufrère, 2002) which can hold true for all types of conservation production (Frame, Charlton, Laidlaw, 1998). The process of ensiling

¹ that measures most structural components in plant cells like lignin, hemicellulose and cellulose

red clover forces it to experience protein degradation causing it to have significant amounts of readily available protein, by which carbohydrates need to be fed in order to add energy and fully utilise all of the available protein and avoid N losses (Vanhatalo, Kuoppala, Ahvenjärvi, & Rinne, 2009). Many chemical constituents are similar to that of lucerne. When compared to other legumes higher levels of Mg can be found, while N, Co, Mg, Fe, Ca, pectin and lignin levels are generally higher than grasses (Frame, 2005; Spedding & Diekmahns, 1972).

2.6. Red Clover Limitations

2.6.1. Bloat

The digestive disorder ruminal tympany (bloat) is associated with many forage legumes, including red clover and lucerne, which has a much stronger effect on cattle than sheep (Frame, 2005; Kemp et al., 1999). In leafy productive legumes there are high levels of plant proteins that cause frothy gas content in the rumen. The occurrence of bloat occurs when the animal's production of gas exceeds its ability to release the gas (Majak, McAllister, McCartney, Stanford, & Cheng, 2003). There are various preventative techniques to avoid the digestive disorder such as supplementary minerals and other additives. Management techniques are used including monitoring the grazing herd, as well as using mixed swards containing grass and a given legume species, which is commonly practiced throughout New Zealand to avoid this problem (Frame, 2005).

2.6.2. Persistence

Longevity of red clover stands could be considered the greatest limitation especially as persistence of a pasture is the basis of profitability (Ford & Barrett, 2011). This is why great importance should be placed on the management of a stand to avoid unfavourable conditions. Generally persistence can be strong for 2 to 3 years in a pasture stand followed by a steep decline (Frame, 2005; Kemp et al., 1999). As a result compensatory growth occurs by remaining plants in a stand as population numbers decrease which can emphasise plant loss when it is discovered (Frame et al., 1998). Persistence is further limited in places of low rainfall, especially less than 500-800 mm (Hyslop, 1999). Breeding red clover to gain more vigorous germplasm has been the

most common goal of breeders since red clovers popularity grew as a forage legume (Abberton & Marshall, 2005; Wang, Hampton, & Hill, 1994).

Crown integrity and resistance to external damage among other morphological traits are apparently the largest contributors to susceptibility to pathogens (Abberton & Marshall, 2005). Frequent grazing decreases future persistence of red clover, as carbohydrate reserves are depleted in the tap root (Brock et al., 2003; Kemp et al., 1999), especially under heavy grazing in winter which will further decrease stand population (Kemp et al., 1999). This can be attributed to the degradation of tap root and reliance on the more shallow adventitious root systems (Spedding & Diekmahns, 1972). Grazing when soil has high moisture content can cause treading damage opening opportunity for disease and general plant destruction (Hay, 1985). Tap roots of red clover root as deeply as lucerne or sweet clover and are commonly prominent for 18-24 months; subsequently the rooting systems is largely made up by adventitious roots (Sheaffer et al., 2003; Spedding & Diekmahns, 1972). Grazing is examined further in the section (Red Clover Growth and Development, 2.4). The survival of the tap root in red clover is a fundamental determinant of the perpetual life of the plant (Abberton & Marshall, 2005; Kemp et al., 1999). Experiments conducted by Skipp & Christensen (1990) illustrated the destruction of functional tap root by root rot or other pest disturbance caused early death to majority of the affected plants. The deterioration of the tap root or the crown is a primary way that population numbers are depleted (Skipp & Christensen, 1990).

2.6.3. Oestrogens

After some confusion in the 1960s over which isoflavone was causing reproductive problems in ewes, 'formononetin' was proven to cause oestrogenic effects related to the conception rates after passing through the rumen (Anwar, 1994; Kemp et al., 1999). A trial undertaken by Shackell, Wylie, and Kelly (1993) indicated that long term exposure to 'Grasslands Pawera' which possessed high levels of phytoestrogens caused significant reduction in ewe reproductive performance. Today, clover induced infertility is a well-documented topic in terms of reproductive issues with ewes (Kelly, Shackell, & Allison, 1980; McDonald, Anwar, & Keogh, 1994; Turnbull, Braden, & George, 1966). Grazing before mating with high levels of formononetin in a pasture is now avoided with ewes (Kemp et al., 1999). When affected by oestrogenic red

clover, ewes can display two variations of infertility which are temporary or permanent infertility (Anwar, 1994). There are also negative reproductive effects that can be seen in cattle but there is no scientific confirmation that a permanent infertility has appeared from continuously grazing of phytoestrogens, and therefore it is suspected to have weaker effects on cattle and deer (Kemp et al., 1999). However, there are now low formononetin level cultivars which are available on the market (Stewart & Charlton, 2003). These low formononetin cultivars include the two red clover cultivars ‘Grasslands Relish’ and ‘Grasslands Sensation’ which are included in this study (Ford & Barrett, 2011).

2.6.4. Pest and Diseases

During establishment there is a low capacity to compete with weeds especially in terms of pure stands. As canopy cover develops red clover stands gain better competitiveness (Frame, 2005). The primary axis for red clover, the crown and roots, are the primary way in which plant persistence can be jeopardised (Skipp & Christensen, 1990). Predominant crown disease (*Sclerotinia trifoliorum*) and root diseases (*Fusarium* and *Rhizoctonia*) are responsible for the rotting of the primary axis (Hyslop, 1999; Skipp, Christensen, & Biao, 1986). Wounds that occur to the plant from grazing or otherwise open up the plant and particularly the crown for fungal and bacterial infections will usually cause death. Variation amongst cultivars also changes susceptibility, for example, late flowering cultivars have often had higher persistence in mixed swards as well as ploidy levels have displayed positive correlated effects for pest and disease resistance and therefore persistence (Hay & Ryan, 1989). There are many insects that feed or attack red clover including nematodes, pea aphid, slugs, grass grub, porina and clover leaf weevil (Hyslop, 1999).

2.7. Lucerne

Originating from South Central Asia (Sheaffer et al., 2003), lucerne is a perennial legume with an erect growth habit and is known around the world by many different names including alfalfa, purple medick, common purple lucerne and purple alfalfa (Frame, 2005). Poor insect resistance in New Zealand in the 1970s led to lucerne cultivars with greater resistance being imported from around the world, but the pest problem couldn't be solved solely from importation of cultivars. New Zealand

breeding efforts were then used to combat the pest problem and resistant cultivars were developed leading to increased New Zealand lucerne production (Janson & Knight, 1985).

The leaves of lucerne are compound trifoliate leaves which have prominent stipules adnate to the petiole (Grove & Carlson, 1972). Having a high nutritional value and high yield potential, it is a prominent contributor of protein in domestic animal diets around the world (Frame, 2005; Radovic, Sokolovic, & Markovic, 2009). It is commonly used as a specialist crop and its erect growth habit allows it to be highly suited for conservation feed, though it can be sown with grasses (Frame, 2005; Kemp et al., 1999). It is cultivated throughout many temperate zones across the world, and is grown in over 80 countries on an area that surpasses 35 million hectares (Radovic et al., 2009). There are a wide range of cultivars available making it suitable for various environments (Frame, 2005).

2.8. Grasslands Torlesse

Bred in New Zealand by AgResearch Grasslands, Torlesse was selected for its winter dormancy along with high productive ability and pest resistance. The high winter dormancy allows for greater persistence especially in cooler areas with high spring and summer production. It is also a dual purpose crop that can be grazed or used for conservation feed Agricom (2012).

2.9. Lucerne Growth and Development

Establishment growth is covered in (Red Clover & Lucerne Establishment, 2.3) section.

Under ideal conditions, i.e., pH of 5.8 to 6.5, well-drained and highly fertile soil, yields can generate over 20 t/DM/ha (Kemp et al., 1999). It has a more narrow range of tolerance for growing conditions than red clover in order to be highly productive. High yields from stands can be expected for 4-6 seasons (Frame, 2005).

The deep tap root facilitates water extraction during establishment, and like red clover, the carbon allocation is predominately for tap root production allowing it to search deep in the soil profile for moisture (Thomas, 2003). The tap root is capable of growing 2-4 metres if soils are well drained which further promotes tap root growth (Frame, 2005). There are also numerous fibrous roots that make up the majority of the rooting system

and are largely responsible for nodulation; these are only found in the top 15 cm of the soil surface (Small, 2011).

Lucerne is very well adapted to growing and competing for light in a pasture because of its erect growth habit. This is especially the case when compared to prostrate legumes like white clover but when grazed, significant losses occur to vegetative buds because of their height in the sward (Thomas, 2003). It should be noted, however, that in the development stages of growth the plant is less able to compete until a canopy is established (Frame, 2005).

Stems originate from the crown buds from which axillary buds on the lower leaf axils produce additional stems, with both functions contributing to form the crown of buds at the base of the plant (Frame, 2005). A cross-section of the primary stem will reveal an almost square tissue with major vascular bundles running parallel to the stem (Grove & Carlson, 1972). Because of the erect structure of the plant after establishment crown buds are predominately responsible for stem production as axillary buds produce branches (Frame, 2005).

The majority of carbohydrates and nitrogen reserves are stored in the roots and crown but also rhizomes and stubble (Frame, 2005). Defoliation depletes these reserves, especially during autumn when reserves are being accumulated, therefore high frequency grazing can cause lasting effects as plant vigour decreases (Li, Volenec, Joern, & Cunningham, 1996).

2.9.1. Harvest and Grazing

Lucerne tolerance for frequent grazing or harvesting is low, like red clover, thus a rotational grazing system is best suited to manage plant persistence (Kemp et al., 1999; Smith, 1972). High frequency defoliation has a direct effect on tap root production in immature stages of growth (Keoghan, 1966), which is one of the key reasons for future lack of persistence. Rest periods are vital for the replenishment of carbohydrates and nitrogen, as nitrogen reserves in roots have significant effect on regrowth (Frame, 2005).

The timing of harvest is normally based on the stage of maturity and an increase in frequency of harvests will increase quality but decrease yield (Frame, 2005). Decreases in seasonal yield occur as stand ages but high frequencies of defoliation decrease

seasonal yield more rapidly (Frame et al., 1998). Preservation of lucerne also usually follows maturity of the plant as immature lucerne is more likely to be grazed and more mature plants will be green chopped, ensiled or made into hay which has the flexibility to be made in late maturity. Hay quality is determined by the speed moisture is reduced to around 20% if the leaves are retained. Silage preservation is concerned more with the storage and fermentation process, which can affect feeding value through lower palatability and digestibility (Barnes & Gordon, 1972).

Grazing management is similar to cutting management but it is important to keep an appropriate stocking rate and grazing time interval to avoid grazing the regrowth too soon which will affect stand persistence. Strategic management is also needed for companion crops as seasonal growth rates will likely vary, palatability of companion crops also need to be high to avoid over grazing lucerne which is also a seasonal concern (Frame et al., 1998).

Height of cutting doesn't have a large effect on persistence of lucerne under appropriate cutting or grazing frequencies if root carbohydrate reserves are able to regenerate after cutting (Smith, 1972). As frequency of harvests increases green leaves from longer stubble may be required to provide additional carbohydrates for regrowth (Keoghan, 1966; Smith, 1972). The importance of root and crown reserves is felt as crown buds are responsible for primary stem production and first annual regrowth, while axillary buds are responsible for regrowth following spring defoliations. However, stubble height governs the ratio of crown buds and axillary buds as longer stubble promotes more axillary bud production (Frame et al., 1998).

2.9.2. Breeding

Common lucerne in New Zealand, *Medicago sativa* L. (included in the field experiment), is a tetraploid species while *Medicago falcata* L. is a diploid species which originated from harsher environments and possesses more tolerant attributes to climatic conditions (Frame et al., 1998; Langer, 1967). There is an availability of a broad range of genotypes available from *M. sativa*, *M. falcata* and hybrids between the two (Frame, 2005), of which various types of bees are responsible for effecting cross-pollination (Frame et al., 1998). Breeding objectives cover varying aspects depending on environmental conditions and management goals, though there have been significant achievements in terms of pest and diseases resistance. Grazing tolerance is

also been a focus of many breeding efforts from which there have been advancements in certain cultivars (Frame, 2005).

2.10. Lucerne Benefits and Limitations

2.10.1. Benefits

Lucerne is able to be sown with grasses that are not overly competitive (Frame, 2005). This can help to avoid weed problems that can occur in a pure sward, make the hay and silage production easier or provide a more balanced feed (Spedding & Diekmahns, 1972). Fresh pasture can be fed to livestock or more commonly as various types of conservation feed; it is highly palatable to cattle however this does vary as there are many varieties (Frame, 2005; Summers & Putnam, 2008).

Animal Performance

Lucerne is readily digestible by ruminants and is high in crude protein and calcium. Quick digestion in the rumen enables ruminant livestock to achieve greater intakes from lucerne (Frame, 2005; Summers & Putnam, 2008). When compared to grass, lucerne has higher animal intake due to a quick rate of passage through the rumen and its structural and chemical composition enabling faster particle breakdown and therefore digestion (Frame et al., 1998). The time of cutting lucerne affects the feeding value as immature lucerne has higher voluntary intake and is more digestible than mature lucerne, but yield is often sacrificed by cutting earlier. Both lowered voluntary intake and plant digestible energy play a part in decreasing digestibility as plants mature, with voluntary intake being a more central determinant (Barnes & Gordon, 1972). Higher daily dry matter intakes were found by Fraser et al. (2004) in lambs grazing lucerne stands in contrast to ryegrass stands. Significantly higher lamb growth rates were achieved with lucerne compared to ryegrass were also recorded giving them the advantage of being ready for slaughter earlier. Similar results have been reported in beef cattle when fed grass silage and lucerne silage; a gain in both liveweight and intake was recorded but liveweight gain was most significant (Frame et al., 1998). The estrogenic compound found in lucerne coumestrol has been proven to have a strong positive effect on weight gain in sheep; however, it is also associated with lowering fertility (Fraser et al., 2004).

Nitrogen Fixation

Variation can be seen in the nitrogen fixation system due to sensitivity to environmental conditions, but lucerne is capable of fixing large amounts of nitrogen, up to 470 kg/ha (Frame et al., 1998; Russelle, 2004). The ability for lucerne to facilitate fixation of atmospheric nitrogen allows it to build up nitrogen rich organic matter in soil. Crops without this ability can benefit from rotational cropping as increased soil fertility is an indirect benefit of lucerne production (Frame et al., 1998).

Unlike clover, lucerne often has the need to be inoculated with rhizobia as a seed especially if the crop has not been grown in the given area in several years (Greenwood & Pankhurst, 1977). Improved crop yields through increase soil nitrogen are a result of inoculation and the fixation process (Black & Moot, 2013). Clover can sometimes avoid this issue as the wide spread production of clover in New Zealand has a presence of rhizobia already in soil (Greenwood & Pankhurst, 1977).

2.10.2. Limitations

Lucerne can be inflexible in terms of management which in times of high feed demand can cause conflict between animal and forage demands (Moot, Brown, Teixeira, & Pollock, 2003). It also doesn't possess the same flexibility to remain highly productive, in terms of range of acceptable growing temperatures and soils types when compared to other legumes including red clover (Frame, 2005).

Lucerne is not a strong competitor with weeds during establishment but as vegetative growth continues so does its ability to compete (Frame, 2005). As a mixture component with grass, it can be difficult to maintain and balance due to defoliation intervals for lucerne having a high likelihood of not suiting the companion grass or vice versa. The seasonality of grass and lucerne growth can also complicate the situation, as optimal temperatures differ are often achieved in spring and summer which generally produces high herbage mass as well as early seasonal growth of lucerne and can limit grass growth. Rooting abilities of lucerne can disadvantage grass in times of drought as grass does not penetrate the soil profile in search of water like the tap root of lucerne (Frame et al., 1998).

There are a wide range of pests that can cause damage to lucerne at any stage of growth with varying degrees of severity. Insects, mites and nematodes are the major sources of

pests that affect the health of lucerne (Frame et al., 1998). Lucerne is susceptible to many diseases which can impair performance at any stage of development, lowering yield and persistence (Frame, 2005). The occurrence and intensity of diseases is determined by the environmental conditions and management. Once the disease is present in the crop there are a few economical control options, such as resistant crops, fungicide application, crop rotation and careful monitoring (Undersander et al., 2011). It is often possible to control disease pressures but there are times when it is not economically nor physically possible to avoid certain pressures (Frame, 2005).

Bloat can be an issue with stock grazing pure swards of lucerne especially with cattle, though effects can be reduced or eliminated by including more fibre in the diet or including bloat safe species to the sward (Frame et al., 1998). Reproductive issues due to plant oestrogens, which fluctuate during growth stages and in response to certain pest attacks, can cause infertility when grazing lucerne around times of conception (Fraser et al., 2004).

CHAPTER 3 - METHODS

The research consists of two experiments one of which was performed in the field and the other inside a glasshouse. The field trial (experiment 1) consisted of two cultivars of red clover, 'Grasslands Relish' and 'Grasslands Sensation', and one lucerne cultivar 'Grasslands Torlesse', which will be referred to simply as Relish, Sensation and Torlesse for the remainder of the thesis. Different first grazing frequencies were tested in the field experiment and various measurements were taken from early establishment until 200 days after sowing when the trial ended, including destructive harvests. Experiment 2 used the same two red clover cultivars as the field experiment with the addition of a third red clover cultivar, 'Grasslands Colenso', which will be referred to as Colenso for remainder of the thesis. Experiment 2 involved various repetitive defoliation frequencies and destructive harvests which are detailed further on.

3.1. Germination Test

A seed sample was taken from each of the three red clover cultivars used in the glasshouse experiment: Relish, Sensation and Colenso. The germination testing was performed using the International Seed Testing Association Rules (ISTA, 2014) to test the viability of seed of each cultivar.

Four replicates of 50 seeds per cultivar were each spread onto two steel blue germination blotters (Anchor Paper Company, Saint Paul, Minnesota, USA) following the top of paper method and placed into air tight plastic containers. The seeds were initially pre-chilled for four days at 5 °C, and then placed at 20 °C ambient temperature for the remainder of the test. Seeds were then assessed after four days to identify how many normal seedlings had sprouted; normal seedlings were removed. A final seedling analysis was performed after a total of ten days and seedlings or seeds were categorised into normal seedlings, abnormal seedlings, hard seeds, fresh ungerminated seeds or dead seeds.

3.2. Experiment 1: Field Trial

Experiment 1 was performed at the Massey University Pasture and Crop Research Unit-No.1 Dairy farm which is located on the Poultry Farm Road, Palmerston North. The

trial was situated on river slowly accumulating Manawatu silt loam soil over sand, with river alluvium as the parent material.

The field trial was sprayed with glyphosate 6 February to kill any plants occupying the area. The site was then ploughed, rolled and power harrowed to prepare a seedbed. Trifluralin herbicide was applied and incorporated by power harrow. Cropmaster 15 fertiliser (N: P: K: S-15.2:10:10:7.7) was applied at 250 kg/ha and dutch harrowed and then rolled again before seeds were sown on 28 February.

Thousand seed weight was calculated for Relish, Sensation and Torlesse. This was performed prior to planting as uniform plant density was important for the trial. Relish was coated including being inoculated which significantly increases seed weight and sowing rate to 14.4 kg/ha while Sensation and Torlesse were not inoculated and sowing rates were 8.4 kg/ha and 8.8 kg/ha respectively, giving an average of 400 seeds per m².

On 12 March 2014 the trial plots were marked out initially with spray paint and shortly after were sown with a 10 row 1.5 m wide cone seeder (15cm apart). Once all plots were sown the area was once again rolled to ensure soil coverage of the seed. Because of the very low rainfall during March (8.8 mm), pipe irrigation was set up 19 March for four hours and 50 mm of water was applied to the trial, this exercise was not repeated (Plate 3.1).



Plate 3.1: Temporary irrigation during early plant establishment

The trial was sprayed with Gallant ultra (active ingredient, 520g/liter haloxyfop-P) at 0.5 litres/ha and uptake spraying oil at 1 litre/ha on 2 May 2014 to prevent competition from grasses for light interception.

3.2.1. Climate Data

All climate data preceding and during the field trial was recorded by AgResearch Grasslands Palmerston North. The location of the AgResearch weather station is approximately 400 metres from the trial location.

Measurements included minimum and maximum daily temperatures, 10 cm soil temperature as well as rainfall and sunshine hours (Table 4.1).

3.2.2. Plot Design

A randomised split plot design was used which included three grazing frequencies, three plant treatments (Relish, Sensation and Torlesse) and four replicates. Due to the constraints of needing a uniform grazing frequency, treatments were only randomised across the entire experimental site. The experimental site was selected for its homogeneity based on previous experience with the site. Each plot was 4.5 by 10 m and the grazing frequencies were 10, 12 and 14 weeks. Randomisation was used when deciding plant treatment location in the trial. The trial was broken into three equal areas and grazing time was also randomised between these three areas (Table 3.1).

Table 3.1: Experimental field design

12 Week Grazing	Torlesse	Torlesse	Sensation	Sensation
	Relish	Sensation	Relish	Relish
	Sensation	Relish	Torlesse	Torlesse
10 Week Grazing	Relish	Torlesse	Sensation	Sensation
	Torlesse	Sensation	Relish	Relish
	Sensation	Relish	Torlesse	Torlesse
14 Week Grazing	Torlesse	Sensation	Torlesse	Sensation
	Relish	Relish	Sensation	Relish
	Sensation	Torlesse	Relish	Torlesse

3.2.3. Experiment 1: Measurements

Plant Density

Plant density was measured on a fortnightly basis from the day of planting until the first grazing, and then subsequently after each grazing. Density was measured by laying a metre ruler alongside a drilled row and counting how many plants were along the given metre which was repeated three times in each plot.

Destructively Harvested Plants

Plants were dug from the trial area fortnightly from the day of planting whereby >20 plants from each replicate were taken.

The entire sample was washed and 5 representative plants from each sample were taken for crown diameter measurement, which was performed with electronic callipers. The same 5 plants from each sample were used for counting the number of leaves and shoots that were on each plant.

The number of leaves was counted for each plant and if a leaf was in an intermediate stage of unfolding it was counted as a half leaf while if a particular leaf was completely folded it was not counted. Stems were counted in terms of a complete regenerating unit, meaning from each stem, depending on age and stage of growth, there were two or more individual petioles (Plate 3.2).



Plate 3.2: Clockwise from the top left, development of a regenerating stem unit (photo 2 shows the first stage of a complete ‘stem’).

Root and shoot measurements were taken from the entire sample from each plot whereby they were fortnightly destructively sampled and separated into either root or shoot material (Plate 3.3). This material was dried at 70 °C for at least 24 hours in a drought oven, which ensured that the material had reached a constant weight. The material was then weighed to obtain a dry weight.



Plate 3.3: Lucerne (left), Red clover (right) during destructive harvest five weeks after sowing.

Grazing measurements

Prior to each of the grazings the sward height was randomly measured 50 times in each plot with the use of a sward stick. At the conclusion of each grazing another 50 random measurements were taken to determine the residual height. At the time of the final grazing the sward stick was unable to be useful as the height of many plants were above the threshold of the equipment; so a one metre ruler was used as an alternative.

Pre- grazing herbage mass was calculated by taking three 0.25 m² quadrat cuts from each plot. The samples were cut to ground level then washed and dried in a drought oven at 70 °C for 24 hours then weighed.

3.2.4. Grazing

The trial consisted of three different grazing treatments at 10, 12 and 14 weeks after sowing (Table 3.1; Plate 3.4); there was also a second and final grazing performed in spring (28th September, 28 weeks after sowing). All grazings aimed for an average residual height of 5cm which was variable only in areas of weed pressure, as sheep would often avoid hard grazing of these areas. Grazing treatments were fenced with temporary electric fences for the duration of each grazing (12-24 hours). The entire trial was grazed over a period of four days at the final spring grazing, whereby the sheep were free to roam over the complete trial area.

All grazings were performed by ewe hogget's which were accompanied by lambs for the final grazing at a relatively high stocking rate (> 925 sheep per hectare).



Plate 3.4: Top to bottom, 10 week, 12 week and 14 week first grazing

3.3. Experiment 2: Glasshouse Trial

There were three cultivars in the glasshouse experiment which were Relish, Sensation and Colenso. Colenso was used as a benchmark cultivar as it had a strong industry presence when it was available on the market (1986-2008), along with it being a diploid variety similar to the other two cultivars. Each treatment was replicated three times for each of the three cultivars.

3.3.1. Glasshouse Design

The design of the glasshouse experiment was a randomised complete block with three cultivars, three defoliation frequencies, four times of harvest and three replicates (Table 3.2).

Table 3.2: Experimental Glasshouse Design

C2b	R2a	S1c	S2c	C4b	S2b	C4b	S2b	S1b	S4a	R1b	C2a
R4b	S4b	C2a	Ece	R2b	C1a	R4a	C1c	R1c	R2a	C1a	S1c
Ecd	Harvest 1					Harvest 3					Esd
S4a	R4c	S1a	C2c	S1b	R1c	S4b	R4b	S4c	C2c	Esf	R1a
S2a	C1c	R1b	R4a	R2c	C1b	R2c	S1a	Ecb	C1b	C4c	S2c
Erd											Erf
Ere	R1a	C4c	S4c	C4a	Ecc	Esc	C2b	R2b	S2a	C4a	R4c
S1b	C4a	R1c	C1a	S2a	R2b	C1a	R2b	S2b	C2c	R1c	C4a
Ese	Harvest 2					Harvest 4					
C4b	S4b	C1b	S1c	C2c	R1a	R4c	R1a	C1b	S1c	S4c	R2c
C2a	S1a	Erb	R4a	R4b	S4a	C1c	C4b	Era	C2b	C4c	Esa
Esb	S2c	R2a	R1b	S2b	Eca	S2c	R4a	S4a	R1b	S1a	S2a
C4c	R4c	S4c	C1c	C2b	R2c	Erc	C2a	S1b	R2a	S4b	R4b

Capital letter indicates Cultivar, (Relish, Sensation, Colenso, E indicating extra pots), Number indicates defoliation frequency (weekly), lowercase letter indicates replicate (a,b,c).

3.3.2. Glasshouse environment

Growing medium used for the experiment was 50% s Manawatu silt loam soil and 50% sand, which was combined using a concrete mixer. During the mixing process a 3 part combination of Woodace long term fertiliser N:P:K–18:2.2:8.3 (200g/50 litres of media) short term fertiliser N:P:K–20:20:20 (100g/50 litres of media) as well as Dolomite N:P:K–14:14:14 (150g/50 litres of media) fertiliser was added before soil was transported to individual pots (30cm high, 25cm diameter). A weekly liquid fertiliser (Peter's Professional allrounder, N:P:K–20:8.7:16.6+TE) was also applied to the experiment and administered to each pot in the experiment with a dosatron and diluted 100:1.

NETAFIM compensating drippers were used for irrigation whereby 19 mm lateral tubing was used for irrigation lines and 3 mm from the dripper to irrigation spike. The applied water quantity gradually increased over the time of the trial to maintain field

capacity as plants grew and demanded greater moisture which started with 60 mm/day and ended at 460 mm/day. The temperature regulation in the glasshouse was kept between 16-26 °C.

Predators used to suppress aphid populations were *Aphidius*, a small parasitic wasp, and Dusky Ladybird, a small predatory ladybird. The natural predator used for Mite control was also a species of Mite 'Mite-E' (Bioforce, 2012).

3.3.3. Glasshouse Measurements

Defoliation

Defoliation frequencies for the experiment were at 1, 2 and 4 week intervals. Before each pot was cut to the residual height, a ruler was used to measure the height of the plants in each pot. The cut was undertaken by holding the bulk of plant mass in each pot and cutting the plants to a residual of 5 cm as used by Claydon et al. (1993). Herbage from the pots was collected, washed and dried in a drought oven at 70 °C for 24 hours. These samples were then weighed on a dry weight basis.

Harvesting and Destructive Sampling

There were four destructive harvests over the four months starting 2 July, with a quarter of the total plants destructively harvested each month until no plants remained.

The pots were taken from the glasshouse and individually emptied into a large sieve while remaining largely intact. The soil was washed from the roots until the plant stubble, root and coarse soil remained in the sieve, the plant stubble and roots were separated and coarse soil was submerged in water where any broken root material was collected. Plant material was submerged a second time whereby any debris or soil material was discarded and any floating plant material was collected.

Petioles were counted from each plant (Figure 2.1); incomplete regenerating stem unit(s) (Plate 3.2), as well as crown diameter for each plant was measured, and root and stubble biomass was separated. The two separated pieces of biomass were dried and weighed on a dry material basis.

3.3.4. Statistical Analysis

The effect of week of first grazing and plant species and their interactions on dry matter (DM), height, density, stem number, petiole number, leaf number, crown diameter, root/shoot ratio, herbage mass root mass and stubble mass were analysed using the general linear model (GLM). Data on DM, height, density, stem number, petiole number, leaf number, crown diameter, root/shoot ratio, herbage mass, root mass and stubble mass between days/weeks after sowing and plant species were analysed using an ANOVA with means compared using a LSD (least significant difference) multiple test. All data analyses were performed using the Procedure GLM in SAS version 9.3 (SAS, 2011), and rejection level was set at $p < 0.05$. Stated differences or changes within this study were less than the rejection level (i.e., < 0.05), while no change or similarities were > 0.05 .

To analyse the effect that timing of first grazing had in spring of experiment 1, first grazing treatments were separated (Table 3.1) to allow for the analysis of any variation between plant species treatments.

CHAPTER 4 - RESULTS

4.1. Climate

Climate data collected from the Palmerston North AgResearch site indicated that rainfall was considerably lower (8.8 mm) than the long term average in March (67 mm) when the trial was sown. During the trial, April was the only month that rainfall was higher than the long term average with the period June-September were (172mm) below the average including July which was substantially below (Table 4.1).

Soil temperature was slightly lower than the long term average during July and August, through the other months of the trial soil temperature was higher than the average (Table 4.1).

Table 4.1: Maximum and minimum air temperature (°C), 10cm soil temperature (°C), total monthly rainfall (mm) and monthly sunshine (hours) at Palmerston North AgResearch weather station from February to September, 2014

		Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.
Maximum	1929-2012	21.0	18.2	15.2	12.8	12.2	13.2	14.8
	2014	22.2	20.5	16.7	15.3	12.8	13.6	16
Minimum	1929-2012	11.7	9.4	7.0	4.9	4.2	5.1	6.7
	2014	9.7	11.2	7.2	7.1	3.7	4.4	7.3
Soil temp (10 cm)	1929-2012	16.2	13.1	10.3	7.9	6.8	7.8	9.9
	2014	16.5	15.1	10.9	9.5	6.7	7.6	10.7
Rainfall (mm)	1929-2012	67.0	76.0	87.5	97.5	89.2	86.6	79.6
	2014	8.8	100.8	85.4	66.4	16.2	64.6	66
Sunshine (hours)	1929-2012	173.2	141.9	115.0	92.3	105.8	124.8	132.8
	2014	191.7	110.8	113.1	118.2	123.9	140.4	128.8

4.2. Experiment 1: Herbage Production

4.2.1. Dry Matter (DM)

Total DM as an accumulation of the two grazings in late autumn and spring were highest for Relish and Torlesse treatments (LSD = 677.16, $P < 0.0001$) that received

the 12 week to first grazing treatment (Figure 4.1). However, Sensation had its highest total DM from the 14 week to first grazing treatment which was 30% greater than the 12 week to first grazing treatment and over 45% greater than the 10 week to first grazing treatment. All species that had the 10 week to first grazing treatment produced less total DM than that from 12 or 14 week to first grazing treatments.

Relish and Sensation for weeks 12 and 14 to first grazing as well as Torlesse for week 12 to first grazing showed the highest DM yields (LSD = 249.69, $P < 0.0001$). From the 10 week to 12 week to first grazing treatments all plant treatments had a significant increase in DM; 156%, 147% and 123% increase for Sensation, Relish and Torlesse, respectively (Figure 4.2). No change was seen in the red clover cultivars between 12 and 14 week to first grazing treatments but the lucerne cultivar, Torlesse, produced significantly reduced DM.

DM production in spring (day 200 after sowing) showed that Relish with both 10 week and 12 week to first grazing treatments was higher than other treatments (LSD = 711.63, $P < 0.0001$). Relish and Sensation that received the 14 week grazing frequency treatment were similar in DM yield (Figure 4.3). However, Sensation that received 10 or 12 week grazing frequency treatments was lower than all other Sensation and Relish DM yields. DM produced by Torlesse for all treatments was significantly lower than both of the red clover cultivars.

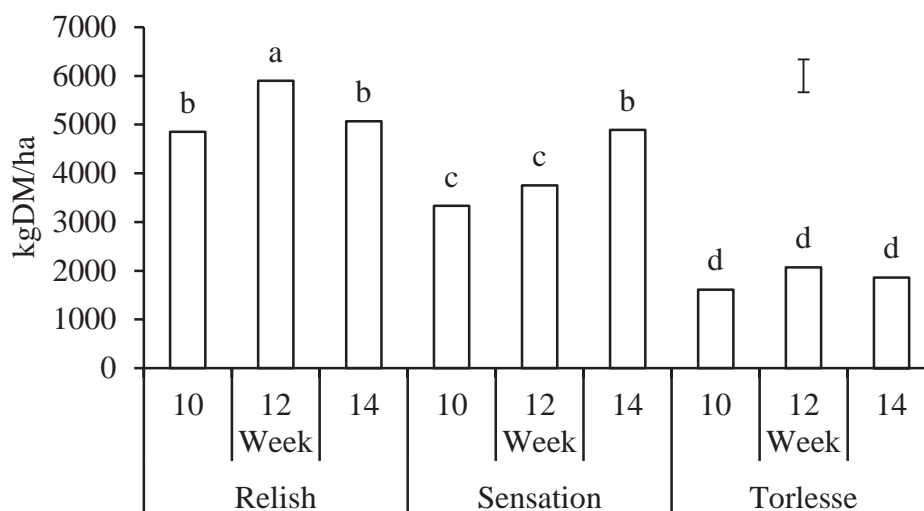


Figure 4.1: Total pre-grazing dry matter (kg/ha) for Relish, Sensation and Torlesse first grazed in autumn 10 weeks (22 May), 12 weeks (4 June), 14 weeks (20 June) after sowing and in spring. The bar indicates the LSD value. Means with the same letter are not significantly different to each other.

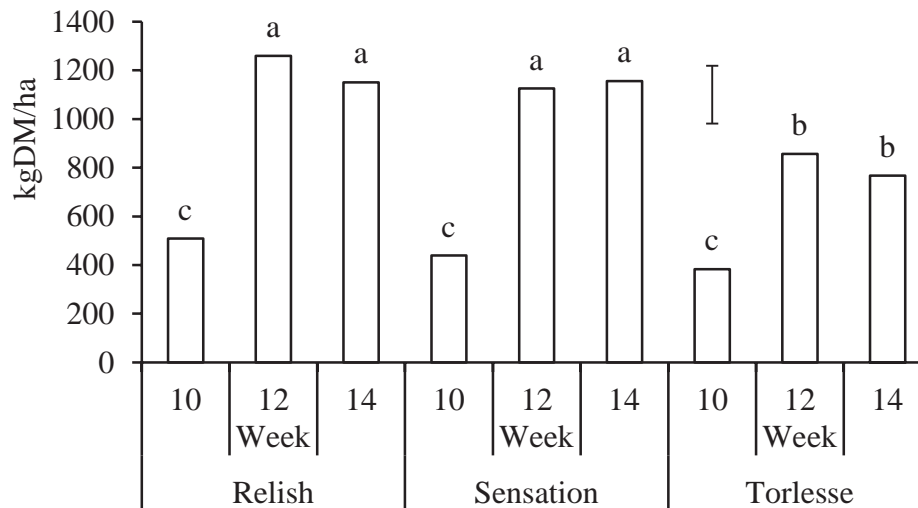


Figure 4.2: Pre-grazing dry matter (kg/ha) for Relish, Sensation and Torlesse at three first grazing treatments 10 weeks (22 May), 12 weeks (4 June), 14 weeks (20 June) after sowing. The bar indicates the LSD value. Means with the same letter are not significantly different to each other.

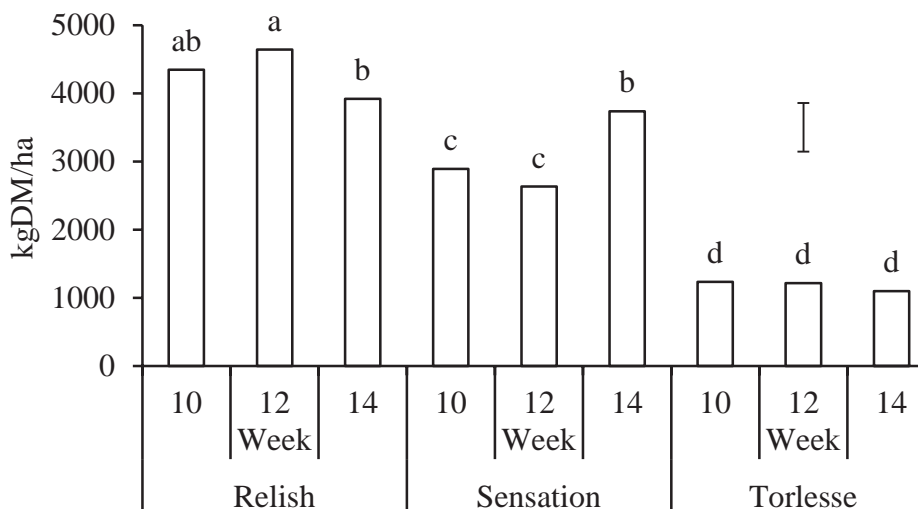


Figure 4.3: Pre-grazing dry matter (kg/ha) at second and final grazing 200 days after sowing for Relish, Sensation and Torlesse first grazed 10 weeks (22 May), 12 weeks (4 June), 14 weeks (20 June) after sowing. The bar indicates the LSD value. Means with the same letter are not significantly different to each other.

4.3. Experiment 1: Plant Morphology

4.3.1. Pre-Grazing Height

The pre-grazing heights of the three grazing treatments had no significance between species or between timing of first grazing.

The pre-grazing height for Relish and Sensation at the final harvest (200 days), amongst the time to first grazing treatments did not differ significantly within each cultivar (Figure 4.4). Sensation subjected to 10 and 12 week first grazing treatments showed no similarity to and was shorter than Relish while in contrast Sensation's 14 week treatment was similar to Relish that received 10 and 14 week first grazing treatments (LSD= 5.59, $P < 0.0001$). Relish subjected to 12 week treatment (46.9cm) was significantly taller than that of Sensation (37.7cm). Torlesse was significantly shorter than the two red clover cultivars over all previous treatments.

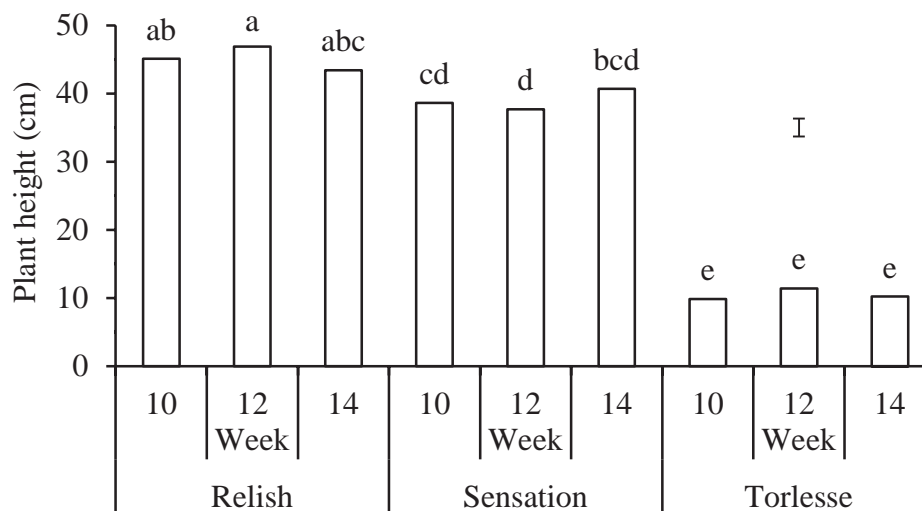


Figure 4.4: The effect of first grazing timing on pre grazing height (cm), 200 days after sowing for Relish, Sensation and Torlesse first grazed at 10 weeks (22 May), 12 weeks (4 June), 14 weeks (20 June) after sowing. The bar indicates the LSD value. Means with the same letter are not significantly different to each other.

4.3.2. Plant Density

After the initial trial establishment period (day 35 after sowing), Torlesse lucerne was denser than both red clover cultivars and Relish was denser than Sensation (Table 4.3). By 10 weeks (day 71) after sowing when the first grazing treatment occurred, the density had fallen and there were no significant differences among cultivars (av. = 213 plants/m²). By the 12 week first grazing treatment (day 84) plant density was no less than at week 10 and again there were no cultivar differences among treatments. However, by the 14 week grazing treatment (day 100) the plant density of Sensation (152) was significantly lower than both Relish and Torlesse (av. 194). Plant density of Relish, Sensation and Torlesse at final harvest (day 200) had not significantly changed from 100 days after sowing.

There was no effect on plant density at final harvest (day 200) among the three timings for the first grazing ($F_{2,63}=0.94$, $P<0.3976$) or interaction between timing of first grazing \times plant species/cultivar treatment ($F_{4,63}=0.63$, $P<0.6444$). However, the plant species/cultivar treatment had an effect on plant density ($F_{2, 63}=3.59$, $P=0.0335$) as Relish and Torlesse had a significantly higher plant population than Sensation at the end of the trial (Table 4.2).

Table 4.2: The effect of first grazing timing on plant density (plants/m²) for Relish, Sensation and Torlesse, first grazed at 71 (10 week), 84 (12 week) and 100 (14 weeks) days after sowing.

Days	Relish	Sensation	Torlesse
35	311 \pm 18.73 ^{aB}	255 \pm 12.40 ^{aC}	370 \pm 22.87 ^{aA}
43	284 \pm 16.87 ^{bA}	216 \pm 12.93 ^{bB}	266 \pm 17.40 ^{aA}
50	246 \pm 12.73 ^{bA}	198 \pm 13.53 ^{bB}	278 \pm 15.53 ^{bA}
71	216 \pm 15.73 ^{bcAB}	193 \pm 8.40 ^{bcA}	231 \pm 12.13 ^{bcA}
84	196 \pm 9.40 ^{cdA}	184 \pm 11.13 ^{bcdA}	210 \pm 11.40 ^{cA}
100	187 \pm 9.67 ^{cdA}	152 \pm 9.67 ^{cdB}	200 \pm 5.53 ^{cA}
200	174 \pm 6.93 ^{dA}	151 \pm 6.20 ^{dB}	169 \pm 5.67 ^{cA}

Means with the same low case letters in each column or the same capital letters in each row are not significantly different ($P > 0.05$).

4.3.3. Stem Number and Leaf Number

Differences in morphology of the red clover and lucerne meant that lucerne presented many more stems ($>3\times$) and leaves ($>2\times$) at each destructive harvest and for this reason only the two red clover cultivars were compared (Appendix 1)

Both Relish and Sensation showed a notable increase in stem number and leaf number from the first and second grazing treatment to the third grazing treatment (84 and 100 days respectively) (Table 4.4 and Table 4.5). There was no variation in the number of stems between the two red clover cultivars at any stage of the trial (LSD 0.13-0.74, $P>0.05$). Though there was an instance of significant difference in leaf number early in the trial all other measurements showed no significant differences between the cultivars. There was no interaction between timing of first grazing \times plant species treatment ($F_{2,98}=0.09$, $P=0.917$).

Table 4.3 Number of stems (stems/plant) of Relish and Sensation. The time of first grazing treatments were 71 (10 week), 84 (12 week) and 100 (14 weeks) days after sowing.

Day	Relish	Sensation
35	1.05 ± 0.03 ^e	1.13 ± 0.06 ^e
50	2.40 ± 0.12 ^d	2.55 ± 0.11 ^d
71	3.43 ± 0.20 ^c	3.25 ± 0.19 ^c
78	3.75 ± 0.24 ^b	3.60 ± 0.27 ^b
84	3.85 ± 0.12 ^b	3.73 ± 0.14 ^b
100	5.02 ± 0.15 ^a	4.70 ± 0.16 ^a

Means with the same lower case letters in each column are not significantly different ($P > 0.05$).

Table 4.4: Number of leaves (leaves/plant) of Relish and Sensation. The time of first grazing treatments were 71 (10 week), 84 (12 week) and 100 (14 weeks) days after sowing.

Day	Relish	Sensation
35	2.14 ± 0.07 ^{eB}	2.43 ± 0.09 ^{dA}
50	5.26 ± 0.22 ^{dA}	5.91 ± 0.28 ^{cA}
71	8.53 ± 0.51 ^{cA}	8.83 ± 0.53 ^{bA}
78	11.4 ± 0.70 ^{bcA}	10.31 ± 0.58 ^{bA}
84	12.93 ± 0.86 ^{bA}	10.95 ± 0.71 ^{bA}
100	16.00 ± 0.75 ^{aA}	14.65 ± 1.12 ^{aA}

Means with the same lower case letters in each column or the same capital letters in each row are not significantly different ($P > 0.05$).

4.3.4. Crown Diameter

Relish and Sensation both showed significantly larger crown diameters than Torlesse from 10 week first grazing treatment (day 71) until the end of the trial (Table 4.6). Sensation at day 78 and the 14 week first grazing treatment (day 100) had a significantly wider crown diameter than Relish and Torlesse.

Crown diameter at the time of final harvest (day 200) showed no interaction between timing of first grazing × plant species treatment ($F_{2,171}=2.2$, $P=0.0710$) or effect among first grazing treatments ($F_{2,171}=2.51$, $P=0.0839$). However, plant cultivar/species treatments did have an effect on crown diameter ($F_{2,171}=94.04$, $P<0.0001$) as Relish and Sensation both showed significantly wider crowns than Torlesse at the last harvest (Table 4.7). The 14 week first grazing Sensation treatment showed a significantly wider

(1.1 mm) crown diameter than Relish. Relish and Sensation that received the 12 week first grazing had significantly larger crown diameters than with their 10 week first grazing treatment.

Table 4.5: Crown diameter (mm) of Relish, Sensation and Torlesse across all treatments. The time of first grazing treatments were 71 (10 week), 84 (12 week) and 100 (14 weeks) days after sowing.

Day	Relish	Sensation	Torlesse
35	0.98 ± 0.03 ^{eA}	0.95 ± 0.02 ^{fA}	0.86 ± 0.05 ^{eA}
50	1.34 ± 0.07 ^{dA}	1.45 ± 0.08 ^{eA}	1.52 ± 0.05 ^{dA}
71	2.49 ± 0.08 ^{cB}	2.75 ± 0.09 ^{dA}	2.00 ± 0.08 ^{cC}
78	2.49 ± 0.07 ^{cB}	2.77 ± 0.11 ^{dA}	2.11 ± 0.07 ^{cC}
84	3.59 ± 0.15 ^{bA}	3.50 ± 0.15 ^{cA}	2.78 ± 0.12 ^{bB}
100	3.77 ± 0.08 ^{bB}	4.53 ± 0.13 ^{bA}	2.93 ± 0.08 ^{bC}
200	7.82 ± .021 ^{aA}	8.1 ± 0.28 ^{aA}	4.25 ± 0.17 ^{aB}

Means with the same lower case letters in each column or the same capital letters in each row are not significantly different ($P > 0.05$).

Table 4.6: The effect of timing of first grazing on crown diameter (mm) at final harvest (200 days) with Relish, Sensation and Torlesse first grazed 10 weeks, 12 weeks and 14 weeks after sowing.

Weeks after sowing	Relish	Sensation	Torlesse
10 week	7.28 ± 0.25 ^{bA}	7.40 ± 0.66 ^{bA}	4.32 ± 0.46 ^{aB}
12 week	8.52 ± 0.47 ^{aA}	8.13 ± 0.33 ^{abA}	3.85 ± 0.13 ^{aB}
14 week	7.67 ± 0.32 ^{abB}	8.77 ± 0.38 ^{aA}	4.59 ± 0.16 ^{aC}

Means with the same lower case letters in each column or the same capital letters in each row are not significantly different ($P > 0.05$).

4.3.5. Root and Shoot to Total Plant Biomass Ratio

The ratio of root to total plant biomass and shoot to total plant biomass are presented in (Figure 4.5 and Figure 4.6). Root ratio increased in all plant species/cultivar treatments from 50 days after sowing to 71 days in Relish (27 to 35%), Sensation (24% to 33%) and Torlesse (19 to 32%). Conversely, a high shoot ratio measurement for a plant treatment will inevitably result in a low root ratio, as they are the only two components compared.

Shoot ratio at day 71 (week 10) and 100 (week 14) was low for Relish and Sensation. Relish showed the lowest shoot ratio 71 days after sowing (65%) and Sensation showed

the lowest shoot ratio on day 100 (65%) along with Torlesse (58%). Percentage of shoot to total plant biomass was highest in the trial 200 days after sowing for both of the red clover cultivars Relish and Sensation (85%), (79%), respectively. Torlesse showed the highest shoot ratio in the early establishment (85%) 35 days after sowing and 81% on day 50. Torlesse showed the highest root ratio at the 14 week grazing (day 100) (42%).

At the final harvest (day 200) root ratio and shoot ratio showed little fluctuation within plant species/cultivar treatments across the three timings of first grazing. Relish had the highest shoot ratio of the three plant treatments at 84 days (week 12) (86%), while Torlesse had the lowest shoot ratio over all previous first grazing treatments (Figure 4.7).

There was no interaction between timing of first grazing \times plant species treatments for root ratio ($F_{4,27}=0.34$, $P=0.8469$) or shoot ratio ($F_{4,27}=0.37$, $P=0.8310$) or effect between first grazing treatments on root ratio ($F_{4,27}=0.27$ $P=0.7683$) and shoot ratio ($F_{4,27}=0.23$, $P=0.7961$). However, plant species/cultivar treatments had an effect on root and shoot to total plant biomass ratio ($F_{4,27}=79.41$, $P<.0001$) ($F_{4,27}=80.64$, $P<.0001$) for root and shoot ratio respectively. This showed as significant differences among all plant species/cultivar treatments as Sensation had a larger root ratio than Relish and Torlesse had a higher root ratio than both other plant species/cultivar treatments, gaining the largest root ratio in the trial in the 71 day (10 week) first grazing treatment ($36.2 \pm 1.86\%$).

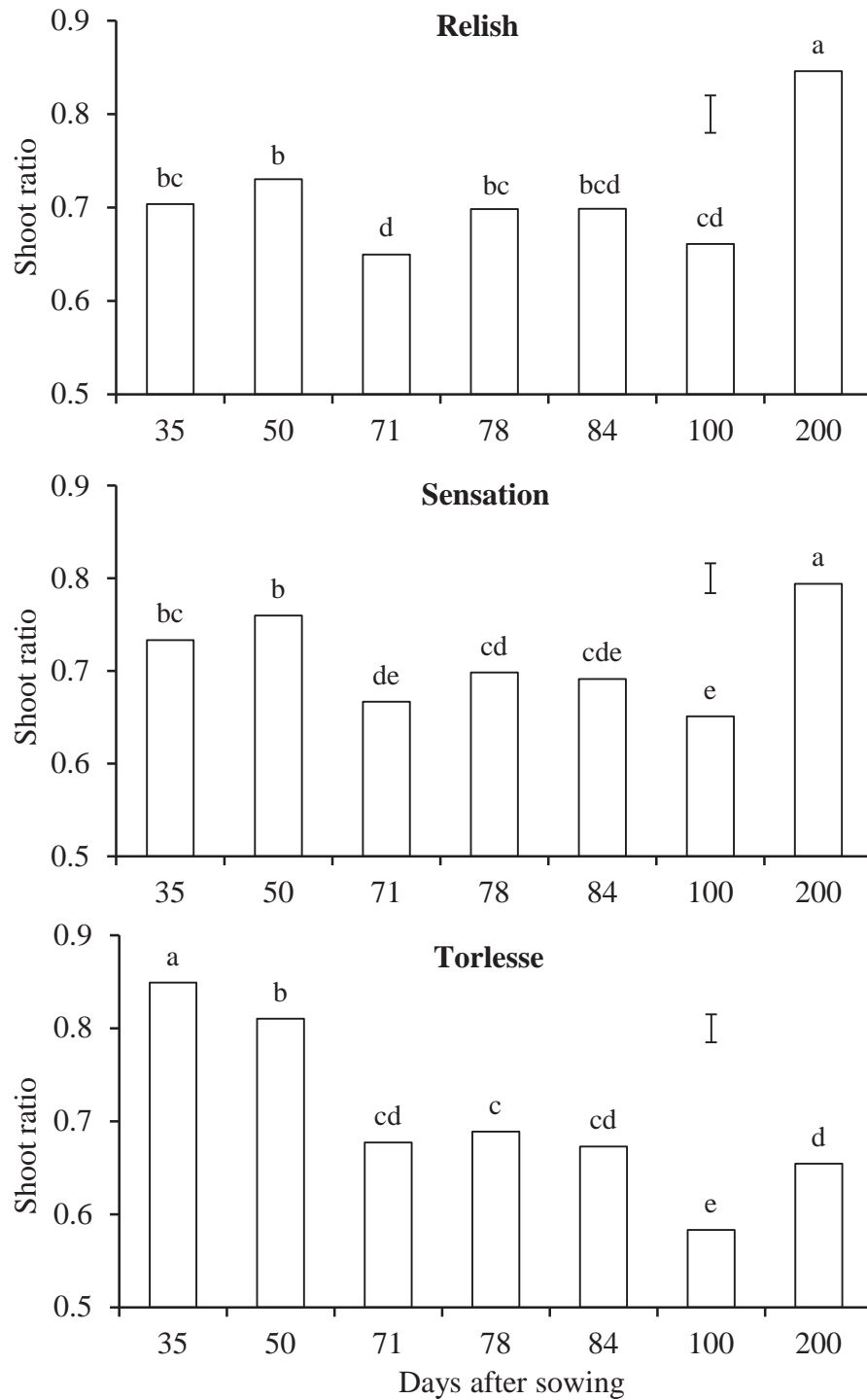


Figure 4.5: Percentage of shoot biomass to total plant biomass of Relish, Sensation and Torlesse. The time of first grazing treatments was 71 (10 weeks), 84 (12 weeks) and 100 days (14 weeks) after sowing. The bar indicates the LSD value. Means with the same letter are not significantly different to each other.

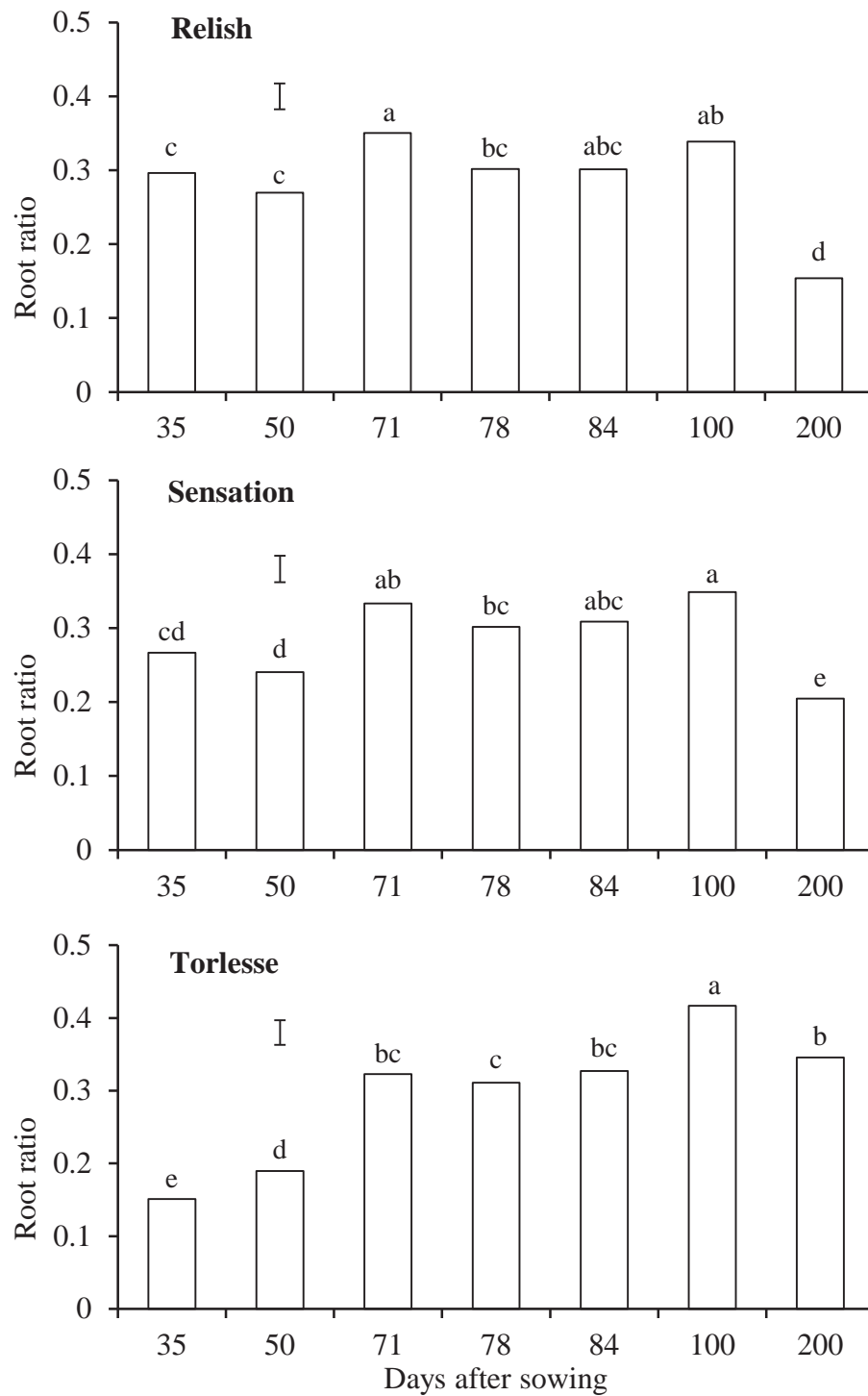


Figure 4.6: Percentage of root biomass to total plant biomass for Relish, Sensation and Torlesse. The time of first grazing treatments was 71 (10 weeks), 84 (12 weeks) and 100 days (14 weeks) after sowing. The bar indicates the LSD value. Means with the same letter are not significantly different to each other.

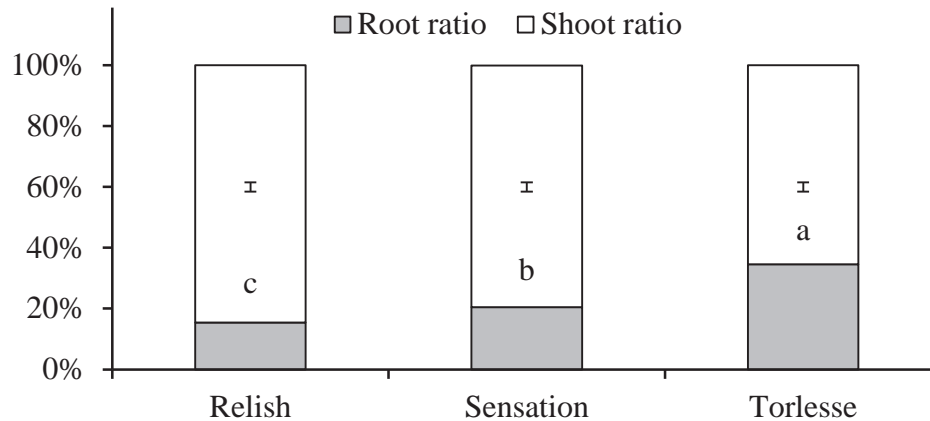


Figure 4.7: The effect of first grazing time on percentage of root biomass and shoot biomass to total plant biomass 200 days after sowing with Relish, Sensation and Torlesse. The bar indicates the LSD value. Means with the same letter are not significantly different to each other.

4.4. Experiment 2: Biomass accumulation

Images from all harvests and defoliation frequencies can be found in (Appendix 2). First harvest occurred at ‘week 8’ whereby all plant treatments had grown from seed for 8 weeks with no restriction or any defoliation treatments, therefore all treatments were uniform at this point in the trial. For this reason the same week 8 data was used when subsequently comparing to later defoliation data.

4.4.1. Germination test

Germination of the three red clover cultivars used in experiment 2 showed only moderate viability of seed which required a heavier sowing rate to ensure effective plant numbers. Abnormal seedlings were apparent in every cultivar and highest in Sensation (Table 4.7).

Table 4.7: Germination test for Relish, Sensation and Colenso including the percentage of abnormal seedlings from the final germination count.

Cultivar	Final germination	Interim germination	Abnormal seedlings
Relish	82%	79.5%	6%
Sensation	79%	75.5%	10%
Colenso	80%	69%	5%

4.4.2. Herbage weight

An increase in herbage mass over the time of the trial occurred in the plant treatments that received the four week defoliation frequency (Figure 4.8). Similar herbage mass accumulation was recorded for all plant treatments that received the four week defoliation treatment during the early stages of the trial, though toward the end of the trial both Relish and Sensation had heavier herbage weights than Colenso. Though Sensation and Colenso were similar at the last harvest there was a large variation in Colenso herbage weights. The two week and one week defoliation frequency herbage mass remained rather static and significantly less than the four week defoliation frequency. Colenso gained a higher herbage weight than at least one of the other plant treatments that received the one week defoliation frequency in over half of the harvests. An interaction was seen between cultivar \times frequency of defoliation \times week of harvest ($F_{18,42}=1.86$ $P=0.0180$) as a result of the dynamic relationships between the various treatments.

4.4.3. Root weight

Plant treatments that received the four week defoliation frequency had similar root weights until the last harvest (20 weeks after sowing) where Relish and Sensation had a significantly higher root weight over Colenso (Figure 4.9). Plant treatments that received a four week defoliation frequency also showed heavier roots than the other two defoliation frequencies ($LSD=3.91-5.05$, $P<0.05$). Colenso under the most intensive defoliation frequency (1 week) showed significantly heavier root biomass over the other two plant treatments toward the end of the trial. There was an interaction between defoliation frequency \times week ($F_{6,90}=49.28$, $P=<0.0001$). However, cultivar \times week of harvest ($F_{6,90}=0.93$, $P=0.477$), cultivar \times frequency of defoliation ($F_{4,90}=1.74$, $P=0.0697$), and cultivar \times frequency of defoliation \times week of harvest ($F_{12,90}=1.46$, $P=0.1563$) showed no interaction.

4.4.4. Stubble weight

Stubble weights at the harvests in the second half of the trial plants that were intensively defoliated (one and two week defoliation frequencies) resulted in Colenso and Relish treatments gaining heavier stubble than Sensation on a number of occasions (Figure 4.10). Plant treatments that received four week defoliation frequency

(LSD=2.46-3.18, $P<0.05$) showed significantly heavier stubble weight than both of the other defoliation treatments. The increase in stubble weight was more rapid in the less frequently cut treatments especially from week 12 to 16 as 1 week defoliation frequency had a 57% increase, while 2 and 4 week defoliation frequencies had 112% and 128% increases, respectively. An interaction was seen between frequency of defoliation \times week of harvest ($F_{6, 90}=63.17$, $P<0.0001$). However, no interaction was seen between cultivar \times week of harvest ($F_{6, 90}=1.54$, $P=0.1741$), cultivar \times frequency of defoliation ($F_{4, 90}=0.08$, $P=0.987$) and cultivar \times frequency of defoliation \times week of harvest ($F_{12, 90}=0.55$, $P=0.8736$).

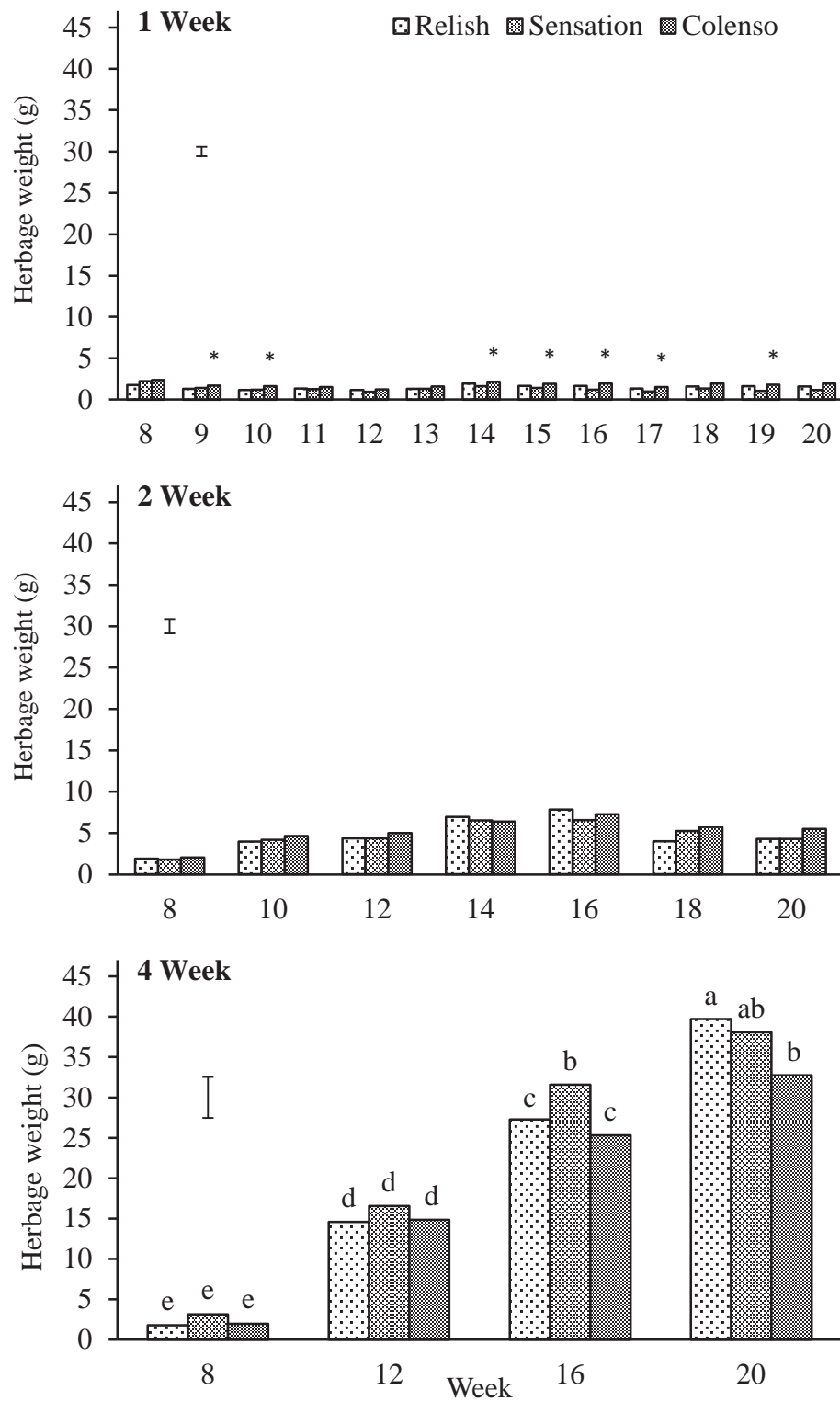


Figure 4.8: The effect of 1 week, 2 week or 4 week defoliation frequencies on herbage weight (g) of Relish, Sensation and Colenso over 20 weeks after sowing. The bar indicates the LSD value. * Indicates that the treatment is significantly greater than at least one of the others in the week. Means with the same letter are not significantly different to each other.

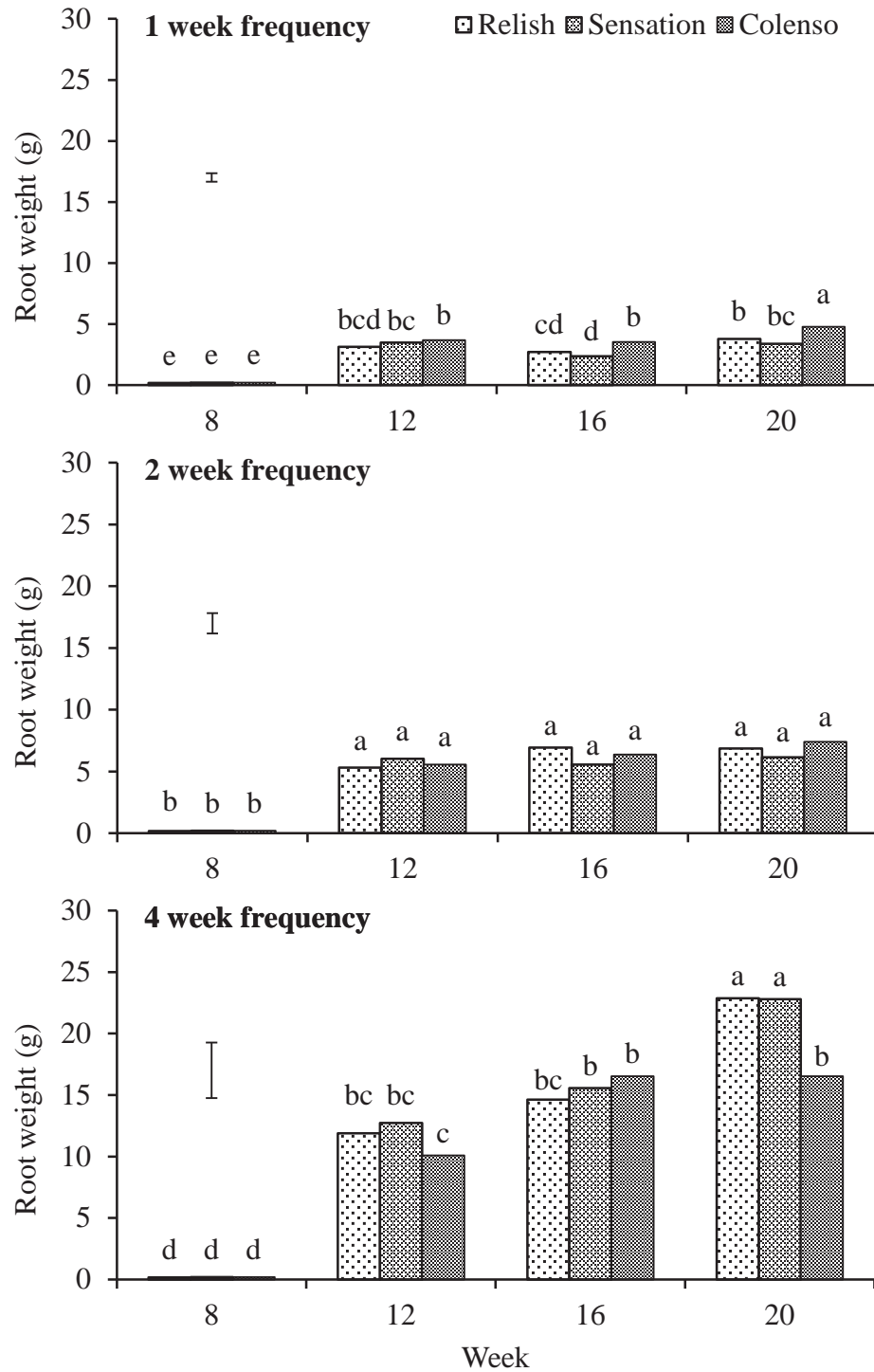


Figure 4.9: The effect of 1 week, 2 week or 4 week defoliation frequencies on root weight (g) of Relish, Sensation and Colenso over 20 weeks after sowing. The bar indicates the LSD value. Means with the same letter are not significantly different to each other.

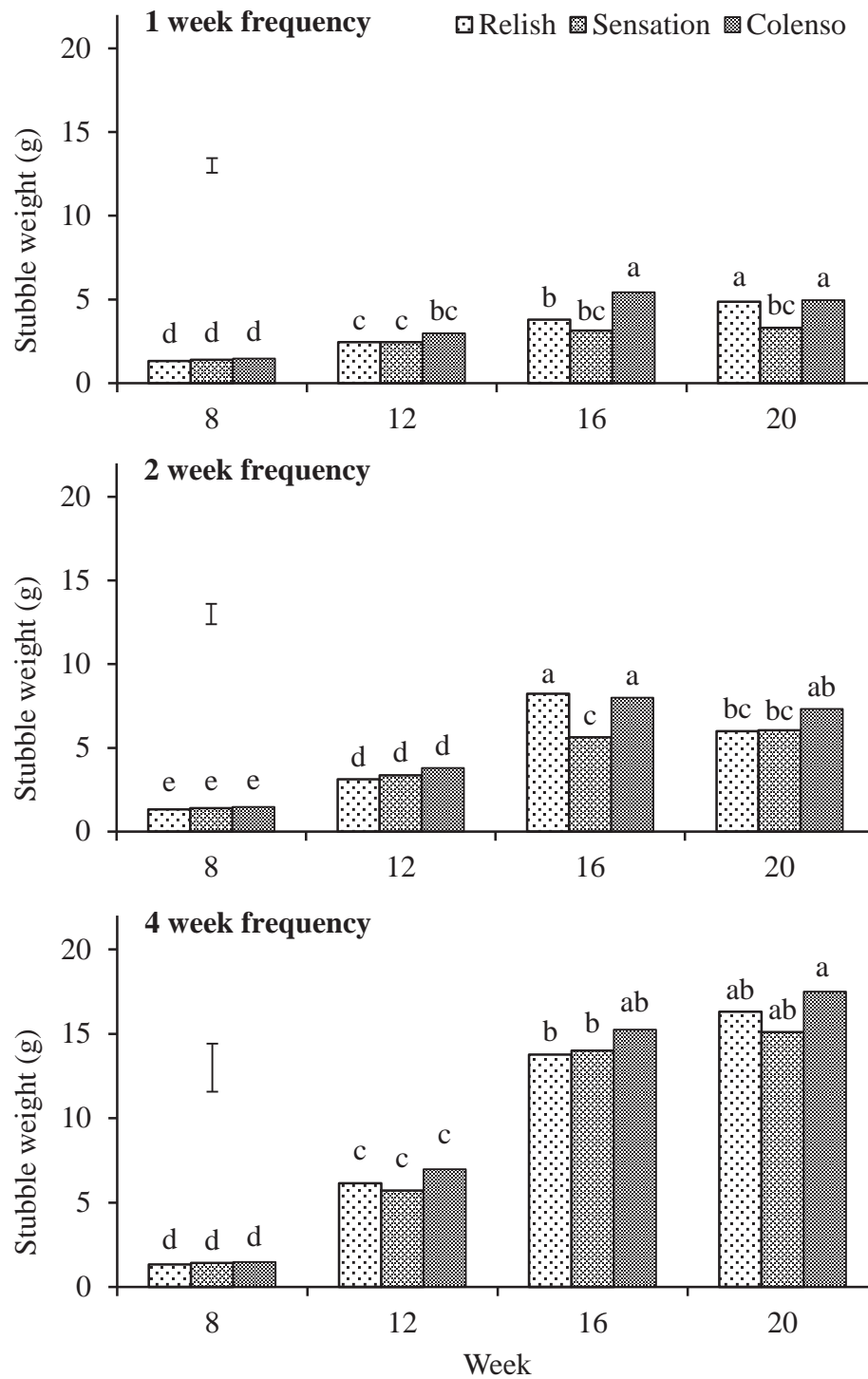


Figure 4.10: The effect of 1 week, 2 week or 4 week defoliation frequencies on stubble weight (g) of Relish, Sensation and Colenso over 20 weeks after sowing. The bar indicates the LSD value. Means with the same letter are not significantly different to each other.

4.5. Experiment 2: Plant Morphology

4.5.1. Petiole number

Over all treatments Colenso had the highest or highest equal number of petioles (Figure 4.11). The plant treatments that received four week defoliation treatments showed that there was similar number of petioles when compared to the one week defoliation treatments. An interaction was seen between the cultivar \times week of harvest, as well as, defoliation frequency \times week of harvest ($F_{6, 90}=2.74$, $P=0.0172$) and ($F_{6, 90}=7.44$, $P<0.0001$), respectively. However, cultivar \times frequency of defoliation, cultivar \times frequency of defoliation \times week of harvest showed no interaction ($F_{6, 90}=1.06$, $P=0.4001$). Relish and Sensation showed similar behaviour in terms of petiole number in all the defoliation treatments throughout the trial.

4.5.2. Crown diameter

Plant treatments that received the four week defoliation treatment showed an increase in crown diameter of 5-6 mm over the length of the trial, and were significantly greater than the one (2-3 mm) and two week (3-4 mm) defoliation frequencies (Figure 4.12). Cultivars that were subjected to one week defoliation frequency were static (Sensation) or showed only small increases (Colenso and Relish) over the length of the trial. Cultivars that had the two week defoliation frequency showed an increase in crown diameter up to 16 weeks and then was static to the end of the trial. An interaction was seen between frequency of defoliation \times week of harvest ($F_{6, 90}=10.07$, $P<0.0001$). However, cultivar \times week of harvest ($F_{6, 90}=2.07$, $P=0.0646$), cultivar \times frequency of defoliation ($F_{4,90}=0.70$, $P=0.5957$) and cultivar \times frequency of defoliation \times week of harvest ($F_{12,90}=0.76$, $P=0.6893$) showed no interaction.

4.5.3. Plant height

The four week defoliation frequency showed that plant height significantly increased over the length of the trial, but especially for all cultivars from week 16 to 20 (Figure 4.13). Sensation showed that it was taller than the other plant treatments that received the 4 week defoliation frequency from the 16 week measurement until the conclusion of the trial (20 weeks). In addition while Sensation was occasionally significantly taller in other less frequently defoliated treatments, relatively short increases in height or static heights was observed among cultivars when contrasted with the 4 week defoliation frequency. An interaction was seen between as cultivar \times frequency of defoliation \times week of harvest ($F_{18, 400}=2.19$, $P=0.0034$). There was a significant difference between all the defoliation frequencies through the entire trial, which at the last harvest (20 weeks) showed the 4 week defoliation frequency height averaged 1.9 and over 3 times taller than the 2 week and 1 week defoliation frequencies, respectively.

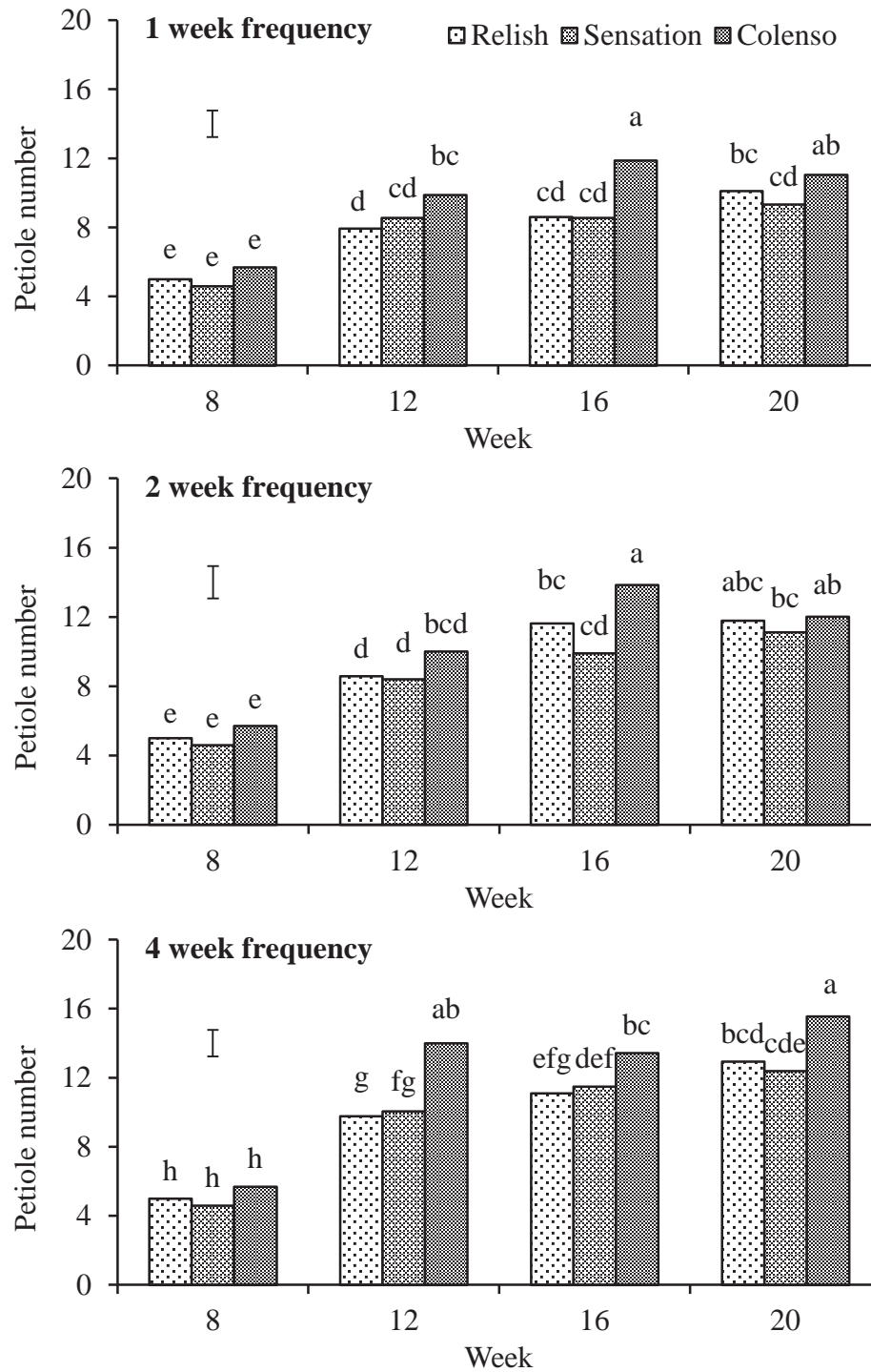


Figure 4.11: The effect of 1 week, 2 week or 4 week defoliation frequencies on petiole number (petioles/plant) of Relish, Sensation and Colenso over 20 weeks after sowing. The bar indicates the LSD value. Means with the same letter are not significantly different to each other.

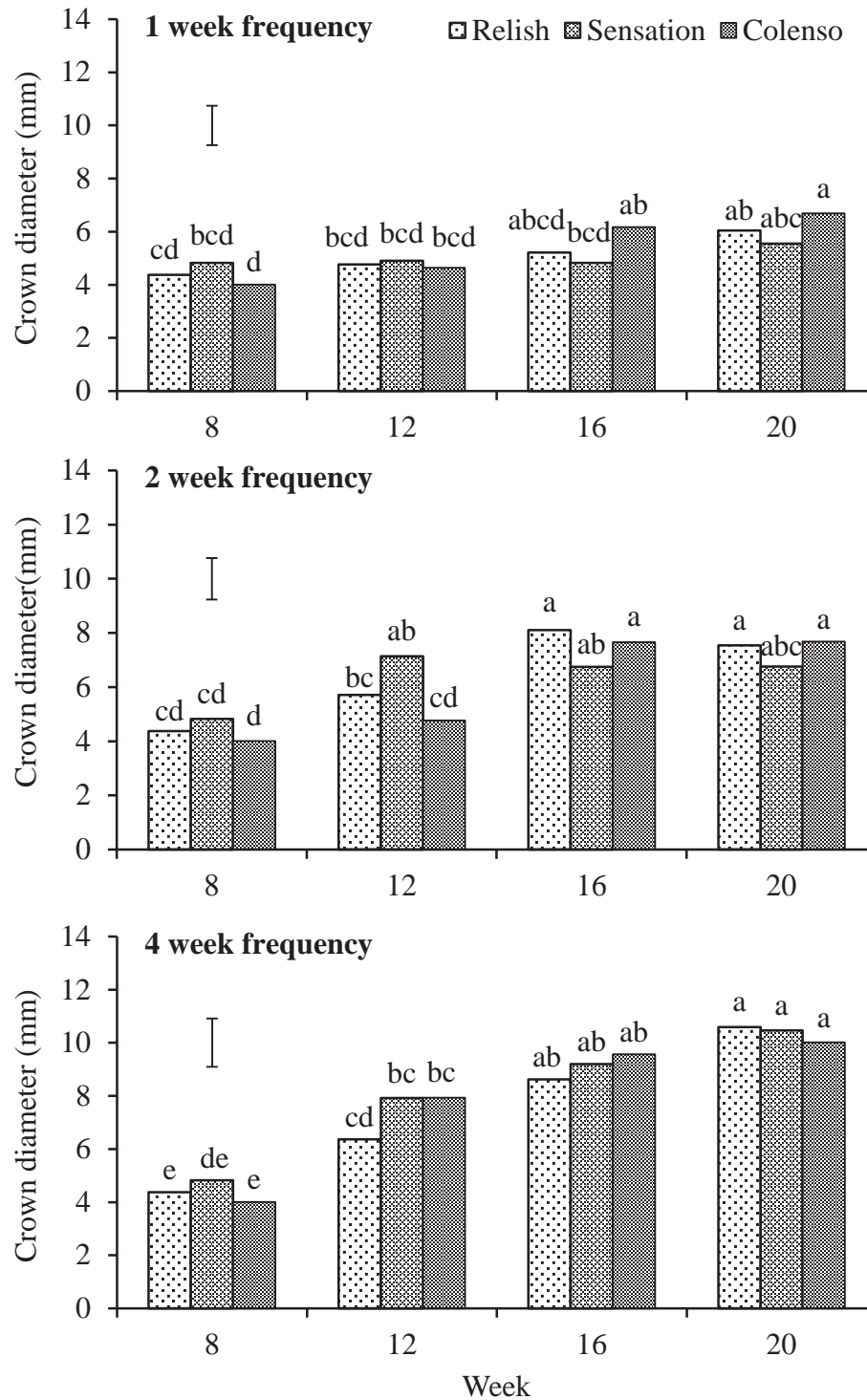


Figure 4.12: The effect of 1 week, 2 week or 4 week defoliation frequencies on crown diameter (mm) of Relish, Sensation and Colenso over 20 weeks after sowing. The bar indicates the LSD value. Means with the same letter are not significantly different to each other.

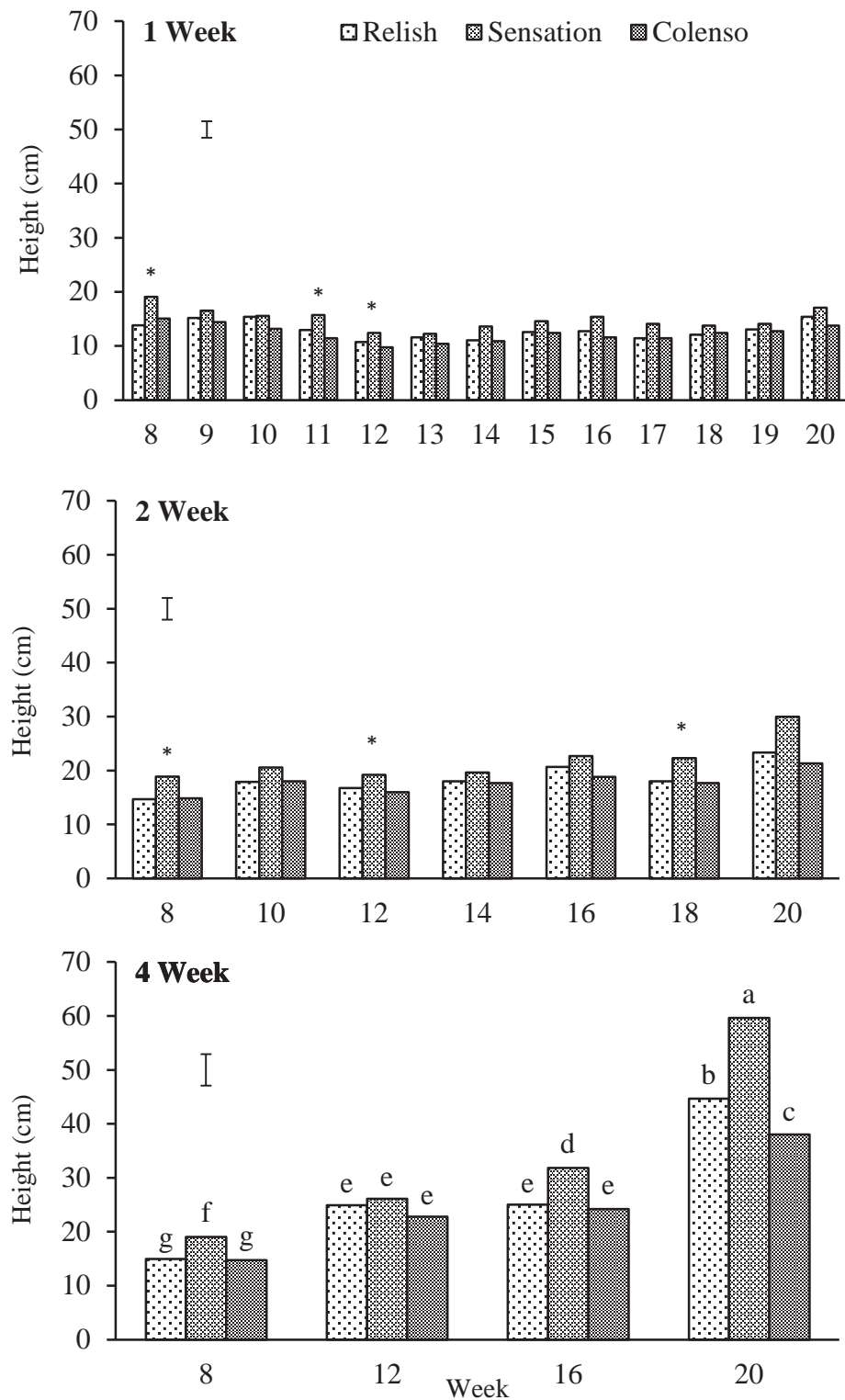


Figure 4.13: The effect of 1 week, 2 week or 4 week defoliation frequencies on plant height (cm) of Relish, Sensation and Colenso over 20 weeks after sowing. The bar indicates the LSD value. * Indicates that the treatment is significantly greater than at least one of the other treatments in the same week. Means with the same letter are not significantly different to each other.

CHAPTER 5 - DISCUSSION

5.1. Introduction

This study aimed to compare different grazing strategies in the early growth and developmental stages of recent red clover cultivars with either lucerne or an older red clover cultivar as benchmarks. Understanding the impact that grazing management has on short and long-term productivity of a pasture can help improve management techniques.

Experiments were conducted to analyse the timing of first grazing and the effects of various grazing frequencies. The effects of timing of first grazing on subsequent growth over a period of 28 weeks from sowing was analysed in a field trial, while repetitive defoliation frequencies were carried out in a glasshouse over a 16 week period.

Experiment 1 (field trial) hosted two cultivars of red clover ‘Grasslands Relish’ and ‘Grasslands Sensation’ and one cultivar of lucerne ‘Grasslands Torlesse’ with particular interest paid to red clover due to many differences being apparent between the behavior and morphology of each species (Thomas, 2003). Experiment 2 (glasshouse trial) also included the two red clover cultivars ‘Grasslands Relish’ and ‘Grasslands Sensation’ with the addition of a third older red clover cultivar ‘Grasslands Colenso’. Discussion points of this section will aim to show the herbage production of each experiment as a result of different first grazing treatments or different grazing frequency treatments. Following the herbage production discussion the various plant components that affected herbage production will be discussed in further detail.

5.2. Herbage production

Relish during experiment 1 accumulated the highest total DM production after being first grazed 12 weeks after sowing, followed by a second grazing in spring (Figure 4.1). Total Sensation DM produced from the 14 week first grazing treatment was comparable to 10 and 14 week first grazing Relish treatments. Ford and Barrett (2011) described similar results in the first year of growth as Relish had a clear growth advantage over Sensation. However, 10 and 12 week first grazing Sensation treatments produced far less total DM than the Relish treatments. Earlier first grazing of an autumn crop enables a longer period before winter for regrowth which can help replenish

energy storage heading into a low to absent winter growth period, which Li et al. (1996) reported in red clover and lucerne during winter hardening in autumn. During winter these root reserves are depleted then regenerated in spring. Reserves are assumed responsible for initial spring growth. This utilisation of the autumn growth period is a likely cause of relatively high DM production for Relish as well as a high resilience to early grazing. Sensation did not show these resilient characteristics at this stage of maturity. The plant having adequate accumulation of reserves looks to be the key determinant to sustaining high DM yields.

Total DM in experiment 1 showed Relish that received a 10 or 14 week first grazing and Sensation that received a 14 week first grazing had similar DM to Hyslop (1999) who used red clover cultivar Astred also autumn sown in Palmerston North but not subjected to a first grazing until the spring (insert date). Relish that received a 12 week first grazing saw a total DM accumulation higher than Astred (Hyslop, 1999) over a similar time frame of 28 weeks. However, all of the red clover treatments in this study saw higher total DM accumulation than what was found by Hyslop (1999) for Grasslands Pawera.

DM production at the time of the three first grazing treatments of experiment 1 showed that 10 weeks after sowing yielded substantially less than the two other treatments (12 and 14 weeks after sowing). Both Relish and Torlesse showed slightly greater DM accumulation at 12 weeks after sowing while Sensation had slightly higher DM at 14 weeks after sowing. This shows that 10 weeks after sowing was too early for all cultivars of the two species used as the grazing 12 weeks after sowing that followed had a 220% to 255% increase in DM across all cultivar/species. Powell et al., (2007) reported early first grazing DM yields of 'Redmore' red clover similar to what was found in this study.

The final harvest of experiment 1 at 200 days after sowing showed the timing of first grazing had a greater detrimental effect on Sensation than Relish. The 14 week first grazing treatment received by Sensation was the only treatment with a similar DM production to any treatments received by Relish. This gives further evidence that Sensation needs a longer growth period before first grazing while Relish was less dependent on the timing of first grazing. The 14 week first grazing of Relish showed the least spring DM accumulation, likely due to being closer to the cool season as plant

reserves were starting to decrease, as Haagensohn et al. (2003) found in their autumn sown lucerne trial. After a high 12 week first grazing production reported in 'Redmore' red clover by Powell et al. (2007) the second pre-graze DM in spring was less (1410-1708kg) than that generated by Relish that received a 10 or 12 week first grazing.

For the experiment 1 first grazing at 14 weeks after sowing treatment, Sensation produced a significantly larger crown diameter compared to Relish. This could be considered a strong contributor to the additional DM gained by Sensation between 12 week and 14 week first grazing treatments, as a larger crown allows for more growing points and possibly a greater plant energy reservoir (Table 4.7). Sensation produced a higher DM after the morphological changes regardless of the less favourable climatic conditions, which could indicate that a more mature stage had been reached. Furthermore this suggests that when compared to Relish, Sensation's ability to produce high yields is limited to a longer establishment phase. Relish did not show the same dependence on stage of maturity for early DM accumulation, which may suggest a greater resistance to depletion of energy reserves in early establishment.

Torlesse had a lower DM yield than the two red clover cultivars across all of the first grazing treatments in experiment 1. Various factors could have contributed to these lower yields such as pest intrusion during the early stages of the experiment by rabbits. Damage was seen on lucerne plants but no damage to red clover was observed. The damage to the lucerne plants during establishment, though not severe, may have damaged growing points causing an unrecoverable setback to the lucerne as weed pressure and general slow growth ensued. Furthermore, normally spring-sown lucerne, does not have the same ability as red clover to be sown in autumn suggesting the sowing date was late giving thermal time before winter as Grassland Torlesse is highly winter dormant (Stewart, Kerr, Lissaman & Rowarth, 2014; Hampton et al., 1999). After receiving a 10, 12 or 14 week first grazing treatment, when contrasted with the two red clover cultivars, lucerne showed that the poor DM yield was somewhat amplified by the spring.

Often the relationship between field and glasshouse trials is not strong because of the high level of environmental control that can be achieved in a glasshouse (Caradus, 1991). However, this added control allows for precise measurements and considerations

that can sometimes prove difficult or unachievable in the field, demonstrated by Crush (2005) in a clover root weevil experiment. In many experiments that have included clover, outside diurnal temperature fluctuations can vary by as much as 12-18 °C (Crush, 2005; Ford & Barrett, 2011), a range which is not within the threshold of ideal growth (Frame, 2005). However, in a glasshouse temperature extremes are avoided especially through winter months.

Herbage mass in experiment 2 showed that the least intensive 4 week defoliation frequency produced the greatest accumulations of herbage mass (Figure 4.8). Relish and Sensation produced high herbage yields towards the end of the four month experiment, although there was no statistical difference between the final herbage weight of Sensation and Colenso due to Colenso having a wide variation among samples, with no obvious reasons for the variation. It could be assumed that the rate of herbage accumulation of Colenso is less than the more recent cultivars Sensation and especially Relish (Claydon et al., 2003). This growth rate variation was also seen in the root weight as the two recent cultivars surpassed Colenso but only at the final trial measurement. This result is covered in more detail further on in the discussion. The increase in stable early total plant biomass production of the recent cultivars Relish and Sensation demonstrates the successful breeding efforts for attributes such as less cool season dormancy, faster establishment and pest resistance of red clover (Tucak et al., 2013).

had herbage weights heavier than at least one of the cultivars on numerous occasions when subjected to the most intensive defoliation frequency in experiment 1. Results showed intensive defoliation gave little variation among different cultivars as energy demands from biomass removals saw minimal vegetative replenishment while stunting and static growth across cultivars was the result (Kemp et al., 1999). As outlined by Richards (1993) defoliation causes the mobilisation of reserve carbohydrates as carbon allocation is directed to leaf meristematic zones. Lowered radiation use efficiency could also be a limiting factor as leaf area is frequently reduced with intensive repetitive defoliation (Teixeira, Moot, & Brown, 2008)

5.3. Plant components

All plant treatments in experiment 1 had similar heights when first grazings occurred. However, both red clover cultivars developed much taller plants than lucerne by spring.

The red clover cultivar treatments that gained the tallest heights also gained the highest DM yield. Relish showed significantly taller plants than Sensation in the 10 week and 12 week treatments in spring contrary to expectations as the growth habit and genetic background of the more erect Sensation suggests it would grow taller in the sward than Relish (Claydon et al., 2003; Ford and Barrett, 2011).

Results from experiment 2 suggested growth habit differences, as Sensation towards the end of the 4 week defoliation frequency Sensation showed a clear height advantage over both Relish and Colenso (Figure 4.13). This is likely due to the natural growth habit of Sensation being able to be exercised as there was less energy limitation from fewer biomass removals (Kemp et al., 1999; Richards, 1993). It cannot be determined from the present study why Sensation grew shorter than Relish, when subjected to 10 and 12 week first grazing treatments during experiment 1, though it could suggest premature first grazing which DM accumulation also reflected (Figure 4.4).

Frequently defoliated plant treatments (1 and 2 week frequency) in experiment 2 showed static height throughout the trial, while the four week defoliating frequency resulted in an increase in height especially towards the end of the trial. This is likely due to natural growth patterns trying to recover from such frequent defoliation along with observed elongation of petioles due to reproductive processes becoming apparent by the final harvest.

From 84 days (12 weeks after sowing) to the end of the experiment 1 there was little variation in plant density within each of the species. This was likely due to the populations coming closer to the predicted self-thinning rule of Westoby (1984), as plant populations reach an equilibrium of biomass accumulation and population. During the trial Relish and Torlesse both started with higher densities than Sensation though they were sown at the same rate. It was not confirmed why the Sensation population density was low in the early stages of the trial but it was likely that poor establishment was the cause not poor germination. This could be due to the low rainfall in March (though irrigated, 18 March) and the above average soil temperature in the early months of the trial, as decreasing soil moisture and high soil temperatures contribute to these symptoms (Hampton et al., 1999), or because seed vigour differences were not detected. At 84 days (12 weeks after sowing) Sensation, Relish and Torlesse had similar plant density, though at 100 days (14 weeks after sowing) Sensation's plant

population was again lower than the other plant treatments, which continued until the end of the trial. These results indicate a stronger plant survival of Relish during early establishment.

At the final harvest for experiment 1 the spring plant density was not affected by the timing of first grazing treatments, indicating the timing of first grazing did not have an effect on plant density after low growth/dormancy during winter. However, Relish and Torlesse continued to show a substantially higher plant density than Sensation in spring, suggesting a continuation of greater persistence performance from early establishment. The early persistence results of Relish support the results of Ford and Barrett (2011) who found long term survival after 4 years was both high in comparison to Sensation as well as in terms of red clover as a species.

and leaf number were collected for all plant treatments in experiment 1 but it was apparent from early in the trial that the differences in morphology between the red clover and lucerne was not giving a fair comparison of above ground foliage. The findings of Thomas (2003) were that primary stems elongate in lucerne, which causes relatively small petioles to those of red clover. This caused lucerne to have a greater number of petioles produced off the primary stem, and, therefore more leaves than red clover. Results from experiment 2 showed Colenso also had a higher number of petioles per plant than either Relish or Sensation. This was likely due to the prostrate growth habit and low lying biomass production of Colenso and the more vertical biomass allocation of Sensation, and intermediate growth habit of Relish (Claydon et al., 2003; Ford & Barrett, 2011).

The two red clover cultivars, Relish and Sensation showed very similar characteristics for both number of stems/petioles and number of leaves throughout experiment 1 and experiment 2. Experiment 1 had a steady increase in both stem and leaf throughout the trial for both cultivars, which caused the interaction between the timing of first grazings. There was a significant increase in stem and leaf number in both of the red clover cultivars from 84 days (12 weeks after sowing) to 100 days (14 weeks after sowing). The root ratio also increased during this period, though it is unlikely that the entire plant grew, as DM production remained unchanged. The morphological change could be due to natural plant preparation heading into the relative dormant months of winter (Li et al., 1996). A progressive increase in petiole

number for all plant treatments throughout the duration of experiment 2, along with previously mentioned high petiole number produced by Colenso resulted in an interaction between cultivars and week of harvest, and was most prominently seen in the infrequently defoliated (4 week) plant treatments. Over the majority of the trial the two week and four week defoliation frequencies showed similar petiole numbers per plant, but the four week defoliation frequency treatments ended the trial with more petioles per plant. Increased energy utilisation from greater biomass removal is the likely cause of the slower increase and fewer numbers of petioles that were produced as a result of higher frequency of defoliation, causing the interaction between defoliation frequency and week of harvest (Kemp et al., 1999; Richards, 1993).

Experiment 1 and 2 showed Relish and Sensation had many similarities in crown diameter throughout the experiments. However, in experiment 1 Sensation had a significant increase in crown diameter from 84 days to 100 days (12-14 weeks after grazing) that was not seen in Relish. Along with yield implications, which have been previously mentioned, the morphological crown diameter change that occurred in Sensation would indicate a greater biomass accumulation in the crown before winter compared to Relish.

Gaining crown growth at 14 weeks after sowing enabled Sensation to compete directly with most of the yields of Relish in experiment 1 and retain a larger crown diameter than Relish into spring. Sensation that received a first grazing earlier (10 and 12 weeks after sowing) had comparable crown diameter to Relish throughout the experiment. However, DM yield of Sensation in spring that received the 10 and 12 week first grazing treatments was less than for Relish. Sensation carried the larger crown diameter from the 14 week first grazing into spring while Relish gained its widest crown diameter in spring from plant treatments that received a 12 week first grazing. Results suggest that the plant species treatments were most productive at their respective first grazings times as they produced the widest crown diameter in autumn as well as DM yields in both autumn and spring, further showing the importance of appropriate timing of first grazing (Hampton et al., 1999).

Experiment 2 showed wider and increasing crown diameters throughout the experiment from less frequently defoliated plant treatments. Increases in the crown diameter over the time of the trial were low in frequently defoliated plant treatments (one and two

week defoliation frequencies) resulting in an interaction between frequency of defoliation \times week of harvest. Plant treatments that had a two week defoliation frequency did show an increase in crown diameter but growth was halted towards the end of the experiment, likely due to a threshold of energy demands being met at this point and therefore simulating the effect of overgrazing a red clover sward (Kemp et al., 1999; Richards, 1993). However, plant treatments receiving a 4 week defoliation frequency produced a continuous increase in crown diameter. This may be due to adequate time for energy allocation of the plant treatments to regenerate essential vegetative components, as well as energy storage being used to facilitate regeneration and possibly greater reserves causing a wider crown.

Biomass allocation to roots in experiment 1 and root weight in experiment 2 both showed close similarities between Relish and Sensation throughout both experiments. This could be due to the fact that some similar genetic background and breeding pools have been used to develop both the recent red clover cultivars Sensation and Relish (Ford & Barrett, 2011).

Similar root weights were seen in experiment 2 between all cultivars that received the four week grazing frequency but by the final harvest Relish and Sensation continued to increase root biomass while Colenso remained unchanged. As mentioned earlier, this could indicate Relish and Sensation accumulated total biomass quicker than the older cultivar Colenso. Plant treatments receiving the four week defoliation frequency also showed an increase in root weight at each harvest while the other more frequent defoliation frequencies showed little increase after halfway through the trial causing an interaction between, week of harvest and defoliation frequency. Once again this suggests an energy limitation caused by high frequency biomass (Kemp et al., 1999; Richards, 1993). Colenso showed a dominant root biomass over the other two cultivars when subjected to an intensive (1 week) defoliation frequency during the second half of the trial. Also as mentioned, this along with other morphological characteristics of the cultivar may be considered to have a higher resilience to intensive defoliation.

In experiment 1, Relish at 10 and 14 weeks after sowing showed its lowest shoot ratios, while at week 12 Relish produced the highest DM yield. However, Sensation had its lowest shoot ratio at 14 weeks after sowing and produced its highest DM yield at this treatment. This indicates the entire plant had grown substantially

during this time, including a greater proportion of root growth which is further suggested by the increase in crown diameter. Experiment 2 further confirmed that an increase in root or shoot weight often corresponded with an increase in total plant biomass (Appendix 3). This also shows morphological variation between red clover cultivars as biomass allocation of Sensation is more directed towards roots with regards to high DM yields when contrasted with Relish. Torlesse showed similar shoot ratios at the 10 week and 12 week (highest DM accumulation) grazing treatments while the 14 week first grazing treatment was significantly lower.

Grazing treatments had virtually no effect on root and shoot ratio when measured in spring, however, differences between plant treatments were apparent. This likely means that the stage of development of red clover or lucerne when first grazed has little to no effect on biomass allocation for regeneration. Relish collected more biomass to above ground vegetation than Sensation, which may partially contribute to it significantly out producing Sensation in the 10 week and 12 week first grazing treatments. Torlesse had a much larger root ratio than both of the red clovers and this could be attributable to the poor vegetative regrowth that was produced in spring.

For the majority of the experiment 2 all cultivars showed similar stubble weights during the early months of growth likely due to their comparable morphological characteristics. Towards the end of the trial, the Sensation treatments receiving the more intensive 1 and 2 week defoliation frequency showed lighter stubble weight than the other cultivars. This could be due to the erect growth habit of Sensation producing a less dense plant base (Claydon et al., 1993) as greater plant height was unable to be achieved with frequent biomass removal. The effect of the lighter stubble of Sensation may expose a greater area of bare soil therefore increasing the likelihood of grazing damage (Sheath & Boom, 1997), which also reinforces the pure sward, 'cut and carry type' breeding background of Sensation (J.Ford, personal communication, April 15, 2014). Less intensive (4 week) defoliation frequency showed much heavier stubble as well as an increasing stubble weight at each progressive harvest that was not shown by the more intensive frequent defoliation frequencies, and resulted in an interaction between defoliation frequency and week of harvest.

5.4. Practical Recommendations

The results of this study show that Relish performed best when grazed with 13 true leaves or 4 regenerating stem units, however, Sensation performed best when it was able to gain greater than 14 leaves as found by Powell et al., (2007) or 5 regenerating stem units. Height of plants in this study did not prove to be a useful indicator of grazing time, as height between first grazing times were similar and were well below the recommended 20-25 cm suggested by Kemp et al. (1999) and Frame et al. (1998). Residual heights below 5 cm should be avoided, but the recommended residual height of 8 cm by Kemp et al. (1999) may be impractical at first grazing.

Autumn sown legumes, especially if a grazing before winter is targeted should be planted as early as possible during early autumn or late summer if soil moisture is adequate or irrigation will be required. This will minimise risk of grazing with inadequate maturity, while enabling a short period before winter for regrowth and energy replenishment, which will later be utilised for initial spring growth (Li et al., 1996).

Repetitive defoliation results from this study showed that performance is reduced significantly with increasing intensity of defoliation. Favourable characteristics of cultivars were less prominent or non-existent when defoliations were intensive, indicating the importance of adequate grazing intervals to ensure the beneficial attributes of species are achieved. Ideally a 6 week grazing rotation should be targeted, with no less than a 4 week rotation to avoid production reduction and subsequent lower morphological variation between older and more recent cultivars (Kemp et al., 1999).

5.5. Future research

The long term effects that first grazing management has on persistency would be beneficial to learn, as persistency could be considered red clover's key limitation, especially in New Zealand. The effects of first grazing of recent red clover cultivars in a mixed sward with grass would also add beneficial knowledge as current research is limited.

CHAPTER 6 - CONCLUSION

Relish established earlier and gained a greater herbage mass than Sensation at both the first grazing and in spring. The swift establishment of Relish allowed more flexible time of first grazing than Sensation. Sensation had a lower plant population than Relish; however, timing of first autumn grazing did not have an effect on the persistency of either red clover or lucerne in spring but yield was highly affected. Both the recent cultivars Relish and Sensation showed higher above and below ground biomass production than the benchmark red clover and lucerne species. Frequent defoliation showed suppression of unique cultivar characteristics between cultivars and relative uniformity was the result. Slower morphological progress and less total herbage mass was also the result of frequent defoliation, as energy balance eventually limits all plant biomass allocation.

Recommendation

Under the climatic conditions in this study establishing an autumn sown red clover by winter, a sowing date earlier than 12 March should be targeted to ensure adequate maturity is reached, especially if a grazing before winter is expected. Stage of maturity is more important than the number of weeks after sowing that grazing occurs, but following an autumn sowing a period before winter should be left for energy regeneration. Relish needs to develop 13 true leaves whereby when this threshold of maturity is reached the sward should be grazed as prolonging first grazing can sacrifice spring yield. However, Sensation requires more than 14 true leaves before first grazing. If red clover was to be planted in a mixed pasture with a grass as a replacement to the typical white clover, Relish would be recommended over Sensation because of the quick maturity and flexibility of first grazing time, beneficial morphological traits, as well as higher persistence and yield.

CHAPTER 7 - REFERENCES

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APPENDICES

APPENDIX 1

Table: Number of obvious main stems (>10cm) from red clover treatments Relish and Sensation stem number 200 days after sowing.

Date	Relish	Sensation	F	P
28 Sep	5.55± 0.31	5.55± 0.22	0	1

Table: Number of stems (stems/plant) and leaves (leaves/plant) of Torlesse over time. The time of first grazing treatments were 71 (10 week), 84 (12 week) and 100 (14 weeks) days after sowing.

Leaf number		Stem number	
Day	Torlesse	Day	Torlesse
35	5.62 ± 0.25	35	3.27 ± 0.11
50	13.73 ± 0.53	50	6.15 ± 0.16
71	19.12 ± 0.87	71	13.03 ± 0.53
78	28.48 ± 1.22	78	17.83 ± 0.72
84	31.20 ± 1.97	84	18.20 ± 0.96
100	35.25 ± 2.40	100	21.05 ± 1.24

APPENDIX 2

Harvest 1, no defoliation treatments applied,
left to right, Colenso, Relish, Sensation



Harvest 2, 1 week defoliation frequency
left to right, Colenso, Relish, Sensation.



Harvest 2, 2 week defoliation frequency
left to right, Colenso, Relish, Sensation.



Harvest 2, 4 week defoliation frequency
left to right, Colenso, Relish, Sensation



Harvest 3, 1 week defoliation left to right,
Relish, Sensation, Colenso.



Harvest 3, 2 week defoliation left to right,
Relish, Sensation, Colenso



Harvest 3, 4 week defoliation left to right,
Relish, Sensation, Colenso



Harvest 4, 1 week defoliation left to right,
Relish, Sensation, Colenso



Harvest 4, 2 week defoliation left to right,
Relish, Sensation, Colenso



Harvest 4, 4 week defoliation left to right,
Relish, Sensation, Colenso

Plate 7.1: Effects of 1, 2 and 4 week defoliation frequency of experiment 2 over four harvests for Relish, Sensation and Colenso.

APPENDIX 3

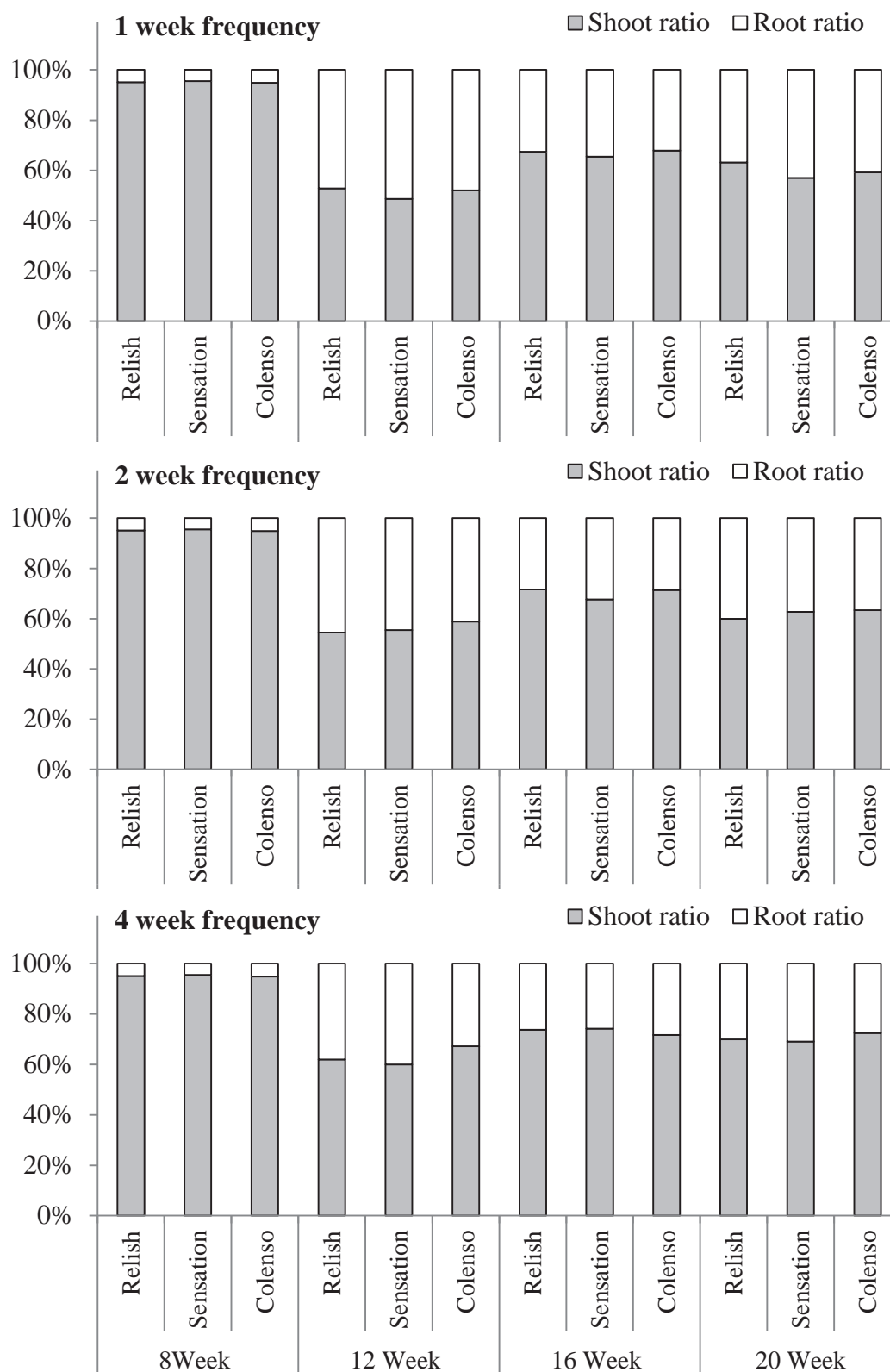


Figure: Root and shoot ratio of 1, 2 and 4 week defoliation frequency of experiment 2 over four harvests for Relish, Sensation and Colenso.