# Chicory (*Cichorium intybus*) and plantain (*Plantago lanceolata*); physiological and morphological responses to water stress, defoliation, and grazing preference with implications for the management of the Herb and Legume Mix

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# Abstract


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Herb and legume sward mixes containing chicory (*Cichorium intybus*), plantain (*Plantago lanceolata*), red clover (*Trifolium pratense*) and white clover (*T. repens*) are being increasingly used by farmers to improve animal performance compared to perennial ryegrass and white clover swards. However, little is known about the agronomic properties of this Herb and Legume Mix. The objectives of this research were to examine key factors likely to affect the success of the Herb and Legume Mix as a perennial sward mix. This thesis included a series of glasshouse experiments, a grazing experiment (examining plant parameters and animal grazing preference) and a mowing experiment. The glasshouse experiments indicated that chicory and plantain have different strategies for coping with moisture stress. The results suggest plantain may be more productive under moderate drought due to its greater shoot mass fraction, whereas chicory may be more productive and persistent under severe drought due to its greater root mass and taproot diameter. The Herb and Legume Mix accumulated greater annual dry matter when removed under Hard grazing (post-grazing residual of 4cm) compared to Lax grazing (post-grazing residual of 8cm). Hard grazing favoured plantain growth and persistence, while Lax grazing favoured red clover growth and resulted in chicory with a larger taproot diameter. It was concluded that grazing management decisions should be determined by ensuring optimal management of chicory. Ewe lambs displayed grazing preference for species within the Herb and Legume Mix; however this varied between seasons and was affected by the species availability, vertical access and palatability. The Herb and Legume Mix had a greater herbage nutritive value than the ryegrass and white clover sward and had a more stable composition over time than pure swards of chicory and plantain under a wide range of defoliation regimes. The results suggest the Herb and Legume Mix might be a more flexible perennial forage option than pure swards of chicory and plantain. Overall the results of this thesis indicated that the Herb and Legume Mix can be successfully utilised in most New Zealand grazing systems as a perennial forage sward.

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# Foreword

This thesis is written so that the chapters can be readily converted into papers for publication. Therefore, each chapter contains a full and thorough discussion and the overall discussion chapter provides a succinct overview of the entire thesis content. Throughout the thesis the convention used for stating significance in tables is $P = 0.05$, as done in the journal Animal Production Science. The references from each chapter are combined and presented at the end of the thesis. It must be pointed out that this work is only focused on the performance of the plant species in the Herb and Legume Mix and not animal performance or weed control. Ultimately, this thesis aims to provide answers for grassland farmers utilising the Herb and Legume Mix.

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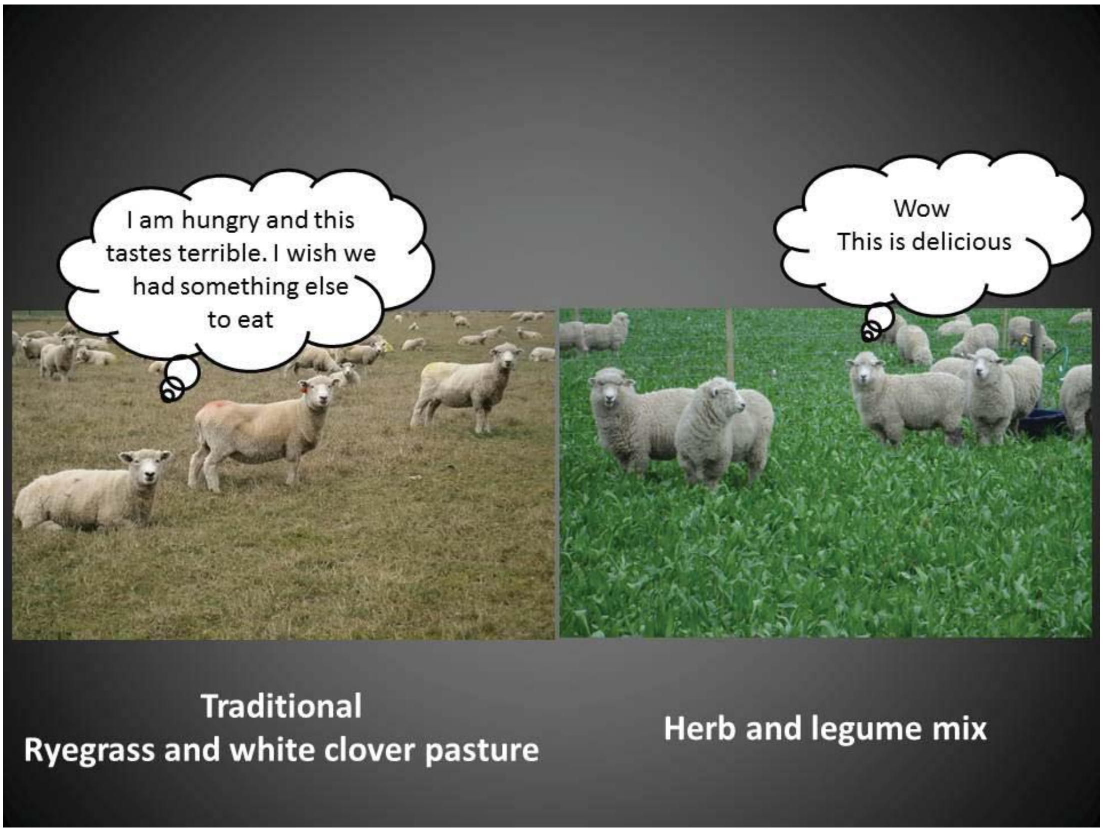
# Chapter 1 Introduction



New Zealand pastoral systems have traditionally been based on grass species with the addition of clover. The predominant sown pasture mix is perennial ryegrass (*Lolium perenne*) with a minor component of white clover (*Trifolium repens*) (Kemp *et al.* 2002b). However, throughout the summer and autumn months production of ryegrass and white clover pasture can be limited in both quality and quantity, which can lead to reduced feed intakes and animal performance (Hughes *et al.* 1980; Burke *et al.* 2002; Moorhead *et al.* 2002). Therefore, during these periods alternate herbage species are of interest.

Chicory (*Cichorium intybus*) and plantain (*Plantago lanceolata*) have long been found in natural grassland (Stewart 1996; Li and Kemp 2005), however, more recently the development of commercial cultivars has resulted in these species being used as both forage crops and components of perennial swards. In the last 10 years the use of chicory in New Zealand has steadily increased, while in the last 5 years the use of plantain as a forage crop has increased monumentally (A. Moorhead; pers. comm).

Both the herbs chicory and plantain can be high yielding (Hare *et al.* 1987; Brown *et al.* 2000; Powell *et al.* 2007), and of high nutritive value (Barry 1998; Burke *et al.* 2006; Hayes *et al.* 2010), high mineral content (Sanderson *et al.* 2003b; Harrington *et al.* 2006) and can support greater live weight gain in sheep, cattle and deer than traditional perennial ryegrass and white clover swards (Fraser *et al.* 1988; Scales 1995; Moorhead *et al.* 2002; Hoskin *et al.* 2005; Judson *et al.* 2009). The major agronomic shortcomings of chicory and plantain are their apparent limited persistence of 3-7 years (Hume *et al.* 1995; Li *et al.* 1997b; Belesky *et al.* 2000; Sanderson *et al.* 2003a) and their difficultly of managing in winter, due to low growth rate (Kemp 1996; Li *et al.* 1997b; Ayala *et al.* 2011b). Red clover (*Trifolium pratense*) and white clover (*Trifolium repens*) are nitrogen fixing legumes, also known to have high nutritive value (Barry 1998; Burke *et al.* 2006) and can produce impressive live weight gains in lambs (Fraser *et al.* 2004; Marley *et al.* 2005; Lindsay *et al.* 2007) and deer (Soetrisno *et al.* 1994).

In the last few years the use of multi-species herb and legume sward mixes has increased, especially since the exclusion of grass species from the mixes. The Herb and Legume Mix; containing chicory, plantain, red clover and white clover is now being used by some farmers as it offers the potential of improved persistence, a longer grazing season and can produce a greater herbage yield than mono-species herb swards and results in improved animal performance (Golding *et al.* 2011; Hutton *et al.* 2011; Sinhadipathige *et al.* 2012). However, overall little is known about the agronomic properties of the Herb and Legume Mix and its potential to be used as a perennial sward mix. Therefore, the general objectives of this thesis are to:

i. Examine the performance of chicory and plantain under dry conditions.
ii. Observe and further understand the diet selection and grazing preference of lambs offered the Herb and Legume Mix.
iii. Examine the sward dynamics, plant density and yield of the Herb and Legume Mix under various defoliation and grazing regimes.

# Chapter 2 A review of chicory and plantain and their potential to be utilised in a mixed sward for livestock production

## 2.1 Introduction

This review, examines the agronomic characteristics, herbage production, grazing management, persistence under grazing, nutritive value, and animal performance of livestock grazing forage chicory and plantain when sown separately and their potential when used in a sward mix with red and white clover.

## 2.2 Plantain (*Plantago lanceolata*)

Plantain is a narrow-leaved perennial herb of the Plantaginaceae family and in some countries it is commonly known as flat weed (Stewart 1996). It is taprooted with erect leaves and a rosette of broad leaves produced from a crown base (Stewart and Charlton 2006). This review will focus on the relatively recent cultivars ‘lancelot’ and ‘Ceres Tonic’.

### 2.2.1 Soil requirements

Plantain establishes rapidly, and grows on a wide range of agricultural soils, being both drought and pest tolerant (Stewart and Charlton 2006). However, plantain does not persist well on water-saturated soils (Mook *et al.* 1989). Plantain does not require high fertility soil and grows under a wide range of soil acidity (pH 4.2-7.8) (Stewart 1996). It is more likely to have its greatest contribution to yield in low fertility dryland pastures (Stewart 1996).

### 2.2.2 Sowing time and establishment

Plantain can be autumn or spring sown. Compared to perennial ryegrass, plantain requires a greater length of time after sowing before it can be grazed for the first time (Stewart 1996). Furthermore, seedling development of plantain is slower in autumn than in spring (Sanderson and Elwinger 2000; Powell *et al.* 2007), probably due to lower mean temperature and decreasing day lengths. Sanderson and Elwinger (2000) suggested that seedlings require more than two fully expanded leaves for winter survival. Powell *et al.* (2007) determined that plantain should not be grazed until it has six fully developed leaves and is approximately 300 mm high, in order to minimise plant loses.

### 2.2.3 Herbage production and pattern of growth

Herbage production of 7.5-12 t DM/ha per year is very common for pure plantain swards over two to four years under grazing (Table 2.1). Whilst, under optimal conditions, plantain can yield up to 17 t DM/ha per year in pure swards (Powell *et al.* 2007). Powell *et al.* (2007) found a pure sward of plantain to be as productive as perennial ryegrass and white clover pasture over a year and more productive than chicory, except during the summer period. Rumball *et al.* (1997) concluded the DM production of plantain seems to be equivalent to that of major grass species, however, this may be further reduced by the frequent grazing needed to retain palatability.

**Table 2.1 Annual herbage yield (t DM/ha) for pure swards of plantain across a number of studies.**

| Cultivar | Region and Country | Average herbage yield[a] | Study |
|---|---|---|---|
| Lancelot | Canterbury, NZ | 7.6 | Stewart (1996) |
| Tonic | Canterbury, NZ | 8.4 | Stewart (1996) |
| Lancelot[b] | Manawatu, NZ | 7.5 | Rumball *et al.* (1997) |
| Tonic | Manawatu, NZ | 17 | Powell *et al.* (2007) |
| Tonic | NSW, Victoria, SA, WA, Australia | 9.5 | Reed *et al.* (2008) |
| Tonic | Treinta y Tres, Uraguay | 9.1 | Ayala *et al.* (2011a) |
| **Average of studies** | | **9.9** | |

[a]Average if reported or calculated from years given

[b]Plantain only yield, total mixed sward of plantain/white clover yielded 11.7 t DM/ha

Plantain is summer active and accumulates most dry matter over the December to February period, with little production during winter (Stewart 1996; Labreveux *et al.* 2006; Moorhead and Piggot 2009). Furthermore, plantain begins growing earlier in spring, than chicory, red clover and white clover (Kemp *et al.* 2010). Plantain remains highly productive over autumn producing greater shoot DM, percentage leafiness and shoot density compared to chicory (Yu *et al.* 2008). Consequently, plantain can be used in a herb sward mix to help extend the length of the grazing season. Moorhead and Piggot (2009) found a plantain based sward which included plantain, red and white clover and ryegrass yielded significantly more than a ryegrass based sward by 1.8 t DM/ha and 0.9 t DM/ha during summer and autumn, respectively. Furthermore, production during winter and spring did not differ significantly, resulting in annual yields of (17.6 t DM/ha vs. 14.3 t DM/ha, for plantain based sward and ryegrass based sward, respectively) (Moorhead and Piggot 2009).

### *2.2.4* Root structure, drought tolerance and freezing tolerance

Plantain has a smaller taproot diameter than chicory (Yu *et al.* 2008). Although it has a less dominant primary root and its lateral roots tend to form a more fibrous system (Sanderson and Elwinger 2000). Consequently, under Australian conditions, Nie *et al.* (2008) found plantain maintained a higher green-leafiness over summer than most temperate grasses with high summer dormancy and had similar green-leafiness to chicory. Likewise, an experiment in Uruguay under a rainfall regime which was 25% under the long term average of the experimental site, showed plantain had an adequate degree of adaptation and tolerance to drought conditions (Barrios 2006 as cited in Ayala *et al.* 2010). Mook *et al.* (1989) reported high plant death rates in summer as a result of drought are an uncommon phenomenon in plantain. Ivins (1952) suggested that the outstanding palatability of plantain observed in a dairy cow grazing experiment resulted from plantain maintaining green and succulent herbage while other species suffered from drought.

Conversely, Hayes *et al.* (2010) concluded that plantain was not a viable species in drought-prone environments due to its inferior productivity and persistence, compared to chicory. Somewhat in support of this, Sanderson *et al.* (2003a) observed a 26% reduction in dry matter yield in a dry summer, indicating plantain exerts only a moderate tolerance to moisture stress. Similarly, a glasshouse experiment showed the extension rate of plantain leaves under water stress treatment dropped below the control treatment on day 12 and reached an extension rate of zero on day 18 and did not recover from the stress treatment (Davis 1995). Compared to perennial ryegrass, natural ecotype plantain performed very poorly under conditions of water stress and did not survive (Davis 1995).

Plantain has a very poor freezing tolerance. Under harsh freezing conditions (-12 to -14°C) studies have shown very low survival rates (Skinner and Gustine 2002; Skinner 2005), thus confirming that plantain is not adapted for harsh winter conditions as seen in north-eastern USA and Canada. However, within the vast majority of New Zealand regions, persistence is unlikely to be limited by low winter temperatures.

### 2.2.5 Nutritive value

#### *2.2.5.1 Crude Protein*

The percentage of crude protein (CP) specifies how fast the protein in a particular forage, passes through the rumen to the small intestine before degradation, with increased flow resulting in increased live weight gain (Hodgson and Brookes 2002). A minimal CP concentration of 90 g CP/kg DM is essential in forage in order to meet the nitrogen requirements of rumen bacteria (Waghorn and Barry 1987). However, CP levels should be between 150-180 g CP/kg DM, or 15-18%, to enable high lamb live weight gain (Hodgson and Brookes 2002).

Across a number of studies, the average CP concentration of plantain was 205 g/kg DM which was higher than the CP concentration of perennial ryegrass (187 g/kg DM) (Table 2.2;Table 2.3). However, the CP concentration of plantain differs greatly between vegetative and reproductive growth. Fraser and Rowarth (1996) reported a CP content of 202 g CP/kg DM for plantain leaf and 138 g CP/kg DM for plantain stem. Thus the CP concentration of plantain is likely to vary between seasons, as during late summer and autumn stem can make up approximately 60% of the plantain on offer (Fraser and Rowarth 1996). Corkran (2009) found the CP content of plantain was higher in samples observed to be 'eaten' than in samples which were 'not-eaten' during early and late autumn. Eaten and Not-eaten samples had CP contents of 129 *vs* 90 g CP/kg DM during early autumn and 175 *vs* 148 g CP/kg DM during late autumn (Corkran 2009).

#### *2.2.5.2 Digestibility*

The neutral detergent fibre (NDF) component of forage estimates the total cell wall material (cellulose, hemicellulose and lignin) and provides an indication of rumen outflow rates and voluntary intakes (Hodgson and Brookes 2002). The acid detergent fibre (ADF) component of forage estimates the cell wall portions that are made up of cellulose and lignin (Waghorn *et al.* 2007). Plantain has a lower NDF and ADF content to perennial ryegrass, (average of a number of studies; Table 2.2;Table 2.3), indicating that it is more digestible than perennial ryegrass.

**Table 2.2 Chemical composition and nutritive value data of perennial ryegrass: (total nitrogen (Total N), dry matter (DM), cruc neutral detergent fibre (NDF), organic matter digestibility (OMD), in vitro dry matter digestibility (IVDMD) and metabolisable studies.**

| Study | Total N (g/kg DM) | DM (g/kg DM) | CP (g/kg DM) | ADF (g/kg DM) | NDF (g/kg DM) | OMD (%) |
|---|---|---|---|---|---|---|
| Suckling (1960) | | 219 | 188 | | | |
| Derrick *et al.* (1993) | | | | 301 | | 62.90 |
| Hoskin *et al.* (1995) | 32.3 | 209 | | 251 | 467 | |
| Scales (1995) | | | | | 508 | 67.30 |
| Jackson *et al.* (1996) | 45.2 | | | 194 | 406 | 85.00 |
| Kusmartono *et al.* (1996) | 29.7 | | | | | 74.00 |
| Barry *et al.*(1998) | | | | | | |
| Burke *et al.* (2000) | | 188 | 155 | | 487 | 77.30 |
| Burke *et al.* (2002) | | | | 255 | 487 | |
| Moorby *et al.* (2004) | | 163 | 187 | 303 | 510 | |
| Marley *et al.* (2005) | | 187 | 186 | 363 | 198 | |
| Burke *et al.* (2006) | | 190 | 160 | | 490 | |
| Harrington *et al.* (2006) | | | 232 | 281 | | |
| Lindsay *et al.* (2007) | | | 159 | | 433 | 63.30 |
| Fulkerson *et al.* (2008) | | | 243 | 232 | 489 | |
| Pacheco *et al.* (2010b) | | | 205 | 251 | 450 | |
| Jacobs and Woodward (2010) | | 263 | 119 | 328 | 559 | 66.10 |
| Pacheco *et al.* (2010a) | | | 224 | | 492 | |
| **Average of studies** | **36** | **203** | **187** | **276** | **460** | **70.8** |

In studies which had more than one measurement, measurements were averaged

**Table 2.3 Chemical composition and nutritive value data of plantain: (total nitrogen (Total N), dry matter (DM), crude protei detergent fibre (NDF), organic matter digestibility (OMD), in vitro dry matter digestibility (IVDMD) and metabolisable energy c**

| Study | Total N (g/kg DM) | DM (g/kg DM) | CP (g/kg DM) | ADF (g/kg DM) | NDF (g/kg DM) | OMD (%) |
|---|---|---|---|---|---|---|
| Suckling (1960) | | 207 | 164 | | | |
| Derrick *et al.* (1993) | | | | 340 | | 48.9 |
| Jackson *et al.* (1996) | 17.5 | | | 166 | 231 | 85.9 |
| Burke *et al.* (2000) | | 130 | 247 | | 283 | 85.0 |
| Burke *et al. (2006)* | | 130 | 250 | | 280 | |
| Harrington *et al.* (2006) | | | 283 | 256 | | |
| Al-Mamun *et al.* (2008) | | | 158 | | | |
| Fulkerson *et al.* (2008) | | | 276 | 282 | 439 | |
| Hayes *et al*. (2010) | | | 173 | 243 | 373 | 77.5[a] |
| Corkran (2009) | | | 136 | | 179 | 86.2 |
| Johnston (2010) | | | 156 | 300 | 478 | |
| **Average of studies** | **18** | **156** | **205** | **265** | **323** | **76.5** |

In studies which had more than one measurement, measurements were averaged

[a] value converted from DMD using the formula OMD = 1.017 DMD + 1.90

The organic matter digestibility (OMD) component of forage estimates the portion of organic matter eaten which is digested and absorbed (Hodgson and Brookes 2002). The average organic matter digestibility of plantain was slightly higher than that found in perennial ryegrass (Table 2.2;Table 2.3). Conversely, Derrick *et al.* (1993) reported that the digestibility of plantain is often 10 – 20% lower than that of perennial ryegrass. The stem portion of plantain has very low digestibility owing to a higher cell wall fraction (Wilman et al. 1997; Burke et al. 2000; Labreveux et al. 2004). Fraser and Rowarth (1996) reported differences between the digestibility of the leaf and stem components, with leaf having a much greater digestibility than stem (80.4 and 58.7% respectively). Similarly, Wilman and Riley (1993) found the NDF content to be 296 and 235 g/kg DM for plantain stem and leaf respectively.

Plantain contains higher levels of water soluble carbohydrates and starch than perennial ryegrass (Table 2.4;Table 2.5). Furthermore, it has a higher ratio of readily fermentable carbohydrates to structural carbohydrates than perennial ryegrass (Jackson *et al.* 1996). Thus, it can be digested more readily in the rumen than perennial ryegrass, potentially supporting greater levels of animal production.

**Table 2.4 Carbohydrate and lignin content (soluble carbohydrates, soluble sugars and starch, pectin, cellulose, hemicellulose and lignin) of perennial ryegrass across a number of studies.**

| Study | (g/kg DM) | | | | | |
|---|---|---|---|---|---|---|
| | Soluble carbohydrates | Soluble sugars and starch | Pectin | Cellulose | Hemicellulose | Lignin |
| Derrick *et al.* (1993) | | | | 261 | | |
| Hoskin *et al.* (1995) | 80 | | 12 | 229 | 216 | 22 |
| Scales (1995) | | | | | | 26 |
| Jackson *et al.* (1996) | 74 | | 10 | 184 | 212 | 10 |
| Burke *et al.* (2000) | 91 | | | | | |
| Burke *et al.* (2002) | | | | | | 35 |
| Moorby *et al.* (2004) | 117 | | | | | |
| Marley *et al.* (2005) | 166 | | | | | |
| Burke *et al. (2006)* | | 210 | | | | |
| Fulkerson *et al.* (2008) | 78 | | | | | |
| Pacheco *et al.* (2010b) | | 168 | | | | |
| Pacheco *et al.* (2010a) | 152 | | | | | |
| **Average of studies** | **108** | **189** | **11** | **225** | **214** | **23** |

In studies which had more than one measurement, measurements were averaged

**Table 2.5 Carbohydrate and lignin content (soluble carbohydrates, soluble sugars and starch, pectin, cellulose, hemicellulose and lignin) of chicory, plantain, red clover and white clover across a number of studies.**

| Species and study | (g/kg DM) | | | | | |
|---|---|---|---|---|---|---|
| | Soluble carbohydrates | Soluble sugars and starch | Pectin | Cellulose | Hemicellulose | Lignin |
| ***Chicory*** | | | | | | |
| Hoskin *et al.* (1995) | 134 | | 98 | 148 | 60 | 30 |
| Scales (1995) | | | | | | 43 |
| Jackson *et al.* (1996) | 111 | | 98 | 104 | 44 | 20 |
| Burke *et al.* (2000) | 118 | | | | | |
| Burke *et al. (2006)* | | 370 | | | | |
| Fulkerson *et al.* (2008) | 53 | | | | | |
| **Average of studies** | **104** | **370** | **98** | **126** | **52** | **31** |
| ***Plantain*** | | | | | | |
| Derrick *et al.* (1993) | | | | 249 | | |
| Jackson *et al.* (1996) | 170 | | 60 | 115 | 65 | 51 |
| Burke *et al.* (2000) | 140 | | | | | |
| Burke *et al. (2006)* | | 270 | | | | |
| Fulkerson *et al.* (2008) | 72 | | | | | |
| **Average of studies** | **127** | **270** | **60** | **182** | **65** | **51** |
| ***Red Clover*** | | | | | | |
| Freudenberger *et al.* (1994) | | | | 181 | 94 | 28 |
| Jackson *et al.* (1996) | 95 | | 36 | 115 | 54 | 12 |
| Burke *et al.* (2000) | 85 | | | | | |
| Moorby *et al.* (2004) | 43 | | | | | |
| Marley *et al.* (2005) | 74 | | | | | |
| Burke *et al. (2006)* | | 220 | | | | |
| **Average of studies** | **74** | **220** | **36** | **148** | **74** | **20** |
| ***White Clover*** | | | | | | |
| Burke *et al.* (2000) | 121 | | | | | |
| Burke *et al.* (2002) | | | | | | 58 |
| Marley *et al.* (2005) | 90 | | | | | |
| Burke *et al. (2006)* | | 310 | | | | |
| **Average of studies** | **106** | **310** | | | | **58** |

In studies which had more than one measurement, measurements were averaged

#### 2.2.5.3 Metabolisable energy

Metabolisable energy (ME) represents the energy reaching the tissues in the form of adsorbed nutrients, after accounting for energy losses in faeces, urine and methane gas (Hodgson and Brookes 2002). The ME content of a herbage should be at least 10-11 MJME/kg DM to enable high lamb live weight gain (Hodgson and Brookes 2002). The ME content of plantain has been shown to range between 9.5 - 11.7, with an average of approximately 10.9 MJME/kg DM (Table 2.3). This is similar to the average ME content of perennial ryegrass (Table 2.2) and slightly less than that of chicory (Table 2.6). However, this could result from differences between seasons. Golding *et al.* (2008) reported that pastures that contained plantain had an improved ME value of 10.2 MJME/kg DM during summer compared to a recently sown perennial ryegrass and white clover sward (9.0 MJME/kg DM).

**Table 2.6 Chemical composition and nutritive value data of chicory: (total nitrogen (Total N), dry matter (DM), crude protei detergent fibre (NDF), organic matter digestibility (OMD) and metabolisable energy content (ME)) across a number of studies.**

| Study | Total N (g/kg DM) | DM (g/kg DM) | CP (g/kg DM) | ADF (g/kg DM) | NDF (g/kg DM) | OMD (%) |
|---|---|---|---|---|---|---|
| Hoskin *et al*. (1995) | 28.9 | 110 | | 178 | 238 | |
| Scales (1995) | | | | | 371 | 64.90 |
| Jackson *et al.* (1996) | 19.7 | | | 124 | 158 | 89.70 |
| Kusmartono *et al*. (1996) | 36.2 | | | | | 84.80 |
| Barry *et al.* (1998) | | | | | | |
| Burke *et al.* (2000) | | 143 | 193 | | 238 | 83.90 |
| Burke *et al*. (2002) | | | | 212 | 238 | |
| Brown and Moot (2004) | | | 180 | | | |
| Burke *et al.* (2006) | | 140 | 190 | | 240 | |
| Harrington *et al*. (2006) | | | 307 | 261 | | |
| Fulkerson *et al*. (2008) | | | 272 | 235 | 314 | |
| Hayes *et al*. (2010) | | | 199 | 218 | 354 | 79.9[a] |
| Jacobs and Ward (2011) | | | 161 | | 352 | |
| Average of studies | 28 | 131 | 215 | 205 | 278 | 80.8 |

In studies which had more than one measurement, measurements were averaged

[a] value converted from DMD using the formula OMD = 1.017 DMD + 1.90

#### *2.2.5.4 Mineral content*

Plantain's taproot allows it to extract more nutrients, especially trace elements, from the soil to a greater extent and depth than perennial ryegrass (Moorhead *et al.* 2002; Kemp *et al.* 2010). Plantain contains a high content of minerals relative to perennial ryegrass (Table 2.7; Table 2.8; Table 2.9). The ash content of plantain has been reported to be higher than that found in perennial ryegrass, red clover and white clover but lower than that of chicory, with a range across studies of 107-160 g/kg DM (Table 2.7; Table 2.8; Table 2.9).

**Table 2.7 The macronutrient content (Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Sodium (Na), Sulfur (S)) and ash content of perennial ryegrass across a number of studies.**

| Study | (g/kg DM) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | N | P | K | Ca | Mg | Na | S | Ash Content |
| Wilman and Riley (1993) | 39.1 | 5.42 | 42.4 | 6.4 | 2.56 | 3.3 | | |
| Wilman and Derrick (1994) | 38.2 | 4.1 | 41.2 | 5.4 | 1.8 | 3.5 | | |
| Hoskin *et al.* (1995) | | | | | | | | 117 |
| Scales (1995) | 22.5 | 3.6 | 25.5 | 6.6 | | 0.8 | 2.8 | |
| Kusmartono *et al.* (1996) | | | | | | | | 104 |
| Barry (1998) | | | | | | | | 105 |
| Barry *et al.* (2001) | | 3.1 | 11.7 | 5.6 | 2.2 | 1.8 | 2.2 | |
| Marley *et al.* (2005) | | | | | | | | 91 |
| Burke *et al. (2006)* | | | | | | | | 110 |
| Harrington *et al.* (2006) | 37.7 | 3.7 | 38 | 4.2 | 1.7 | 1.8 | 3.5 | 117 |
| Pacheco *et al.* (2010a) | | | | | | | | 107 |
| Marley *et al.* (2013) | 23.2 | 2.6 | 9.9 | 7.6 | 2.3 | 4.2 | 1.6 | |
| **Average across studies** | **32** | **4** | **28** | **6** | **2** | **3** | **3** | **107** |

In studies which had more than one measurement, measurements were averaged

**Table 2.8 The macronutrient content (Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Sodium (Na), Sulfur (S)) and ash content of chicory and plantain across a number of studies.**

| Species and study | (g/kg DM) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | N | P | K | Ca | Mg | Na | S | Ash Content |
| ***Chicory*** | | | | | | | | |
| Crush and Evans (1990) | 30.2 | 3.3 | 68.7 | 13.2 | 2.8 | 3.5 | | |
| Hoskin *et al.* (1995) | | | | | | | | 188 |
| Scales (1995) | 21.1 | 3.4 | 36.4 | 14.9 | | 2.1 | 3.9 | |
| Kusmartono *et al.* (1996) | | | | | | | | 153 |
| Barry (1998) | | | | | | | | 149 |
| Barry *et al.* (2001) | | 2.6 | 15.8 | 11.7 | 3.2 | 5.4 | 2.8 | |
| Sanderson *et al.* (2003b) | | 4.7 | 36 | 18 | 4.8 | | | |
| Burke *et al. (2006)* | | | | | | | | 170 |
| Harrington *et al.* (2006) | 43.5 | 6.6 | 38 | 11.8 | 3.9 | 5.9 | 6.3 | 147 |
| Hayes *et al.* (2010) | | | | | | | | 151 |
| Jacobs and Ward (2011) | | 3.5 | 37.6 | 15 | 4.5 | 13 | 4.2 | |
| Marley *et al.* (2013) | 26.8 | 3.4 | 16.5 | 13.4 | 4.4 | 3.4 | 1.9 | |
| **Average across studies** | **30** | **4** | **36** | **14** | **4** | **6** | **4** | **160** |
| ***Plantain*** | | | | | | | | |
| Wilman and Riley (1993) | 36.5 | 4.54 | 43.8 | 20.1 | 2.39 | 7.2 | | |
| Wilman and Derrick (1994) | 42 | 4.02 | 35 | 18.6 | 2.45 | 3.15 | | |
| Sano *et al.* (2002) | | 3.4 | 43 | 20 | 1.8 | | | |
| Sanderson *et al.* (2003b) | | 3.9 | 25 | 19 | 3.5 | | | |
| Burke *et al. (2006)* | | | | | | | | 160 |
| Harrington *et al.* (2006) | 33.7 | 4.8 | 19.7 | 17.7 | 2.5 | 6.2 | 5.3 | 119 |
| Al-Mamun *et al.* (2008) | | | | | | | | 138 |
| Corkran (2009) | | | | | | | | 114 |
| Hayes *et al.* (2010) | | | | | | | | 107 |
| **Average across studies** | **37** | **4** | **33** | **19** | **3** | **6** | **5** | **128** |

In studies which had more than one measurement, measurements were averaged

**Table 2.9 The macronutrient content (Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Sodium (Na), Sulfur (S)) and ash content of red clover and white clover across a number of studies.**

| Species and study | (g/kg DM) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | N | P | K | Ca | Mg | Na | S | Ash Content |
| ***Red Clover*** | | | | | | | | |
| Barry (1998) | | | | | | | | 104 |
| Todorova (2001) | | | | | | | | 94 |
| Marley *et al.* (2005) | | | | | | | | 106 |
| Burke *et al. (2006)* | | | | | | | | 130 |
| Drobná (2006) | | 2.8 | 20.8 | 18.8 | 6.0 | | 1.8 | |
| Vasiljevic *et al.* (2011) | | 3.0 | 24.2 | 18.6 | | 0.8 | | 81 |
| Marley *et al.* (2013) | 38.8 | 2.7 | 11.4 | 18.4 | 4.2 | 2.3 | 1.6 | |
| **Average across studies** | **39** | **3** | **19** | **19** | **5** | **2** | **2** | **103** |
| ***White Clover*** | | | | | | | | |
| Anderson (1973) | 53.0 | 4.2 | 38.4 | 15.3 | 3.8 | | 2.0 | |
| Marley *et al.* (2005) | | | | | | | | 101 |
| Burke *et al. (2006)* | | | | | | | | 130 |
| Harrington *et al.* (2006) | 45.6 | 3.5 | 28.3 | 11.9 | 2.4 | 2.1 | 2.1 | 129 |
| Marley *et al.* (2013) | 44.5 | 3.5 | 12.9 | 16.1 | 2.8 | 3.3 | 1.9 | |
| **Average across studies** | **48** | **4** | **27** | **14** | **3** | **3** | **2** | **120** |

In studies which had more than one measurement, measurements were averaged

Plantain contains measurably higher calcium content than perennial ryegrass (Table 2.7; Table 2.8). Stewart (1996) suggested that a small proportion of plantain in the sward could increase the calcium retention by animals. Across a range of studies, plantain had a higher copper and selenium content than both perennial ryegrass and chicory (Table 2.10). Furthermore, studies have found lambs grazing plantain have greater intakes of trace and other elements due to minerals being present in the leaves (Rumball *et al.* 1997; Moorhead *et al.* 2002).

**Table 2.10 The micronutrient content (Manganese (Mn), Copper (Cu), Zinc (Zn), Boron (B), Selenium (Se), Cobalt (Co), Iron (Fe), Molybdenum (Mo) of perennial ryegrass, chicory, plantain and white clover across a number of studies.**

| Species and study | Micronutrients (mg/kg DM) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mn | Cu | Zn | B | Se | Co | Fe | Mo |
| ***Perennial ryegrass*** | | | | | | | | |
| Scales (1995) | | 6 | | | | 0.83 | | |
| Barry *et al.* (2001) | 183 | 7.6 | 45.2 | | 0.1 | 0.55 | | 0.23 |
| Morehead *et al.* (2002) | | 11.3 | | | 4.8 | | | |
| Harrington *et al.* (2006) | 99 | 7.9 | 22 | 19 | 0.023 | 0.193 | 151 | 0.64 |
| **Average across studies** | **141.0** | **8.2** | **33.6** | **19.0** | **1.6** | **0.5** | **151.0** | **0.4** |
| ***Chicory*** | | | | | | | | |
| Scales (1995) | | 11 | | | | 0.51 | | |
| Barry *et al.* (2001) | 201 | 10.5 | 55.8 | | 0.1 | 1.38 | | 0.18 |
| Sanderson *et al.* (2003b) | 170 | 32 | 45 | 33 | | | | |
| Harrington *et al.* (2006) | 161 | 18.6 | 57.7 | 38.3 | 0.043 | 0.273 | 167 | 0.42 |
| **Average across studies** | **177.3** | **18.0** | **52.8** | **35.4** | **0.1** | **0.7** | **167.0** | **0.3** |
| ***Plantain*** | | | | | | | | |
| Morehead *et al.* (2002) | | 35.4 | | | 8.5 | | | |
| Sanderson *et al.* (2003b) | 89 | 22 | 31 | 25 | | | | |
| Harrington *et al.* (2006) | 109 | 15.1 | 37.7 | 23.3 | 0.053 | 0.36 | 182 | 0.27 |
| **Average across studies** | **99.0** | **24.2** | **34.4** | **24.2** | **4.3** | **0.4** | **182.0** | **0.3** |
| ***Red Clover*** | | | | | | | | |
| Drobná (2006) | 47.0 | 10.6 | | | | | 483.0 | |
| ***White Clover*** | | | | | | | | |
| Harrington *et al.* (2006) | 55 | 8.6 | 22 | 28.7 | 0.073 | 0.173 | 109 | 0.223 |

### 2.2.6 Palatability

There is conflicting evidence surrounding the palatability of plantain. Numerous experiments have reported plantain to be less palatable than chicory, red clover, white clover and perennial ryegrass (Fraser and Rowarth 1996; Golding *et al.* 2008; Corkran 2009; Horadagoda *et al.* 2009). Other studies have reported plantain to be highly palatable in mixed pastures, being selectively grazed before most legumes and grasses, by both sheep (Milton 1933; Clark and Harris 1985; Stewart 1996) and dairy cows (Ivins 1952). An experiment which compared the grazing preferences of dairy cows offered 14 different forage species showed plantain was the least consumed (3.2 minutes out of 60 minutes) (Horadagoda *et al.* 2009). Conversely, Rumball *et al.* (1997) found plantain (*cv.* Lancelot) to be at least as palatable as white clover with pre- and post-grazing residuals of 1390 *vs.* 150 kg DM/ha and 1340 *vs.* 360 kg DM/ha for plantain and white clover, respectively.

Plantain palatability changes with plant maturity. Early research on plantain showed its palatability was greater when herbage was restricted to a sward height of 70-100 mm (Milton 1943). Reproductive stems and old plantain leaves are less palatable than fresh material (Stewart 1996). This was confirmed by Ayala *et al.* (2011b) as sheep tended to avoid the oldest plantain leaves. Similarly, Ivins (1952) reported dairy cows were less eager to graze plantain stem and flowering heads compared to leaf. Consequently, previous studies have reported that lambs experience palatability problems with plantain during late summer and early autumn (Fraser and Rowarth 1996; Swainson and Hoskin 2006). Corkran (2009) found lambs preferentially selected chicory ahead of plantain when grazing a mixed herb sward in autumn. Golding *et al.* (2008) observed mature plantain leaves became prominent in a plantain, ryegrass, white clover mix as lambs preferred ryegrass and white clover to plantain. Conversely, in an indoor study, Pain *et al.* (2010) found lambs displayed no preference between plantain and chicory during both spring and summer.

### 2.2.7 Grazing management and persistence

Plantain is moderately tolerant of treading and soil compaction (Stewart 1996) and hence is best suited to rotational grazing (Kemp *et al.* 2002b). Yu *et al.* (2008) found the shoot dry matter of plantain increased with longer defoliation frequencies (1, 2, 4 weeks). Sanderson *et al.* (2003a) recommended the use of a four to five week grazing interval to attain maximum yields of plantain. Labreveux *et al.* (2004) found grazing every three weeks versus five weeks reduced dry matter yield in summer by more than twofold (1.7 vs 4.5 t DM/ha). Whilst, Ayala *et al.* (2011b) found grazing every three weeks during spring and summer resulted in significantly greater yields than grazing every six weeks. Furthermore, Sanderson *et al.* (2003b) reported that more frequent harvests of three weeks versus five weeks tended to improve herbage nutritive value, resulting in *in vitro* true digestibility (IVTD), NDF and CP being one to two percentage units higher. Additionally, mineral concentrations were higher in herbage from a three week harvest interval compared to a five week harvest interval (Sanderson *et al.* 2003b).

Labreveux *et al.* (2004) recommended a resting period of no longer than three weeks so as to prevent the accumulation of reproductive stems. However, if plantain does become reproductive, mechanical topping can be used to control stem and seed head to keep plantain in a vegetative state (Milton 1943; Stewart 1996).

Once established, plantain should not be grazed during winter, as grazing then has been reported to result in decreased leaf to stem ratio in spring, decreased spring production and 32.2% fewer plants in the summer following winter grazing (Ayala *et al.* 2011b).

Powell *et al.* (2007) reported after a year of establishment and seven grazings, plantain showed better persistence than chicory with plant density rates of 67 and 40 plants/m$^2$, respectively. Similarly, Yu *et al.* (2008) reported the taproot diameter of plantain was unaffected by cutting height, whereas that of chicory was reduced under hard cutting, thus showing plantain is less sensitive to cutting height than chicory.

### 2.2.8 Animal performance

#### *2.2.8.1 Voluntary Feed intake*

In a diet preference study, where lambs were offered the same amount of each herbage lambs offered plantain had similar rates of feed intake compared to lambs offered perennial ryegrass, however this was significantly lower than intake rates observed on chicory, red clover and alfalfa (lucerne) (Pain *et al.* 2010).

#### *2.2.8.2 Live weight gain*

Lambs have shown variable live weight gain when grazing plantain (Table 2.11;Table 2.12). Most experiments (carried out in spring, summer and autumn) show plantain will support live weight gain in excess of 150g/day which is higher than lambs grazing perennial ryegrass (Table 2.11;Table 2.12). An experiment carried out in Uruguay during a dry summer (rainfall 25% under the long term average) reported lambs grazing plantain had live weight gain of between 158 – 226 g/day under stocking rates varying between 18.4 – 10.5 lambs per hectare (Barrios 2006 as cited in Ayala *et al.* 2010). Studies which have reported low lamb growth rates are a result of grazing plantain at an advanced stage of maturity when nutritive value is low (Robertson *et al.* 1995; Fraser and Rowarth 1996; Niezen *et al.* 1998). Thus, it is vital that plantain is maintained in a vegetative state in order to ensure high live weight gain.

Deer have shown superior live weight gains when grazing plantain during autumn but not during spring (Hoskin *et al.* 2005; Appendix 1- Table 1). Hoskin *et al.* (2005; 2006) concluded that for deer plantain offers little advantage over perennial ryegrass based pasture during spring; however it is advantageous during autumn.

**Table 2.11 Lamb live weight gain (g/day) during spring on various pasture species. Means within rows with different letters are**

| Country | Year of study or note | Perennial ryegrass | Chicory | Plantain | White Clover | Red Clover | Alfalfa (lucerne) | Study |
|---|---|---|---|---|---|---|---|---|
| New Zealand | 1 | 113a | | | 240b | | 227 | McLean *et* |
| | 2 | 141a | | | 308b | | 290 | |
| New Zealand | | 227a | | | 331b | | | Ulyatt (19 |
| New Zealand | | 227a | | | 321b | | 308b | Cruickshar |
| Australia | | | 243 | | | | 233 | Hopkins *e* |
| Wales | | 184a | | | | 305c | 243b | Fraser *et a* |
| Wales | | 201a | | | 282b | 292b | 210a | Marley *et* |
| New Zealand | Ø | 269 | 268 | | | | | Athanasia |
| | Ő | 219 | 262 | | | | | |
| New Zealand | 1 | 296a | | 376b | | | | Judson *et* |
| | 2 | 309a | | 346b | | | | |

Ø dam drenched with an anthelmintic at lambing; ő dam not drenched

**Table 2.12 Lamb live weight gain (g/day) during summer and autumn on various pasture species. Means within rows with dif** ***P*** **= 0.05.**

| Season and country | Year of study or note | Perennial ryegrass | Chicory | Plantain | White Clover | Red Clover | Alfalfa (lucerne) | Rape | Leaf turnips | Cocksf |
|---|---|---|---|---|---|---|---|---|---|---|
| ***Summer*** | | | | | | | | | | |
| New Zealand | 1 | 39 | 74 | | | | 86 | | | 53 |
| | 2 | 104 | 146 | | | | 146 | | | 115 |
| New Zealand | 1 | 169-195a † | 311b | | | | 222a | | | |
| | 2 | 120-148a † | 250b | | | | 250b | | | |
| New Zealand | 1 | 128a | 182b | 141a | 219b | | | | | |
| | 2 | 98a | 181b | 84a | 225b | | | | | |
| | 3 | 136a | 214b | 102a | 233b | | | | | |
| New Zealand | | 135a | | 222b | | | | | | |
| Wales | | 182b | | | | 228a | 200ab | | | |
| New Zealand | | 160a | | | 311c | | | | 222b | |
| New Zealand | € | 140bcd | | 130de | | 110bd | | 310a | 60bf | |
| | £ | 160cd | | 90bdf | | 150d | | 320a | 200c | |
| ***Autumn*** | | | | | | | | | | |
| New Zealand | | 170a | | | 213b | | 159 | | | |
| New Zealand | | | 236b | | | | | 165a | | |
| New Zealand | | 166b | | 51a | | | 243c | | | |
| Wales | | 219 | | | | 263¥; 215β | | | | |

€ ewe lambs; £ ram lambs

† liveweight gain range for average of cocksfoot, perennial ryegrass and tall fescue pasture

¥ liveweight gain on a cultivar containing high formononetin content; β liveweight gain on a cultivar containing low formononetin cont

## 2.3 Chicory (*Cichorium intybus*)

This aspect of the review will focus on three chicory cultivars; ‘Puna’, ‘Puna II’ and ‘Choice’.

### 2.3.1 Soil requirements and fertiliser

Chicory is a perennial pasture herb with a deep taproot and large, shiny hairless leaves growing from a basal rosette (Kemp *et al.* 2002b; Stewart and Charlton 2006). Chicory performs well on most soil types; however it is more persistent on light or free draining soils with medium to good fertility (Kemp *et al.* 2002b). Standard nutrient requirements of chicory are: Olsen phosphorus (P) 20-30 mg/kg, potassium (K) >8 mg/kg and sulphur (S) > 10 mg/kg (Moloney and Milne 1993).

Chicory is tolerant of a wide range of soil pH but grows best within the pH range 5.5 - 6.0 (Crush and Evans 1990). In an evaluation of perennial pasture legumes and herbs across a range of Australian sites, chicory persisted well under both neutral and acid soil conditions (Li *et al.* 2008).

Chicory yield increase per unit of nitrogen applied is comparable to that of temperate grasses (Collins and McCoy 1997). Alemseged (2000) reported chicory dry matter yields increased with increasing application rates of nitrogen between 0 and 120 kgN/ha. Furthermore, Collins and McCoy (1997) observed applications of nitrogen are quickly taken up by chicory, which suggests that the timing of nitrogen fertilization could have a large effect on the seasonal distribution of DM production.

### 2.3.2 Sowing time and establishment

Chicory can be autumn or spring sown. However, chicory requires warm temperatures (>10°C) while seedlings are establishing, otherwise seedlings can be outcompeted by weed species (Barry *et al.* 1998). Exposure to minimum air temperatures of -5°C to -10°C (corresponding soil temperatures 0 to -5°C) will result in seedling death (Barry *et al.* 1998). Consequently, seedling development of chicory is slower in autumn than in spring (Sanderson and Elwinger 2000; Powell *et al.* 2007). Sanderson and Elwinger (2000) suggested that seedlings require more than two fully expanded leaves for winter survival. Powell *et al.* (2007) determined that chicory should not be grazed until it has seven fully developed leaves and is approximately 250 mm high, in order to minimise plant loses.

Spring-summer sowings are recommended in less temperate environments, as autumn sowings only have time to develop a small taproot before winter and are more prone to suffering from weed competition, frost or drought than larger plants (Hare *et al.* 1990). A further advantage of spring sowing is that spring sown crops do not usually produce seed until the second summer, 15-18 months after sowing as chicory must first undergo vernalisation (Hare *et al.* 1990). Conversely, early-autumn sowings may be more reliable in frost-free coastal districts where early summer droughts can be expected (Moloney and Milne 1993).

### 2.3.3 Herbage production and pattern of growth

Herbage production of approximately 7-9 t DM/ha is common for pure chicory swards for the first two or three years under grazing conditions (Table 2.13). When allowed to go reproductive, yields of up to 25 t DM/ha annually have been observed (Lancashire 1978; Hare *et al.* 1987). Compared to perennial ryegrass and white clover pasture, chicory has been shown to be as productive (Powell *et al.* 2007) or more productive over a one year period (Hogh-Jensen *et al.* 2006). Powell *et al.* (2007) found chicory produced 13.7 t DM/ha in its first year of establishment. Under favourable conditions chicory swards can grow at rates in excess of 150 kgDM/ha/day (Lancashire 1978; Matthews *et al.* 1990) and with good grazing management chicory can produce 10 t DM/ha of leaf mass (Kemp *et al.* 2002b).

Chicory has a distinct seasonal growth pattern in response to temperature, being virtually dormant in winter and highly active in summer (Hare *et al.* 1987; Hume *et al.* 1995). In the southern hemisphere chicory is productive from September to May (Lancashire 1978). Brown *et al.* (2005) established chicory swards can produce approximately 70 kg DM/ha/day during November, with an increase to peak in January at approximately 90 kg DM/ha/day and then reduce to approximately 50 kg DM/ha/day during February, with negligible growth over winter. While, MacFarlane *et al.* (1990)

concluded chicory has the ability to generate spring growth rates of up to 300 kg DM/ha/day, with summer growth rates exceeding 50 kg DM/ha/day. Jung *et al.* (1996) observed little herbage growth occurred in chicory when the mean temperature was less than 14˚C. Regardless of temperature, chicory maintains a faster growth rate during spring than autumn, as remobilisation of root stored assimilate supplements photosynthetic production during spring (Brown *et al.* 2000).

### 2.3.4 Drought tolerance, water use efficiency and freezing tolerance

Chicory's deep taproot enables the plant to remain highly productive during dry summer conditions (Li and Kemp 2005; Stewart and Charlton 2006) and tolerates moisture stress to a greater extent than plantain (Nie *et al.* 2008). Thus, high yields have been reported under dryland conditions (Hunter *et al.* 1994; Brown *et al.* 2005). Chicory utilises water more efficiently than other pastures, whereby under warm temperatures chicory can grow at 38 kg herbage mass/ha/day per mm of rainfall (Dowling *et al.* 2006). Consequently, during a dry summer chicory can grow more rapidly (Fraser *et al.* 1999) and maintain higher green-leafiness than both grasses (Kemp *et al.* 2002a; Nie *et al.* 2008) and legumes (Kemp *et al.* 2002a).

An experiment carried out across a range of Australian sites found chicory was highly productive for two to three years on three of five sites, however, under severe drought (270-290 mm rain per annum) chicory swards died (Li *et al.* 2010). Likewise,

**Table 2.13 Annual herbage yield (t DM/ha) for pure swards of chicory across a number studies.**

| Cultivar | Region and Country | Year | | | | | Average[a] |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | |
| Puna[b] | Manawatu, NZ | 8.1 | | | | | 8.1 |
| Puna[c] | Manawatu, NZ | 7.9 | | | | | 7.9 |
| Puna | Canterbury, NZ | 11.2 | 6.0 | 7.0 | | | 8.1 |
| Puna | Manawatu, NZ | 8.8 | | | | | 8.8 |
| Puna | Pennsylvania, USA | 9.4 | 7.9 | | | | 8.7 |
| Puna | Oklahoma, USA | 7.5 | 7.7 | | | | 7.6 |
| Puna[d] | Palmerston North, NZ | 8.5 | 9.4 | - | 4.6 | | 7.5 |
| Puna | West Virginia, USA | 7.8 | 6.3 | 7.6 | | | 7.2 |
| Puna | Prince Edward Island, Canada | 7.1 | 5.8 | 6.4 | | | 6.4 |
| Puna | Canterbury, NZ | 19.8 | 16.4 | | | | 18.1 |
| Puna[e] | NSW, Australia | 2.1 | 4.3 | 6.5 | 9.4 | | 5.6 |
| Puna | Canterbury, NZ | 16.0 | 13.0 | 14.0 | 12.0 | 7.0 | 12.4 |
| Choice | Manawatu, NZ | 13.7 | | | | | 13.7 |
| Grouse | NSW, Victoria, SA, WA, Australia | | | | | | 9.1 |
| Puna II | Ceredigion, Wales | 7.4 | 7.8 | | | | 7.6 |
| **Average of studies** | | | | | | | **9.1** |

[a]Average if reported or calculated from years given

[b]Yield under 30cm row spacing; [c]Yield under 60cm row spacing

[d]Yield from November to April.

[e]Green-leaf dry matter yield, nitrogen fertiliser applied at 100-150 kg/ha year in years 3 and 4.

Matthews *et al.* (1990) found under severe moisture stress and hard grazing chicory displayed negative growth (-12 kg DM/ha/day) due to plant senescence.

Winter survival of chicory under severe freezing conditions (-14°C) is quite high (73-93%) and greater than that of plantain (Skinner and Gustine 2002). In Atlantic Canada, Puna persisted for three production years under the cold winters (Kunelius and McRae 1999). Established stands of chicory will tolerate minimum air temperatures of -5°C to -10°C (corresponding soil temperatures 0 to -5°C) for up to 3 weeks in New Zealand with no adverse effect on persistence (Barry *et al.* 1998). Skinner and Gustine (2002) and Kunelius and McRae (1999) concluded that Puna chicory should survive winters in the north-eastern USA and Canada. Therefore, New Zealand winter conditions should not affect chicory plant persistence.

### 2.3.5 Nutritive value

#### *2.3.5.1 Crude Protein*

Across a number of studies, the average CP concentration of chicory was 214 g/kg DM which was higher than the CP concentration of perennial ryegrass (187 g/kg DM) and similar to that of plantain (213 g/kg DM) (Table 2.2;Table 2.3;Table 2.6).

#### *2.3.5.2 Digestibility*

Chicory has been shown to have both a lower NDF and ADF concentration than perennial ryegrass, across a range of studies as well as a higher OMD (Table 2.2;Table 2.6). Hayes *et al.* (2010) reported chicory herbage had the lowest NDF and ADF (35.4% and 21.8%, respectively) concentrations of a range of perennial pastures.

Across a range of studies chicory has a similar water soluble carbohydrate content to perennial ryegrass (Table 2.4;Table 2.5) Chicory has been found to have a higher ratio of readily fermentable carbohydrates to structural carbohydrates than perennial ryegrass (Hoskin *et al.* 1995; Jackson *et al.* 1996) and perennial ryegrass and white clover pastures (Kusmartono *et al.*) as well as plantain and red clover (Jackson *et al.* 1996).

Nutritive value differs between vegetative and reproductive growth in chicory. Chicory leaf has substantially higher *in vitro* digestibility and nitrogen concentration and lower organic matter content than stem (Li *et al.* 1997b). Clark *et al.* (1990a) reported that the flowers and live leaves of chicory had higher dry matter digestibility (DMD) values (81 and 77%, respectively) compared to the main stem (46.2%). Similarly, Kemp *et al.* (2002b) reported digestibility of 50-64% and 85% for chicory stem and leaf, respectively.

#### *2.3.5.3 Metabolisable energy (ME)*

The ME content of chicory ranges between 9.7 - 13.7, with an average of approximately 11.5 MJME/kg DM (Table 2.6), which is higher than the recommended minimum ME content for finishing lambs (Hodgson and Brookes 2002). The ME content of chicory is significantly greater than that of perennial ryegrass (Table 2.2;Table 2.6), with the difference being most pronounced during the summer months.

#### *2.3.5.4 Mineral content*

Chicory contains higher concentrations of minerals relative to both grasses and legumes (Belesky *et al.* 2001) (Table 2.7; Table 2.8; Table 2.9; Table 2.10). Across a range of studies the average ash content of chicory (160 g/kg DM) has been shown to be higher than that of perennial ryegrass (107 g/kg DM), plantain (128 g/kg DM), red clover (109 g/kg DM) and white clover (129 g/kg DM) (Table 2.7; Table 2.8; Table 2.9).

Van Eekeren *et al.* (2006) found chicory had higher concentrations of sulphur, copper and zinc than both perennial ryegrass and white clover and concluded that it has the potential to be used on farms with copper or zinc deficiencies to combat the problem. In support of this, Barry *et al.* (2001) found deer grazing chicory had higher liver copper concentrations than those grazing perennial ryegrass. Several studies have found chicory to be low in nitrogen and silicon compared with perennial ryegrass and white clover (Rumball 1986; Crush and Evans 1990).

### 2.3.6 Palatability

Chicory is considered to be highly palatable due to its high nutritive value (Barry 1998), high digestibility and low fibre content thus making it highly desirable for growing and/or lactating ruminants (Turner *et al.* 1999). In grazing preference studies, Hunt and Hay (1990) showed that red and fallow deer selected legumes and chicory in preference to perennial grasses. In an indoor study, Pain *et al.* (2010) observed lambs preferentially ate red clover before chicory and plantain, which did not differ during, both spring and summer. Corkran (2009) observed lambs grazing a mixed sward of plantain, chicory and red clover during early autumn preferentially selected red clover before chicory which was preferred before plantain. In Denmark, Nielson *et al.* (2009) found lambs showed negative selection indices throughout the period (July-October) to chicory (*cv.* Puna) when it was included in a ryegrass and white clover sward mix. Similarly, in an experiment which compared the grazing preferences of dairy cows offered 14 different forage species showed chicory was least preferred despite its greater quantity and quality of herbage on offer (Horadagoda *et al.* 2009). This was possibly due to the concentration of bitter sesquiterpene lactones (Foster *et al.* 2006). Sesquiterpene lactones and phenolics found in chicory help to protect the plant from insect feeding but also help deter herbivory (Rees and Harborne 1985).

In chicory palatability changes with plant maturity. During spring, primary leaves make up the main source of feed for livestock, whereas, from December onwards, secondary and axillary leaves grow (Li *et al.* 1998) as chicory progresses into the reproductive state and palatability decreases (Li *et al.* 1997b). Clark *et al.* (1990b) found calves and bulls preferentially grazed chicory leaf before stem. Similarly, Taube *et al.* (2004) found cows preferred young chicory leaves to chicory stems. This was likely due to its low DMD and nitrogen content compared to chicory leaf (Clark *et al.* 1990b).

### 2.3.7 Grazing management and persistence

Chicory has a typical persistence of three to five years (Hume *et al.* 1995; Li *et al.* 1997b; Belesky *et al.* 2000). Chicory persistence is largely dependent on the plant density obtained at establishment as seedling recruitment is unlikely in a mature chicory stand (Volesky 1996; Li *et al.* 1997a). The limited persistence of chicory results from a decline in plant density (i.e. plants/square meter) over time. Chicory loses approximately 30-35% of its plant population per year (Hume *et al.* 1995; Li *et al.* 1995; Li and Kemp 2005) with dry matter production significantly decreasing by the third (Li *et al.* 1997a; Belesky *et al.* 2000) or fourth year (Hume *et al.* 1995). Alemseged (2000) concluded that optimum yields of chicory can be obtained at a density of 50 – 60 plants/$m^2$. Li *et al.* (1997a) observed chicory stands containing more than 50 plants/$m^2$ with two to four shoots per plant yielded approximately twofold more than stands containing 25 plants/$m^2$ with six to seven shoots per plant. Once plant density declines to about 20-30 plants/$m^2$ chicory has low growth rates and allows weed invasion resulting in an unproductive crop (Li *et al.* 1994a). Therefore it is important that grazing management practices minimise plant death.

Once established, chicory should not be grazed during late autumn (Li *et al.* 1997b) and throughout winter (Kemp 1996). Grazing at high stocking rates during heavy rain can result in exposed taproots being damaged by overgrazing and treading, reducing the sward persistence (Rumball 1986; Li and Kemp 2005) by causing the death of twenty percent or more of the plants (Barry *et al.* 1998). High application rates of nitrogen fertiliser can reduce chicory plant density (Clark *et al.* 1990a; Collins and McCoy 1997). Li *et al.* (1998) concluded that grazing management should aim at preserving crown bud development, which occurs during early spring and late autumn in order to maintain productivity and persistence.

Chicory responds poorly to continuous grazing (Alemseged *et al.* 2003) and is best managed using a rotational grazing system (Lancashire and Brock 1983). Grazing management of chicory should aim to keep chicory in the vegetative state (Kusmartono *et al.* 1994), thereby maximising leaf production and minimising stem production (Hume *et al.* 1995; Li *et al.* 1997a). Clark *et al.* (1990a) suggested the desirable proportion of leaf and stem should be 70% leaf and 30% stem. The leaf to stem ratio has been shown to be affected by both defoliation frequency (Clark *et al.* 1990a; Matthews *et al.* 1990; Li *et al.* 1997a; Li *et al.* 1997b) and defoliation height (Matthews *et al.* 1990; Li *et al.* 1994b; Jung *et al.* 1996).

It is well established that chicory produces a greater dry matter yield under a five or six week defoliation frequency than under more frequent defoliation regimes (Belesky *et al.*

1999; Alemseged *et al.* 2003; Sanderson *et al.* 2003a). Kemp *et al.* (2002a) reported chicory persisted for four years without any apparent decline in plant populations and with increasing productivity under a six week harvest and grazing-management regime. Li *et al.* (1997b) found a four week grazing frequency resulted in the highest yield but also a higher stem content compared to a one week frequency, however, the leaf: stem ratio still remained within the target range. Likewise, Jung *et al.* (1996) suggested that prolonged frequent harvesting may adversely affect forage mass and early spring growth in the subsequent year. Defoliation frequency also affects the nutritive value of chicory (Jung *et al.* 1996). In central Pennsylvania, pure chicory swards averaged 230 g CP/kg DM under intensive defoliation and 140 g CP/kg DM when managed under an infrequent defoliation regime (Jung *et al.* 1996).

Post-grazing height also has an effect on chicory dry matter production, persistence and forage quality. Jung *et al.* (1996) found chicory leaf mass was 33% less under lenient than intensive management in the first year, but that this was not observed in the second year. Arias-Carbajal (1994) found hard grazing resulted in leaf yields 53% higher than lax grazing and reduced dead matter production. Li *et al.* (1994b) reported leaf growth rate was greater early in the season under hard grazing, and late in the season under lax grazing, with plant density unaffected by post-grazing height. Overall, medium grazing (100-150 mm stem stubble) represented a reasonable compromise between total herbage production and nutritive value (Li *et al.* 1994b). This result was confirmed in a glasshouse experiment where a cutting height of >100 mm produced more herbage mass than a cutting height <100 mm and a cutting height of <150 mm resulted in significantly lighter root mass (Li *et al.* 1997c). Moreover, Li *et al.* (1995) concluded that severe grazing of chicory in spring (i.e. 50 mm or less stem stubble) is likely to exacerbate the decline in plant density at this time.

Barry *et al.* (1998) advised starting grazing when plants are 300 mm high and grazing down to 100 mm in order to maximize leaf mass without excessively decreasing the persistency of the plants. Contrastingly, other research suggests that when rotationally grazed, chicory can be grazed to ground level at each grazing as new shoots regenerate from the basal crown (Rumball 1986). Li *et al.* (1997c) concluded that the persistence of chicory is more sensitive to cutting frequency than cutting height. In support of this, Alemseged (2000) postulated that the height of stubble as a source of growing points is not important as regrowth is supported entirely through taproot reserves.

Chicory shows a strong tendency to progress into the reproductive state during summer. Reproductive stems can develop from late October (Hare and Rolston 1987; Hare *et al.* 1987; Arias-Carbajal 1994). Jung *et al.* (1996) found stem production increased as grazing frequency reduced. A grazing frequency of no greater than five weeks was recommended by Matthews *et al.* (1990) to prevent reproductive stem becoming mature. In a four year experiment, Li *et al.* (1997a) found a two to three week grazing interval over spring and summer of years one and four, resulted in a better control of primary reproductive stems and a higher leaf: stem ratio than grazing at 5 week intervals during year two.

Arias-Carbajal (1994) advised that chicory should be grazed during summer with a leader/follower grazing system, with young stock grazing green leaf and the follower flock being used to eat the reproductive stems. Kusmartono *et al.* (1994) observed a four to five week rotation allowed stem formation and seed head development to occur in a deer production system, and required a follower mob to maintain the chicory in the vegetative state. Alternatively, chicory can be mechanically topped to keep it in the vegetative phase. However, if it is left to enter the reproductive state, flowering chicory can provide large amounts of herbage for cattle, that is capable of giving live weight gains equivalent to ryegrass and white clover summer pasture, but at a higher stocking rate (Clark *et al.* 1990b).

Overall, Hare *et al.* (1987) and Clark *et al.* (1990a) suggested that rotationally grazing chicory to 30-50 mm above ground level at four to five week intervals appeared to be the best management to achieve the desirable proportion of leaf and stem. However, Li *et al.* (1997c) concluded that provided defoliation is above 150 mm, and at a defoliation frequency at least three weeks, the persistency of chicory will not be detrimentally affected.

### 2.3.8 Animal performance

#### *2.3.8.1 Voluntary feed intake*

Pain *et al.* (2010) reported lambs fed chicory had feed intake rates comparable to lambs fed red clover and alfalfa (lucerne) but were significantly greater than feed intake rates of lambs offered plantain and perennial ryegrass. Similarly, research in deer production has shown higher rates of voluntary feed intake (VFI) when deer are grazing chicory in comparison to perennial ryegrass and white clover pasture (Semiadi *et al.* 1993; Soetrisno *et al.* 1994; Kusmartono *et al.* 1995; Kusmartono *et al.* 1996; Min *et al.* 1997). Kusmartono *et al.* (1996) found the VFI of deer grazing chicory was 55%, 25% and 15% greater than that of deer grazing perennial ryegrass and white clover pasture during summer, autumn and spring respectively. Conversely, Hoskin *et al.* (1999) found no difference in the VFI of deer grazing chicory or perennial ryegrass and white clover pasture during both spring and autumn.

#### *2.3.8.2 Live weight gain*

Lambs grazing chicory exhibit greater live weight gains compared to lambs grazing perennial ryegrass and white clover pasture (Table 2.11;Table 2.12). Most experiments show chicory will support lamb live weight gain in excess of 200g/day which is similar to lambs grazed on alfalfa (lucerne) and white clover and higher than lambs grazed on perennial grasses (Table 2.11;Table 2.12). This is especially evident during summer when perennial grass based pastures lose nutritive value. Holst *et al.* (1998) concluded chicory produced sufficient quantity of high quality forage to finish lambs over summer. Chicory has also been shown to produce superior lamb live weight gain compared to rape, with lambs growing 70 g/day faster on allowances of 3 kg/head/day and at a comparable live weight gain rate to rape when chicory was offered at a lower herbage allowance of 1.5 kg/head/day (Fraser *et al.* 1988). Similarly, deer have been shown to display superior live weight gains when grazing chicory in comparison to perennial ryegrass and white clover pasture (Appendix 1- Table 1). Furthermore, Kusmartono *et al.* (1996) reported this live weight gain response was greater in autumn than in spring. Friesian bull calves grazing Puna chicory at allowances of 50 and 100 g DM/kg live weight achieved live weight gains of 0.39 and 0.88 kg/head/day during February and 0.67 and 0.93 kg/head/day during May (Clark *et al.* 1990b).

## 2.4 Herb and Legume Sward Mixes

### 2.4.1 Development of the mix

In a pure sward, chicory has a consistently high overall nutritive value and high summer growth rates (Table 2.6) (Brown *et al.* 2005). However, it has a short growing season and poor persistence (Hume *et al.* 1995; Li *et al.* 1997b; Belesky *et al.* 2000). Contrastingly, plantain has a variable nutritional composition and is generally comparable to perennial ryegrass with similar levels of gross energy, crude fibre and amino acids (Hodgkinson *et al.* 2009). However, it yields are relatively high and it has a reasonably long growing season (Powell *et al.* 2007).

Both red clover and white clover contain high levels of CP, are readily digestible and highly metabolisable (Table 2.14;Table 2.5). Pure swards of white and red clover can produce average yields of 9.9 t DM/ha per year and 12 t DM/ha per year, respectively (Table 2.15). Red and white clover are both highly palatable forages (Frame *et al.* 1998a; Frame *et al.* 1998b) and in pure swards have been shown to support lamb live weight gains of 250-300 g/day (Table 2.11;Table 2.12).

The seasonal growth patterns of white clover, red clover, chicory and plantain vary but they are all dormant or semi-dormant in winter (Allen *et al.* 1976; Coop 1986; Hare *et al.* 1987; Hume *et al.* 1995; Stewart 1996; Kelly *et al.* 2005; Labreveux *et al.* 2006; Moorhead and Piggot 2009). The combination of chicory, plantain, red clover and white clover provides a pasture with a longer growing season than any one of the species by itself. Plantain begins its growth earlier in spring and continues later into autumn than the other species (Yu *et al.* 2008; Kemp *et al.* 2010). While, chicory and red clover remain more productive as the soil dries out in summer than plantain and white clover (Kemp *et al.* 2002b; Li and Kemp 2005; Stewart and Charlton 2006).

**Table 2.14 Chemical composition and nutritive value data of red clover and white clover: (total nitrogen (Total N), dry matte fibre (ADF), neutral detergent fibre (NDF), organic matter digestibility (OMD), in vitro dry matter digestibility (IVDMD) and me number of studies.**

| Study | Total N (g/kg DM) | DM (g/kg DM) | CP (g/kg DM) | ADF (g/kg DM) | NDF (g/kg DM) | OMD (%) | IVI ( |
|---|---|---|---|---|---|---|---|
| **Red Clover** | | | | | | | |
| Suckling (1960) | | 218 | 215 | | | | |
| Freudenberger *et al.* (1994) | 43.9 | 124 | | | 303 | | |
| Jackson *et al*. (1996) | 46.9 | | | 127 | 181 | 85.50 | |
| Barry *et al*. (1998) | | | | | | | |
| Burke *et al*. (2000) | | 148 | 274 | | 336 | 85.00 | |
| Todorova (2001) | | | 261 | | | | |
| Brown and Moot (2004) | | | 250 | | | | |
| Moorby *et al*. (2004) | | 138 | 243 | 276 | 356 | | |
| Marley *et al*. (2005) | | 150 | 218 | 263 | 198 | | |
| Burke *et al*. (2006) | | 150 | 270 | | 340 | | |
| Vasiljevic *et al.* (2011) | | | 180 | 300 | 383 | | |
| **Average of studies** | **45** | **155** | **239** | **242** | **300** | **85** | |
| **White Clover** | | | | | | | |
| Suckling (1960) | | 231 | 230 | | | | |
| Harrington et al. (2006) | | | 270 | 228 | | | |
| Burke et al. (2000) | | 150 | 269 | | 256 | 82.10 | |
| Burke et al. (2006) | | 150 | 270 | | 260 | | |
| Burke et al. (2002) | | | | 190 | 256 | | |
| Marley et al. (2005) | | 134 | 249 | 223 | 185 | | |
| Lindsay (2006) | | | 228 | | 292 | 71 | |
| Jacobs and Woodward (2010) | | 184 | 209 | 252 | 384 | 75.4 | |
| **Average of studies** | | **170** | **246** | **223** | **272** | **76.2** | |

In studies which had more than one measurement, measurements were averaged

**Table 2.15 Annual herbage yield (t DM/ha) for pure swards of red clover and white clover across a number of studies within New Zealand.**

| Cultivar | Region | Year | | | | | Average[a] | Study |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | | |
| ***Red Clover*** | | | | | | | | |
| Broad | Wanganui | | | | | | 20.2 | Suckling (1960) |
| Montgomery | Wanganui | | | | | | 26.4 | Suckling (1960) |
| G4706[b] | Manawatu | 13.3 | | | | | 13.3 | Anderson (1973) |
| Turoa nucleus[b] | Manawatu | 10.4 | | | | | 10.4 | Anderson (1973) |
| Turoa breeders[b] | Manawatu | 11.2 | | | | | 11.2 | Anderson (1973) |
| Hamua[b] | Manawatu | 12.3 | | | | | 12.3 | Anderson (1973) |
| S123[b] | Manawatu | 11.1 | | | | | 11.1 | Anderson (1973) |
| Local variety[b] | Manawatu | 11.5 | | | | | 11.5 | Anderson (1973) |
| Hamua | Canterbury | 11.6 | 8.0 | | | | 9.8 | Vartha and Clifford (1978) |
| Turoa | Canterbury | 13.2 | 9.0 | | | | 11.1 | Vartha and Clifford (1978) |
| Pawera | Canterbury | 14.2 | 11.4 | | | | 12.8 | Vartha and Clifford (1978) |
| Hamua[c] | Southland | | | | | | 10.2 | Hay and Ryan (1989) |
| G22[c] | Southland | | | | | | 7.9 | Hay and Ryan (1989) |
| Turoa[c] | Southland | | | | | | 7.6 | Hay and Ryan (1989) |
| Pawera[c] | Southland | | | | | | 6.7 | Hay and Ryan (1989) |
| Colenso | Canterbury | 9.0 | 8.8 | 5.0 | | | 7.6 | Hunter *et al.* (1994) |
| Astred[d] | Manawatu | 14.1 | | | | | 14.1 | Hyslop (1999) |
| Astred[d] | Manawatu | 14.2 | | | | | 14.2 | Hyslop (1999) |
| Pawera | Canterbury | 21.6 | 15.3 | | | | 18.45 | Brown *et al.* (2000) |
| Pawera | Canterbury | 16.5 | 15.0 | 3.1 | 2.0 | 0.0 | 7.32 | Brown *et al.* (2005) |
| **Average of studies** | | | | | | | **12.2** | |
| ***White Clover*** | | | | | | | | |
| Wild cultivar | Wanganui | | | | | | 10.5 | Suckling (1960) |
| Huia | Canterbury | 9.5 | 7.0 | | | | 8.3 | Vartha and Clifford (1978) |
| Pitau | Canterbury | 9.9 | 7.1 | | | | 8.5 | Vartha and Clifford (1978) |
| Huia[a] | Manawatu | 12.5 | | | | | 12.5 | Anderson (1973) |
| **Average of studies** | | | | | | | **9.9** | |

[a]Average if reported or calculated from years given

[b]Average of high and low cutting heights

[c]Average of 4, 6 and 9 week grazing frequencies

[d]Average of 4, 6 and 8 week, hard and lax grazing regimes

The optimum grazing management of red and white clover is compatible with that of both chicory and plantain. Red clover responds poorly to both continuous grazing and hard grazing, and is much more persistent under lax rotational grazing (Brock *et al.* 2003; Stewart and Charlton 2006). White clover is able to thrive under both rotational and continuous stocking (Frame *et al.* 1998b) and is tolerant of grazing and treading damage (Lambert *et al.* 2004).

Sinhadipathige *et al.* (2012) concluded that whilst the lower CP content of plantain and chicory did not limit lamb growth in their study, it was important to include legumes in herb sward mixes, as the combined CP content of the four species should ensure this is not the case. Overall the herb and legume sward offers the potential for improved summer growth and greater nutritive value than traditional perennial ryegrass and white clover pasture.

Neither, chicory nor plantain is particularly compatible with grass species due to their very different grazing requirements. Therefore, the development of a perennial forage sward with herbs contributing the majority of DM yield and legumes added to fix nitrogen and maintain a high nutritive value could have great potential.

### 2.4.2 Advantages of a Herb and Legume Sward Mix

Increasing plant species diversity in a sward mix has been found to be positively associated with herbage yield (Ruz-Jerez *et al.* 1991; Daly *et al.* 1996; Todorova 2001; Sanderson *et al.* 2004; Taube *et al.* 2004; Sanderson *et al.* 2005) and negatively associated with weed invasion (Sanderson *et al.* 2004; Taube *et al.* 2004; Sanderson *et al.* 2005). Belesky (1999; 2000) recommended growing chicory in a mixed species sward in order to reduce weed invasion as chicory stands decline. Ruz-Jerez *et al.* (1991) reported a herbal sward mix containing; numerous grasses, white and red clover, plantain and chicory yielded 25-30% more than a perennial ryegrass and white clover pasture over a two year period, with late-spring and summer production being higher. Sward mixtures based on herbs and legumes have superior herbage quality (Daly *et al.* 1996; Goh and Bruce 2005) and greater tolerance of dry summers (Goh and Bruce 2005) compared to perennial ryegrass and white clover pasture. They are advantageous over short term pastures as they have a longer growing season, and maintain pasture production and nutritive value for longer at the beginning and end of the growing season (Nie *et al.* 2008). Moreover, unlike annual or short-term forage species, a perennial sward mix does not require regular cultivation and thus should have lower financial and environmental costs associated with it (Kemp *et al.* 2002b).

The few studies that have examined Herb and Legume Mixed Swards, have shown it generally has a lower fibre content, similar CP content and higher OMD % and ME content than perennial ryegrass and white clover pasture (Table 2.16).

**Table 2.16 Average chemical composition (crude protein (CP), neutral detergent fibre (NDF), organic matter digestibility (OMD), and metabolisable energy content (ME)) of a Herb and Legume Sward Mix compared to perennial ryegrass and white clover pasture across a number of studies. Means within studies and columns with different letters are significantly different at *P* = 0.05.**

| Season, herbage type and study | CP (g/kg DM) | NDF (g/kg DM) | OMD (%) | ME (MJ/kg DM) |
|---|---|---|---|---|
| ***Spring*** | | | | |
| Kenyon *et al. (2010)* | | | | |
| Ryegrass and white clover | 131 | 544 | | 9.2 |
| plantain/chicory/red clover/white clover | 125 | 378 | | 10.5 |
| Hutton *et al. (2011)* | | | | |
| Ryegrass and white clover | 123 | 362 | 72.0 | 10.6 |
| plantain/chicory/red clover/white clover | 152 | 276 | 74.0 | 10.8 |
| Sinhadipathige *et al*. (2012) | | | | |
| Ryegrass and white clover | 228 | 396b | | |
| plantain/chicory/red clover/white clover | 165 | 263a | | |
| plantain/red clover/white clover | 191 | 263a | | |
| ***Summer*** | | | | |
| Kenyon *et al. (2010)* | | | | |
| Ryegrass and white clover | 90.0 | 618 | 64.4 | 8.9 |
| plantain/chicory/red clover/white clover | 89 | 488 | 67.9 | 9.8 |
| Hutton *et al. (2011)* | | | | |
| Ryegrass and white clover | 92 | 502 | | 9.5 |
| plantain/chicory/red clover/white clover | 122 | 384 | | 9.9 |
| Golding *et al*. (2008) | | | | |
| plantain/chicory/red clover/white clover | 158ab | 281a | 82.9c | 11.4b |
| New pasture | 196b | 481b | 64.1b | 9.0b |
| Old pasture | 141a | 537c | 61.2a | 8.8a |

### 2.4.3 Grazing management

Grazing management is the key to ensuring companion species in a mixed sward survive (Briske 1996). The ability of plants to recover from grazing is not only determined by morphological and physiological characteristics, but also by competitive pressures from companion species (Briske 1996). Chicory grows vigorously during spring (MacFarlane 1990). Therefore, grazing early in spring will help companion species to survive strong competition for light by chicory (Arias-Carbajal 1994).

The grazing management of the Herb and Legume Mix will be limited by the requirement of the species most vulnerable to the effects of grazing height and frequency, which differs between seasons. Thus, the choice of grazing frequency and height should generally reflect the optimum grazing management of chicory and red clover, with no grazing during late autumn or winter (Kemp 1996; Li *et al.* 1997b), a grazing frequency of between three and five weeks (Clark *et al.* 1990a; Barry *et al.* 1999) and a post-grazing height of no less than 50 mm (Li *et al.* 1995).

### 2.4.4 Animal performance

#### *2.4.4.1 Live weight gain and animal performance*

Burke *et al.* (2002) concluded that mixed forage diets may be beneficial for animal performance. To date, few studies have examined lamb live weight gain on a Herb and Legume Mix but these have shown it is capable of supporting lamb live weight gains of approximately 250 g/day, which is significantly greater than lamb live weight gain achieved on a perennial ryegrass and white clover sward (Table 2.17).

**Table 2.17 Live weight gain (g/day) of lambs grazing a Herb and Legume Mix; containing chicory, plantain, red and white clover in comparison with perennial ryegrass and white clover pasture during spring and summer. Means within rows with different superscripts are significantly different at *P* = 0.05.**

| Season and study | Perennial ryegrass and white clover | Herb and Legume Mix |
|---|---|---|
| *Spring* | | |
| Sinhadipathige *et al*. (2012) | 322a | 360b |
| *Summer* | | |
| Golding *et al*. (2008) | 119a | 247b |
| Parker *et al*. (2008) | 93a | 192b |
| Wilson (2009) | 59a | 246b |

Several studies have shown that offering a mixed sward containing chicory, plantain, red clover and white clover in late pregnancy and during lactation increased ewe live weight gain, body condition score, ewe milk production, lamb live weight gain and improved lamb survival compared to ewes grazing perennial ryegrass and white clover (Kenyon *et al.* 2010; Hutton *et al.* 2011). Similarly, cows grazing a chicory and white clover mix had higher intakes and milk yields during summer than cows grazing perennial ryegrass and white clover or tall fescue and white clover mixes (Chapman *et al.* 2008).

#### *2.4.4.2 Grazing behaviour*

Grazing behaviour differs when animals are offered differing sward mixes. Sheep preference for plantain is lower than for chicory, red clover and white clover (Corkran 2009), but if used in mixtures animal productivity is maximized (Golding *et al.* 2008). Taube *et al.* (2004) found that cows tend to graze layer by layer resulting in more selection behaviour among the top layers than the bottom layers. Therefore, the grazing preference observed in a herb and legume sward may differ with different pre-grazing sward heights depending on the vertical availability of each species.

## 2.5 Conclusions

Chicory and plantain are both high yielding, summer active herbs which maintain high nutritive values during warm summer conditions. When sown in a combined mix with red and white clover it is expected that dry matter yield and nutritive value will remain high, whilst, the growing season is lengthened. Anecdotal evidence suggests chicory and plantain yield more under dry conditions than pasture based swards. The below-mentioned limitations in the literature on herb and legume sward mixes provide the focus for the experimental chapters in this thesis.

- A comparison of the responses and recovery of plantain and chicory to conditions of moisture stress (Chapter 3).
- An understanding of the physiological and morphological responses of plantain and chicory to moisture stress and defoliation (Chapter 4).
- The potential yield and persistence of the Herb and Legume Mix under various post-grazing heights (Chapter 5).
- Lamb diet selection and grazing preference on the Herb and Legume Mix (Chapter 6).
- Comparison of the Herb and Legume Mix to perennial ryegrass and white clover pasture and pure swards of chicory and plantain under various mowing frequencies and defoliation heights (Chapter 7).

# Chapter 3 Herbage production and biomass allocation of plantain (*Plantago lanceolata*) and chicory (*Cichorium intybus*) in response to defoliation following a period of moisture stress in glasshouse conditions

## 3.1 Abstract

The productivity of New Zealand grazing systems is becoming increasingly dependent on the growth response and survival of forage species under moisture stress as the prevalence of drought conditions increase as a result of climate change. The herbage production and biomass allocation of chicory (*Cichorium intybus*) and plantain (*Plantago lanceolata*) were studied in response to defoliation following a period of moisture stress. A glasshouse experiment was conducted between 29 August and 8 December 2011 at the Plant Growth Unit, Massey University, Palmerston North, New Zealand. Plantain had a greater ($P < 0.05$) relative shoot growth rate than chicory under Optimum and Dry water treatments, but did not differ ($P > 0.05$) from chicory under Very-Dry water treatment. Plantain had a greater ($P < 0.05$) shoot mass fraction than chicory under both optimum and water stress treatments. The root masses of both chicory and plantain were lower ($P < 0.05$) under defoliation compared to No-Defoliation. Furthermore, Late-Defoliation in chicory resulted in a lesser ($P < 0.05$) root mass than Early-Defoliation. The taproot diameter of chicory was lower ($P < 0.05$) under defoliation, while that of plantain was not affected. These findings suggest that plantain is more robust in response to moisture stress and defoliation than chicory. Further work is required to confirm these conclusions and explain the potential mechanisms behind these findings that allow plantain to have a production advantage over chicory when grown in the glasshouse under moisture stress.

## 3.2 Introduction

Plants are often subjected to periods of soil and atmospheric water deficits with drought posing a significant environmental constraint to plant survival and crop productivity (Chaves *et al.* 2003). This will likely become increasingly important as the world is challenged by predicted warmer climates (IPCC 2007). Understanding how plants respond to moisture stress can play a major role in stabilising crop performance under drought conditions.

Forage chicory (*Cichorium intybus*) and plantain (*Plantago lanceolata*) are widely used throughout the world as high feed quality perennial herbages (Sanderson *et al.* 2003a; Labreveux *et al.* 2006; Li *et al.* 2010; Golding *et al.* 2011; Hutton *et al.* 2011). Both plantain and chicory are tap-rooted. The health and carbohydrate storage of tap-roots influence the persistence of plants (Lodge 1991) and also confers a degree of drought tolerance (Nie *et al.* 2008) through accessing water deeper in the soil profile. Consequently, both chicory and plantain are relatively tolerant to moisture stress (Lambert 1963; Mook *et al.* 1989; Nie *et al.* 2008). Nie *et al.* (2008) reported that chicory can tolerate moisture stress to a greater degree than plantain. However, little is known about the effect of moisture stress on plantain and chicory production and persistence under differing defoliation regimes. The objective of this preliminary study was to examine the effects of moisture stress, followed by defoliation on the herbage production, biomass allocation and persistence of Puna Π chicory and Tonic plantain under glasshouse conditions.

## 3.3 Materials and methods

### 3.3.1 Plant establishment

The experiment was conducted at the Plant Growth Unit, Massey University, Palmerston North, New Zealand (latitude 40° 23' S), between 29 August and 8 December 2011. Two plant species, plantain (*Plantago lanceolata*) cv. 'Ceres Tonic' and chicory (*Cichorium intybus*) cv. 'Puna II', were utilised.

Seeds were sown directly into 104 pots (52 chicory and 52 plantain) on 13 September 2011 with pots being hand watered daily with a sprinkler. The pots were 250 mm in diameter and 250 mm in depth and were filled with approximately 10 kg of potting medium. The potting medium consisted of 20% Manawatu silt loam (B Horizon) and 80% sand (3 mm) amended with 125g short term mix (Woodace, flowering plant special; LebanonTurf; 14% N, 6% P, 11.6% K + trace elements) and 75g dolomite for 50L of soil as a base start fertiliser. Plants were initially thinned on 23 September 2011 to approximately 6 plants per pot before being thinned to four plants per pot once well-established on 17 October 2011. Several plants, across both species developed a light infestation of aphids. Affected plants were sprayed with the insecticide Imidacloprid (Confidor; 0.125 g/litre) on 4 November 2011.

An automatic watering system, incorporating an individual dripper in each pot was established on 17 October. All pots were watered six times daily at a rate of 100 mL/pot for 3 minutes. Liquid nutrients (1g/litre) were applied once to all pots (before the dry-down period commenced) until soil was fully saturated and water was running off. The liquid fertiliser used was Peters professional 'Allrounder' (Everris; water soluble NPK fertiliser; 20%, 8.7%, 16.6% plus trace elements (B, Cu, Fe, Mn, Mo, Zn)). Air temperatures were maintained by heating or ventilation at 10°C - 15°C at night and 25°C - 30°C during the day.

### 3.3.2 Experimental design

A 2 × 3 × 3 factorial combination in a randomised complete block design (RCB) with four blocks was used (26 pots per block). The three main factors tested were plant species (Plantain and Chicory) and water treatment (Optimum (control) and Dry and Very-Dry), and defoliation treatment (No-Defoliation (control) and Early-Defoliation and Late-Defoliation). Each treatment combination was replicated once within each block for each harvest date (n = 4 for each treatment combination).

The water treatment began on 22 October 2011 (Day 1). Optimum water treatment provided a set volume of water per pot which was based on the level of sunlight and plant growth, and maintained soil volumetric moisture content above 12%. Under the Dry water treatment, plants received no water until the following conditions occurred: plants were wilting, leaf extension was minimal and soil moisture content reached approximately 8% (Day 7). Under the Very-Dry water treatment, plants received no water until they showed severe wilting, leaf extension stopped and soil moisture content reached approximately 5% (Day 10). These water stress treatments were developed based on previous evidence which suggests a severe water stress normally involves the complete loss of turgor, and thus leaf wilting occurs (Fitter and Hay 2001).

At defoliation plants were cut to 50 mm above potting media level. No-Defoliation treatments were not defoliated. Early-Defoliation was carried out 10 days after the end of the water treatment period, i.e. Day 17 for Dry treatment pots and on Day 20 for Very-Dry treatment pots. Late-Defoliation was carried on Day 31 for both Dry and Very-Dry treatment pots.

One complete treatment set of plants per block was destructively harvested at three dates. These were; before the water treatments were applied (Harvest 1 - Day 1; n = 2 × 4 = 8), at the end of the water treatment period (Harvest 2 - Day 10; n = 6 × 4 = 24) and at the end of the experimental period (Harvest 3 - Day 47; n = 18 × 4 = 72).

### 3.3.3 Measurements

During the water and the defoliation treatment periods the lengths of the two youngest leaves on all No-defoliation plants were measured at 1-3 day intervals (Days; 1, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 17, 18, 19, 20, 21, 22, 24, 25, 26, 27, 28, 31, 32, 33, 35, 36, 38, 40, 41, 42, 45, 46). The mean leaf extension rate (mm/leaf/day) of each species by water treatment combination was calculated by subtracting the sum of the previous measurement of leaf length from the current day's leaf length. The volume of water that each pot contained was monitored daily during the water and the defoliation treatment periods using the time-domain-reflectometry technique (TDR; CS-615, Campbell Sci., Leicestershire, UK) with moisture probes to a 200 mm depth. Probes were inserted into the middle of the pot.

The number of live leaves per plant was recorded at destructive harvests 1 and 3 (Day 1 and Day 47). At destructive harvests taproot diameter at the widest point and root length were recorded. Above ground herbage mass was dissected into live and dead material. There was very little dead material and it was therefore not analysed. The root mass was measured after manually washing off soil media. All mass data represent the mass per pot (i.e. mass from four plants) and are presented on a dry weight basis, oven dried at 60°C for 48 hours.

The shoot mass fraction was calculated as;

$$Shoot\ mass\ fraction = \frac{Total\ shoot\ mass\ per\ pot}{Total\ shoot\ mass + total\ root\ mass\ per\ pot} \times 100$$

The relative shoot and root growth rates between each harvest were calculated as;

$$Relative\ Growth\ Rate\ (RGR) = \frac{lnW_2 - lnW_1}{time}$$

Where:

$W_1$ = weight at time 1

$W_2$ = weight at time 2

Time = number of days between harvest events

### 3.3.4 Statistical analysis

All data were analysed using SAS (SAS Version 9.2, SAS Institute Inc., Gary NC, USA). Data were analysed separately for the three destructive harvest periods (Day 1, Day 10 and Day 47) and for each parameter (absolute shoot mass, relative shoot growth, taproot diameter, root length, absolute root mass, relative root growth, shoot mass fraction, number of live leaves per plant and leaf mass). At Harvest 1, the mixed model included the fixed effects of plant species (chicory and plantain) and the random effect of block. At harvest 2, the mixed model included the fixed effects of plant species (chicory and plantain) and water treatment (Optimum and Dry and Very-Dry) and the two-way interaction between plant species and water treatment and the random effect of block. At Harvest 3, the mixed model included the fixed effects of plant species (chicory and plantain), water treatment (Optimum and Dry and Very-Dry) and defoliation treatment (No-Defoliation and Early-Defoliation and Late-Defoliation) and all two-way and three-way interactions between plant species, water treatment and defoliation treatment and the random effect of block. Interactions were only presented in tables if they were significant ($P < 0.05$).

The mean leaf extension rates of the plants, on an individual plant basis, were calculated using the classic growth analysis method, interval approach, (Hunt 1978). A GLM model including the fixed effect of treatment (combination of plant species and water treatment) was used to compare the treatments on each individual day. An ANCOVA regression model including the fixed effect of treatment (combination of plant species and water treatment) and the covariate of day was used to compare the treatments over the entire experimental period.

## 3.4 Results

### 3.4.1 Day 1 – Harvest 1

Chicory had a greater ($P < 0.05$) absolute shoot mass, taproot diameter and absolute root mass than plantain (Table 3.1). Root length, number of live leaves per plant and the shoot mass fraction did not differ ($P > 0.05$) between plantain and chicory. Plantain had a greater ($P < 0.05$) average leaf mass than chicory.

**Table 3.1 Effect of plant species (Plantain and Chicory) at Day 1 on absolute shoot mass, taproot diameter, root length, absolute root mass, shoot mass fraction, number of live leaves per plant and leaf mass. Data presented as means ± s.e. Means within columns having different letters are significantly different at *P* = 0.05.**

| | Absolute shoot mass (g) | Taproot diameter (mm) | Root length (mm) | Absolute root mass (g) | Shoot/ (shoot + root) (%) | Number of live leaves per plant | Leaf mass (g/leaf) |
|---|---|---|---|---|---|---|---|
| Plantain | 1.4a | 2.4a | 110.5 | 0.2a | 86.4 | 9.3 | 0.06b |
| Chicory | 2.5b | 4.5b | 116.3 | 0.5b | 82.9 | 9.8 | 0.04a |
| s.e. | 0.21 | 0.28 | 5.81 | 0.06 | 1.18 | 0.52 | 0.004 |

### 3.4.2 Day 10 – Harvest 2

There were no ($P$ > 0.05) interactions between species and water treatment for any herbage measure. Absolute shoot mass did not differ ($P$ > 0.05) between species, although relative shoot growth (Day 1 – Day 10) was greater ($P$ < 0.05) for plantain than chicory (Table 3.2). The shoot mass fraction was greater ($P$ < 0.05) for plantain than chicory. Chicory had a greater ($P$ < 0.05) taproot diameter and absolute root mass than plantain. Relative root growth (Day 1 – Day 10) and root length did not differ ($P$ > 0.05) between species.

Optimum water treatment plants had greater ($P$ < 0.05) absolute shoot mass and taproot diameter than Dry and Very-Dry plants, which did not differ ($P$ > 0.05) (Table 3.2). Very-Dry plants had shorter ($P$ < 0.05) roots than Dry and Optimum plants, which did not differ ($P$ > 0.05). Water treatment had no effect ($P$ > 0.05) on relative shoot growth, shoot mass fraction, absolute root mass and relative root growth.

**Table 3.2 Effect of plant species (Plantain and Chicory) and water treatment (Optimum and Dry and Very-Dry) at Day 10 on a rate (Day 1- Day 10), taproot diameter, root length, absolute root mass, relative root growth rate (Day 1- Day 10) and means ± s.e. Means within columns having different letters are significantly different at *P* = 0.05.**

| | Absolute shoot mass (g) | Relative shoot growth (g/g/day) | Taproot diameter (mm) | Root length (mm) | Absolute root mass (g) | Relative root growth (g/g/day) | S (shoo |
|---|---|---|---|---|---|---|---|
| ***Species*** | | | | | | | |
| Plantain | 4.3 | 0.11b | 2.5a | 131.50 | 0.7a | 0.12 | |
| Chicory | 5.0 | 0.06a | 5.3b | 141.40 | 1.7b | 0.11 | |
| s.e. | 0.62 | 0.01 | 0.38 | 10.33 | 0.25 | 0.02 | |
| ***Water*** | | | | | | | |
| Optimum | 5.7b | 0.11 | 4.5b | 222.9b | 1.3 | 0.12 | |
| Dry | 4.1a | 0.08 | 3.7a | 229.8b | 1.1 | 0.11 | |
| VD | 4.0a | 0.07 | 3.5a | 196.2a | 1.2 | 0.11 | |
| s.e. | 0.67 | 0.02 | 0.40 | 5.15 | 0.28 | 0.02 | |

### 3.4.3 Day 47 – Harvest 3

#### *3.4.3.1 Above-ground herbage measures*

All plants survived the entire experimental period. There were no ($P > 0.05$) three-way interactions between species, water treatment and defoliation treatment or two-way interactions between water treatment and defoliation treatment for any above-ground herbage measure (absolute shoot mass, relative shoot growth, number of live leaves per plant, leaf mass) or for the shoot mass fraction.

There were two-way ($P < 0.05$) interactions between species and water treatment and between species and defoliation treatment for absolute shoot mass. Optimum water treatment Plantain and Dry Plantain plants had an absolute shoot mass which did not differ ($P > 0.05$) and which was greater ($P < 0.05$) than Very-Dry Plantain plants and all Chicory water treatments, which did not differ ($P > 0.05$) (Table 3.3).

There was no ($P > 0.05$) two-way interaction between species and defoliation treatment for relative shoot growth (Day 10 - Day 47). However, there was a two-way ($P < 0.05$) interaction between species and water treatment for relative shoot growth (Day 10 – Day 47). Relative shoot growth (Day 10 – Day 47) of Very-Dry plants did not differ ($P > 0.05$) between Plantain and Chicory (Table 3.3). Under Dry and Optimum water treatments, Plantain had a greater ($P < 0.05$) relative shoot growth (Day 10 – Day 47) than Chicory. Very-Dry Plantain had a lower ($P < 0.05$) relative shoot growth (Day 10 –

Day 47) than Dry and Optimum Plantain, which did not differ ($P > 0.05$). Chicory's relative shoot growth (Day 10 – Day 47) was lower ($P < 0.05$) under the Optimum water treatment than under the Dry and Very-Dry water treatments, which did not differ ($P > 0.05$).

There was no ($P > 0.05$) two-way interaction between species and defoliation treatment for shoot mass fraction. However, there was a two-way ($P < 0.05$) interaction between species and water treatment for shoot mass fraction. Across all water treatments Plantain had a greater ($P < 0.05$) shoot mass fraction than Chicory (Table 3.3). Within Chicory, Very-Dry plants had a greater ($P < 0.05$) shoot mass fraction than Optimum plants, with Dry plants not differing ($P > 0.05$) from either water treatment. Within Plantain, Optimum plants had a greater ($P < 0.05$) shoot mass fraction than Dry plants, with Very-Dry plants not differing ($P > 0.05$) from either water treatment. Defoliation treatment had no ($P > 0.05$) effect on shoot mass fraction.

**Table 3.3 Effect of plant species (Plantain and Chicory), water treatment (Optimum and Dry and Very-Dry) and defoliation treatment (No-Defoliation and Early-Defoliation and Late-Defoliation) at Day 47 on absolute shoot mass, relative shoot growth rate (Day 10 – Day 47), shoot mass fraction, number of live leaves per plant and leaf mass. Data presented as means ± s.e. Means within columns and treatments having different letters are significantly different at *P* = 0.05.**

| | Absolute shoot mass (g) | Relative shoot growth (g/g/day) | Shoot/ (shoot + root) (%) | Number of live leaves per plant | Leaf mass (g/leaf) |
|---|---|---|---|---|---|
| ***Species*** | | | | | |
| Plantain | 41.8b | 0.05b | 80.1b | 53.5b | 0.19a |
| Chicory | 29.1a | 0.04a | 57.7a | 22.5a | 0.33b |
| s.e. | 1.32 | 0.001 | 2.01 | 1.34 | 0.01 |
| ***Water*** | | | | | |
| Optimum | 39.6b | 0.039a | 68.0a | 41.2b | 0.27 |
| Dry | 35.3ab | 0.043b | 67.5a | 37.7ab | 0.26 |
| Very-Dry | 31.5a | 0.042ab | 71.2b | 35.1a | 0.26 |
| s.e. | 1.62 | 0.001 | 2.11 | 1.65 | 0.01 |
| ***Defoliation*** | | | | | |
| No-Defoliation | 51.1c | 0.05c | 69.80 | 41.4b | 0.36a |
| Early-Defoliation | 33.8b | 0.04b | 69.80 | 33.8a | 0.27b |
| Late-Defoliation | 21.4a | 0.03a | 67.10 | 38.8b | 0.15c |
| s.e. | 1.62 | 0.001 | 2.11 | 1.64 | 0.01 |
| ***Species*water*** | | | | | |
| Plantain-Optimum | 48.8b | 0.048d | 81.8d | | |
| Plantain-Dry | 42.7b | 0.050d | 77.1c | | |
| Plantain-Very-Dry | 33.8a | 0.043c | 81.5cd | | |
| Chicory-Optimum | 30.5a | 0.030a | 54.2a | | |
| Chicory-Dry | 27.8a | 0.037b | 58.0ab | | |
| Chicory-Very-Dry | 29.2a | 0.041bc | 60.9b | | |
| s.e. | 2.29 | 0.001 | 2.38 | | |
| ***Species*defoliation*** | | | | | |
| Plantain-No-Defoliation | 60.8d | | | 60.4c | 0.25c |
| Plantain-Early-Defoliation | 38.6c | | | 45.2b | 0.22bc |
| Plantain-Late-Defoliation | 25.9b | | | 55.0c | 0.12a |
| Chicory-No-Defoliation | 41.3c | | | 22.5a | 0.47e |
| Chicory-Early-Defoliation | 29.1b | | | 22.4a | 0.32d |
| Chicory-Late-Defoliation | 17.0a | | | 22.7a | 0.19b |
| s.e. | 2.29 | | | 2.32 | 0.02 |

There was no ($P > 0.05$) interaction between species and water treatment for the number of live leaves per plant. However, there was a two-way ($P < 0.05$) interaction between species and defoliation treatment for the number of live leaves per plant. Within all defoliation treatments Plantain had a greater ($P < 0.05$) number of live leaves per plant than Chicory (Table 3.3). Within Plantain, Very-Dry plants had a lower ($P < 0.05$) number of live leaves per plant than Optimum plants, with Dry plants not differing ($P > 0.05$) from either water treatment. Within Chicory, the number of live leaves per plant did ($P < 0.05$) between the defoliation treatments. The number of live leaves per plant was greater ($P < 0.05$) under the Optimum water treatment than the Very-Dry water treatment, while the Dry water treatment did not differ ($P > 0.05$) from either water treatment.

There was no ($P > 0.05$) interaction between species and water treatment for leaf mass. However, there was a two-way ($P < 0.05$) interaction between species and defoliation treatment for leaf mass. Within each defoliation treatment Chicory had a greater ($P < 0.05$) leaf mass than Plantain. Water treatment had no ($P > 0.05$) effect on leaf mass (Table 3.3).

#### *3.4.3.2 Below-ground herbage measures*

There were no ($P > 0.05$) three-way interactions between species, water treatment and defoliation treatment or two-way interactions between species and water treatment or between water treatment and defoliation treatment for any below-ground herbage measure (taproot diameter, root length, absolute root mass, relative root growth).

There was a two-way ($P < 0.05$) interaction between species and defoliation treatment for taproot diameter. Within all defoliation treatments, Chicory had a greater ($P < 0.05$) taproot diameter and absolute root mass than Plantain (Table 3.4). Within Plantain, defoliation treatment had no ($P > 0.05$) effect on taproot diameter. Whereas, within chicory, No-defoliation plants had a greater ($P < 0.05$) taproot diameter than Early or Late-defoliation plants. Water treatment had no ($P > 0.05$) effect on taproot diameter.

There was a two-way ($P < 0.05$) interaction between species and defoliation treatment for root length. Within all defoliation treatments Plantain had a greater ($P < 0.05$) root length than Chicory (Table 3.4). Chicory root length did not differ ($P > 0.05$) between the defoliation treatments. While, Plantain root length was shorter ($P < 0.05$) under Late defoliation than under No or Early-defoliation, which did not differ ($P > 0.05$). Very-Dry plants had a shorter ($P < 0.05$) root length than Optimum and Dry plants, which did not differ ($P > 0.05$).

**Table 3.4 Effect of plant species (Plantain and Chicory), water treatment (Optimum and Dry and Very-Dry) and defoliation treatment (No-Defoliation and Early-Defoliation and Late-Defoliation) at Day 47 on taproot diameter, root length, absolute root mass and relative root growth rate (Day 10 - Day 47). Data presented as means ± s.e. Means within columns and treatments having different letters are significantly different at *P* = 0.05.**

| | Taproot diameter (mm) | Root length (mm) | Absolute root mass (g) | Relative root growth (g/g/day) |
|---|---|---|---|---|
| ***Species*** | | | | |
| Plantain | 4.5a | 272.8b | 10.7a | 0.053 |
| Chicory | 15.1b | 159.8a | 21.7b | 0.052 |
| s.e. | 0.27 | 4.22 | 1.11 | 0.002 |
| ***Water*** | | | | |
| Optimum | 9.9 | 222.9b | 18.0b | 0.053b |
| Dry | 9.5 | 229.8b | 16.7ab | 0.056b |
| Very-Dry | 9.8 | 196.2a | 13.9a | 0.048a |
| s.e. | 0.33 | 5.15 | 1.36 | 0.002 |
| ***Defoliation*** | | | | |
| No-Defoliation | 10.8b | 222.1b | 22.8c | 0.061c |
| Early-Defoliation | 9.6a | 228.0b | 15.1b | 0.052b |
| Late-Defoliation | 9.0a | 198.8a | 10.6a | 0.045a |
| s.e. | 0.33 | 5.16 | 1.36 | 0.002 |
| ***Species*defoliation*** | | | | |
| Plantain-No-Defoliation | 4.4a | 281.1c | 14.2b | |
| Plantain-Early-Defoliation | 4.7a | 292.3c | 9.9ab | |
| Plantain-Late-Defoliation | 4.4a | 244.9b | 7.8a | |
| Chicory-No-Defoliation | 17.1c | 163.1a | 31.4d | |
| Chicory-Early-Defoliation | 14.4b | 163.7a | 20.3c | |
| Chicory-Late-Defoliation | 13.7b | 152.6a | 13.4b | |
| s.e. | 0.47 | 7.36 | 1.93 | |

There was a two-way ($P < 0.05$) interaction between species and defoliation treatment for absolute root mass. Within each defoliation treatment, Chicory had a greater ($P < 0.05$) absolute root mass than Plantain (Table 3.4). Within Chicory, Late-Defoliation plants had a lesser ($P < 0.05$) absolute root mass than Early-Defoliation plants, which was in turn lesser ($P < 0.05$) than No-Defoliation plants (Table 3.4). Within Plantain, Late-Defoliation plants had a lesser ($P < 0.05$) absolute root mass than No-defoliation plants, with Early-Defoliation not differing ($P > 0.05$) from either defoliation treatment. Very-Dry plants had a lesser ($P < 0.05$) absolute root mass than Optimum water treatment plants, with Dry plants not differing ($P > 0.05$) from either water treatment.

There was no ($P > 0.05$) interaction between species and defoliation for relative root growth (Day 10 – Day 47). Relative root growth (Day 10 – Day 47) did not differ ($P > 0.05$) between Chicory and Plantain (Table 3.4). Very-Dry plants had a lower ($P < 0.05$) relative root growth (Day 10 – Day 47) than Dry and Optimum plants, which did not differ ($P > 0.05$). Late-Defoliation plants had a lower ($P < 0.05$) relative root growth (Day 10 – Day 47) than Early-Defoliation plants, which was in turn lower ($P < 0.05$) than No-Defoliation plants.

### 3.4.4 Leaf growth analysis

Differences ($P < 0.05$) in the rates of accumulated leaf elongation between treatments were observed on Days 5-25 (Table 3.5). Between Days 5-6, Dry Chicory plants had a greater ($P < 0.05$) rate of accumulated leaf elongation than Optimum Plantain plants. Between Days 9-24, Very-Dry Chicory had a greater ($P < 0.05$) rate of accumulated leaf elongation than Very-Dry Plantain. After Day 25, no differences ($P > 0.05$) in the rates of accumulated leaf elongation were observed between species by water treatment groups.

Regression analysis shows under Optimum water treatment the rates of accumulated leaf elongation of plantain and chicory do not differ ($P > 0.05$) (Figure 3.1). In plantain, Very-Dry plants had a lower ($P < 0.05$) rate of accumulated leaf elongation than Dry and Optimum plants, which did not differ ($P > 0.05$). In chicory, Very-Dry plants had a greater ($P < 0.05$) rate of accumulated leaf elongation than Optimum plants, which in turn was greater ($P < 0.05$) than Dry plants.

**Table 3.5 Effect of treatment (combination of plant species (Plantain and Chicory) and water treatment (Optimum and elongation (mm) for each day, with the water treatment period taking place between Day 1 and Day 10. Means within ro different at *P* = 0.05.**

| | Optimum | | Dry | | |
|---|---|---|---|---|---|
| Day | Chicory | Plantain | Chicory | Plantain | Ch |
| 1 | 115 ± 7 | 125 ± 5 | 132 ± 8 | 131 ± 8 | 134 |
| 3 | 191 ± 11 | 192 ± 7 | 211 ± 11 | 213 ± 12 | 21 |
| 5 | 360ab ± 18 | 339a ± 10 | 396b ± 15 | 377ab ± 15 | 380a |
| 6 | 450ab ± 19 | 418a ± 11 | 483b ± 16 | 457ab ± 18 | 479a |
| 9 | 745abc ± 32 | 663a ± 16 | 767bc ± 24 | 707abc ± 26 | 768 |
| 12 | 1034ab ± 53 | 938ab ± 24 | 1031ab ± 37 | 962ab ± 45 | 1091 |
| 16 | 1337ab ± 67 | 1216ab ± 27 | 1324ab ± 48 | 1221ab ± 59 | 1407 |
| 20 | 1752ab ± 81 | 1587a ± 36 | 1686ab ± 60 | 1571a ± 74 | 1837 |
| 24 | 2000ab ± 91 | 1890ab ± 52 | 1919ab ± 68 | 1843ab ± 80 | 2106 |
| 25 | 2083ab ± 95 | 1986ab ± 55 | 2002ab ± 72 | 1943ab ± 84 | 2192 |
| 30 | 2329 ± 105 | 2268 ± 63 | 2270 ± 81 | 2223 ± 89 | 245 |
| 35 | 2715 ± 114 | 2691 ± 76 | 2648 ± 85 | 2646 ± 103 | 283 |
| 40 | 3016 ± 118 | 3045 ± 85 | 2992 ± 94 | 2998 ± 113 | 315 |

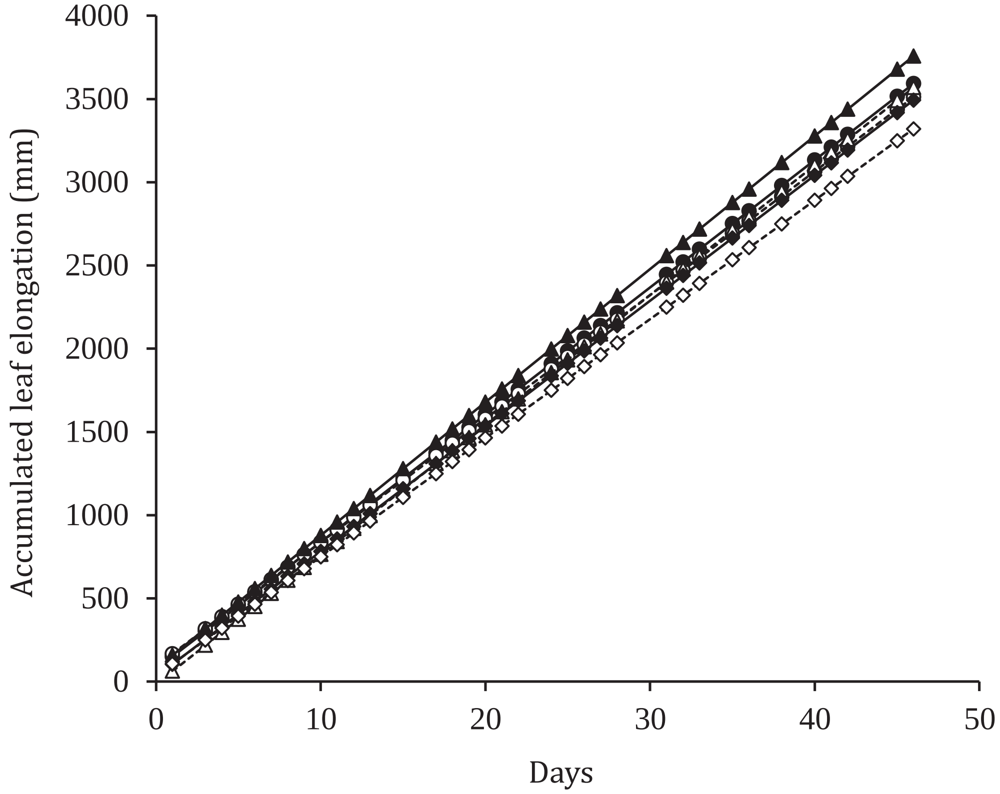



**Figure 3.1 Rates of accumulated leaf elongation of the youngest leaves of chicory and plantain grown as monocultures subjected to three levels of water treatment (Optimum, Dry and Very-Dry) between Days 0 and 10. Linear regression equations are y=76.50*x* + 73.18, $r^2$=0.9236 (Chicory-Optimum; ●, b), y=74.32*x* + 92.85, $r^2$=0.9515 (Chicory-Dry; ○, c), y=80.00*x* + 78.24, $r^2$=0.9743 (Chicory-Very-Dry; ▲, a), y=77.93*x* – 15.69, $r^2$=0.9705 (Plantain- Optimum;△, ab), y=75.33*x* + 28.26, $r^2$=0.9392 (Plantain-Dry; ◆, bc), y=71.40*x* + 35.57, $r^2$=0.9763 (Plantain-Very-Dry; ◇, d). Linear regression equations followed by the same letter indicate that slopes of linear regressions are not significantly different (ANCOVA: *P* > 0.05).**

## 3.5 Discussion

This experiment provides preliminary evidence on how chicory and plantain respond to soil moisture stress and defoliation following a period of moisture stress. All plants (both chicory and plantain) survived the water treatments and the subsequent defoliation period. Mook *et al.* (1989) reported high death rates in a summer drought are uncommon in plantain, while, Lambert (1963) observed the proportion of naturally occurring plantain in a mixed sward increased during drought. Rollo *et al.*(1998) reported chicory displayed consistent production advantages in the first two years after establishment compared to perennial ryegrass in areas prone to summer moisture and heat stress. Plant survival through the drought period has been shown to be related to root density and depth and differs between species (Volaire and Thomas 1995; Bahrani *et al.* 2010; Wedderburn *et al.* 2010). Combined these studies indicate that both chicory and plantain display a degree of drought tolerance and may be utilised successfully in drought prone regions.

At the start of the experimental period chicory had a greater absolute shoot mass than plantain. However, during the water treatment period plantain had a greater relative shoot growth rate and consequently by the end of the water treatment period it had a similar absolute shoot mass to chicory. Furthermore, at the end of the experimental period plantain had a greater absolute shoot mass and relative shoot growth rate (Day 10-47) than chicory under all water treatments except the very dry treatment. Previous research has reported plantain displayed a greater herbage accumulation rate than chicory in all seasons except summer (Powell *et al.* 2007). Somewhat in support of this, Nie *et al* (2008) found chicory yielded more under drought conditions than plantain. Unfortunately, in this experiment shoot material was not collected at times of defoliation. Therefore, it is not possible to determine the full effect of the defoliation treatment on plant growth patterns and shoot dry matter yield. However, leaf extension analysis showed under the very dry water treatment, plantain and chicory displayed contrasting growth responses. Chicory had a greater rate of leaf extension under the very dry water treatment compared to the less severe water treatments. Conversely, plantain had a lower rate of leaf extension under the very dry water treatment compared to the less severe water treatments. Similarly, Davis (1995) showed severe moisture stress reduced the leaf extension rate of plantain considerably. In witloof chicory, Vandoorne *et al.* (2012) reported the rate of leaf appearance was drastically reduced during drought. However, it is likely that forage chicory cultivars would be bred to maintain leaf extension during dry conditions. Overall, this experiment suggests that chicory and plantain likely display different functional responses when under moisture stress. It seems apparent that under mild to moderate moisture stress conditions plantain is able to sustain a greater rate of herbage accumulation than chicory. While, under severe moisture stress chicory may be able to show greater herbage accumulation than plantain, thus supporting anecdotal evidence.

Root carbohydrate reserves in perennial forage plants are an important source of energy necessary for winter survival and initiation of growth in spring (Kust and Smith 1961). Furthermore they can provide the necessary energy to support shoot growth following defoliation (Fankhauser *et al.* 1989). At all harvests, within all defoliation treatments, chicory had a greater absolute root mass than plantain. Sanderson and Elwinger (2000) also showed that chicory accumulated more root mass than plantain during seedling development (0-74 days after planting). The greater shoot mass and lesser root mass of plantain compared to chicory, resulted in plantain consistently having a greater shoot mass fraction under all water treatments. This was also found by Powell *et al.* (2007) under field conditions. Furthermore, Davis (1995) found plantain had a greater shoot:root ratio under both control and water stress conditions when compared to a number of perennial pasture species. This illustrates that plantain has a more aggressive herbage growth strategy than chicory, allocating more energy to above ground biomass, under a range of soil moisture conditions. However, it could be hypothesised that under sustained periods of water stress plantain may struggle to continue allocating greater reserves to above ground biomass and consequently sward persistence may be compromised unless the plants become dormant.

The root mass of chicory decreased incrementally with later defoliation. Whereas in plantain the root mass only decreased in weight with late defoliation compared to no defoliation. This suggests that plantain was able to recover from the early defoliation more quickly than chicory. Furthermore, the taproot diameter of chicory was reduced under defoliation, while that of plantain was not affected, supporting the hypothesis that plantain maybe more robust in response to defoliation following a drought period than chicory. In support of this, Yu *et al.* (2008) observed the taproot diameter of chicory was reduced under hard cutting intensity while that of plantain was unaffected.

Across both species the root length and relative root growth rate (Day 10-47) of the very dry water treatment plants was lower compared to the less severe water treatments from the end of the water stress period onwards. Similarly, the absolute root mass of the very dry water treatment plants was less than the optimum water treatment plants. This suggests that during a severe drought the roots of both chicory and plantain will be vulnerable and less resilient to grazing, and therefore grazing management should take this into account so that plant death rates are minimised.

In conclusion, this experiment highlighted some key differences in the shoot and root morphology of chicory and plantain under water stress conditions. Plantain maintained a greater allocation of energy to above-ground biomass, represented by a greater shoot mass, relative shoot growth rate and shoot mass fraction compared to chicory. However, under very dry conditions chicory displayed a greater rate of leaf extension than plantain. The root characteristics of chicory were more sensitive to the timing of defoliation than plantain. Further experiments need to quantify these differences and investigate whether these differences in production traits are a result of physiological differences between the species (Chapter 4).

# Chapter 4 Morphological and physiological responses of plantain (*Plantago lanceolata*) and chicory (*Cichorium intybus*) to moisture stress and defoliation in glasshouse conditions

## 4.1 Abstract

In New Zealand the frequency and magnitude of droughts is expected to increase due to global warming and consequently a greater understanding of the response of alternative forage species to moisture stress is necessary. Experiment one studied the morphological responses of chicory and plantain to Optimum, Dry and Very-Dry water treatments under Weekly, Fortnightly and Three-Weekly defoliation intervals. Experiment two compared the physiological responses of chicory and plantain under Optimum and Very-Dry water treatments, and Experiment three investigated the rooting depths of chicory and plantain under field conditions. In Experiments one and two, chicory had a greater ($P < 0.05$) root mass and taproot diameter and a lower ($P < 0.05$) shoot mass fraction than plantain. In Experiment one, during the water treatment period, for both species, Very-Dry plants had a lower ($P < 0.05$) absolute shoot mass grown than Optimum and Dry plants, which did not differ ($P > 0.05$). While, during the recovery period, Very-Dry plants under Fortnightly and Three-Weekly defoliation had a greater ($P < 0.05$) absolute shoot mass grown than Dry and Optimum plants which did not differ ($P > 0.05$). In Experiment one, for both species, Three-Weekly defoliation resulted in a greater ($P < 0.05$) taproot diameter (at end of experiment) and a greater ($P < 0.05$) relative shoot and root growth rate than Weekly defoliation. In Experiment two, the photosynthetic and evapotranspiration rates of chicory and plantain did not differ ($P > 0.05$), and were lower ($P < 0.05$) under Very-Dry than optimum conditions. Under Very-Dry conditions, compared to plantain, chicory had greater ($P < 0.05$) leaf water potential on Days 3 and 4. Chicory displayed ($P < 0.05$) osmotic adjustment on Days 4 and 5, while plantain only displayed ($P < 0.05$) osmotic adjustment on Day 5. Experiment three showed under field conditions chicory had a greater ($P < 0.05$) root

mass in the lower soil depth profile (10-20 cm depth) than plantain. These findings suggest that both chicory and plantain can survive and continue to grow under water stress conditions with the main differences between the species being attributable to morphological characteristics (root mass, taproot diameter, shoot mass fraction) rather than differences at a physiological level. Overall, the results suggest plantain may be more productive under moderate drought due to its greater shoot mass fraction, whereas chicory may be more productive and persistent under severe drought due to its greater root mass and taproot diameter.

## 4.2 Introduction

Forage chicory (*Cichorium intybus*) and plantain (*Plantago lanceolata*) are utilised worldwide as high feed quality perennial herbages (Sanderson *et al.* 2003a; Labreveux *et al.* 2006; Li *et al.* 2010; Golding *et al.* 2011; Hutton *et al.* 2011). More recently Herb and Legume Mixes containing; chicory, plantain, red and white clover have grown in popularity as they combine to form a high feed quality, summer active forage, with a lengthened growing season compared to pure swards and with the ability to fix nitrogen (Kemp *et al.* 2010). The Herb and Legume Mix has been shown to support high rates of animal performance including; increased milk yields, higher lamb weaning weights and post weaning live weight gains (Kenyon *et al.* 2010; Golding *et al.* 2011; Hutton *et al.* 2011).

Differences between species in the amount and duration of shoot and root growth inhibition caused by defoliation are likely to be important mechanisms influencing the competitive balance in pastures. Both plantain and chicory are tap-rooted (Stewart and Charlton 2006) and require specific grazing management. This includes rotational grazing at 3-5 weeks intervals to allow plants sufficient time to recover without allowing secondary and axillary stem development in summer (Hare *et al.* 1987; Clark *et al.* 1990a; Sanderson *et al.* 2003a; Labreveux *et al.* 2004) and not grazing below 5 cm regularly (Li *et al.* 1994b; Li *et al.* 1995; Li *et al.* 1997c).

On a global basis, drought, in conjunction with coincident high temperature and radiation, poses the most important environmental constraints to plant survival and crop productivity (Chaves *et al.* 2003). This will become increasingly significant as the world is challenged by predicted warmer climates (IPCC 2007). The occurrence of morphological and physiological responses which may lead to some adaptation to drought stress may vary considerably among species. Stomatal regulation of photosynthesis during water shortage has been well documented (Chaves 1991).

The health and carbohydrate storage of tap roots influences the persistence of plants (Lodge 1991) and also confers a degree of drought tolerance (Nie *et al.* 2008) through accessing water deeper in the soil profile. Both chicory and plantain are relatively tolerant to moisture stress (Lambert 1963; Mook *et al.* 1989; Nie *et al.* 2008), with Nie *et al.* (2008) reporting that chicory can tolerate moisture stress to a greater degree than plantain. In Chapter 3, plantain was found to have a greater shoot mass, relative shoot growth rate and shoot mass fraction than chicory under moisture stress. Furthermore, the root characteristics of chicory appeared to be more sensitive to the timing of defoliation following a period of moisture stress compared to plantain. However, overall little is known about the effects of moisture stress and defoliation frequency on the morphological and physiological responses of both plantain and chicory. Therefore, the objective of this research was to address these issues by studying the effects of moisture stress and defoliation frequency on the herbage production, biomass allocation, persistence and physiological responses of Puna Π chicory and Tonic plantain under glasshouse conditions.

## 4.3 Materials and methods

Two glasshouse experiments were conducted in the Plant Growth Unit, Massey University, Palmerston North, New Zealand (latitude 40° 23' S). In addition, one field experiment was carried out at the Massey University Pasture and Crop Research Unit at Poultry Farm Road, Palmerston North, New Zealand.

### 4.3.1 Experiment 1: Response of plantain and chicory to moisture stress under varying defoliation frequencies

#### *4.3.1.1 Plant establishment*

The experiment was conducted between 24 February and 22 August 2012. Two plant species, plantain cv. 'Ceres Tonic' and chicory cv. 'Puna II', were used. The potting medium consisted of 33% Manawatu silt loam (B Horizon), 33% sand (3 mm) and 33% sand (fine sand-dune; <0.5 mm) amended with 100g short term mix (Woodace, flowering plant special; LebanonTurf; 14% N, 6% P, 11.6% K + trace elements) and 50g dolomite for 30L of potting medium as a base start fertiliser. Prior to seed establishment (17 February 2012) soil samples of the potting medium were taken to determine soil water retention and pF curve (soil moisture retention curve). Field capacity was reached at a gravimetric water content of 11g/100g.

The pots were 250 mm in diameter and 250 mm in depth and were filled with 10.0 ± 0.05 kg of potting medium. Seeds were sown directly into pots (154 in total, 76 chicory and 76 plantain), on 24 February 2012 and pots were hand watered daily with a sprinkler. After seeds germinated, seedlings were thinned to six per pot and then to four per pot when well-established on 22 March 2012 (a total of 304 chicory plants and 304 plantain plants).

An automatic watering system, incorporating an individual dripper in each pot was established on 5 March 2012. All pots were regularly watered to maintain 100% field capacity between 5 March and 15 May (prior to the water treatment period) and 8-22 August (recovery period). Between 30 March and 12 May 2012, all pots were watered daily with Peters professional ‘Allrounder’ water soluble NPK fertiliser (20%, 8.7%, 16.6%) plus trace elements (B, Cu, Fe, Mn, Mo, Zn) at a rate of 1g/litre. Air temperatures were maintained by heating or ventilation at 12°C - 16°C at night and 20°C - 25°C during the day. The plants were cut to 50 mm above media level on 4 April, 18 April and 9 May to ensure all plants were sufficiently established (82 days after sowing).

#### *4.3.1.2 Experimental design*

On 16 May (Day 1; 82 days after sowing), an experiment that consisted of a 2 × 3 × 3 factorial combination of treatments in a randomised complete block design (RCB) with four blocks (19 chicory and 19 plantain pots per block) was begun. The three main factors were plant species (Plantain and Chicory) and water treatment (Optimum (control, 100% field capacity), Dry (63-82% field capacity) and Very-Dry (36-55% field capacity)), and defoliation frequency (Weekly, Fortnightly and Three-Weekly) over the period Day 1 to Day 98 (Figure 4.1). Each treatment combination was replicated once within each of the four blocks for each harvest date (n = 4 for each treatment combination). At defoliation, plants were cut to 50 mm above media level. The length of the water treatment period (Day 1 - 84; 12 weeks) was designed to simulate a severe summer water stress period. Each individual pot was weighed approximately every three days and the amount of water required to keep each pot within its water treatment field capacity range was calculated and applied. Following the water treatment period the plants were given a 2 week recovery period (Day 85 - 98) during which they received Optimum water (100% field capacity) and no further defoliation. A subset of the plants in pots from each treatment combination were destructively harvested at three dates; Day 1, Day 42 (midpoint of the water treatment period) and Day 98 (end of the recovery period).

**Figure 4.1 Experiment 1: Effect of water treatment and defoliation frequency at week two of the treatment period.**

#### *4.3.1.3 Measurements*

The volume of water that each pot contained was monitored approximately every 3 days using the time-domain-reflectometry technique (TDR; CS-615, Campbell Sci., Leicestershire, UK) with moisture probes to a 20 cm depth. Probes were inserted into the middle of the pot at each measurement time.

The number of live leaves per plant was recorded at time of each destructive harvest. At destructive harvest taproot diameter at the widest point and taproot length were recorded and above ground herbage mass was dissected into live and dead material. The root mass was measured after manually washing off media. All mass data represent the mass per pot (i.e. mass from four plants) and are presented on a dry weight basis, oven dried at 60°C for 48 hours.

The shoot mass fraction was calculated;

$$Shoot\ mass\ fraction = \frac{Total\ shoot\ mass\ per\ pot}{Total\ shoot\ mass + total\ root\ mass\ per\ pot} \times 100$$

The relative growth rate of shoot and root between each harvest was calculated as;

$$Relative\ Growth\ Rate\ (RGR) = \frac{lnW_2 - lnW_1}{time}$$

Where:

$W_1$ = weight at time 1

$W_2$ = weight at time 2

Time = number of days between harvest events

#### *4.3.1.4 Statistical Analysis*

All data were analysed using the SAS mixed data procedure (SAS Version 9.2, SAS Institute Inc., Gary NC, USA). Data were analysed separately for the three destructive harvest periods (Day 1, Day 42 and Day 98) and for each parameter (absolute shoot mass, relative shoot growth rate, taproot diameter, root length, absolute root mass, relative root growth rate, shoot mass fraction, number of live leaves per plant and leaf mass). At Day 1 (Harvest 1), the model included the fixed effects of plant species (chicory and plantain) and the random effect of block. At Day 42 (Harvest 2) and Day 98 (Harvest 3), each model included the fixed effects of plant species (chicory and plantain), water treatment (Optimum and Dry and Very-Dry) and defoliation frequency (Weekly, Fortnightly and Three-Weekly) and all two-way and three-way interactions between plant species, water treatment and defoliation treatment and the random effect of block. Interactions were only presented in tables if they were significant ($P < 0.05$).

### 4.3.2 Experiment 2: Physiological responses of plantain and chicory to moisture stress

#### *4.3.2.1 Plant establishment*

The experiment was conducted between 7 January and 16 March 2013. Two plant species, plantain cv. 'Ceres Tonic' and chicory cv. 'Puna II', were used. The potting medium consisted of 50% sand (3 mm) and 50% aggregate stones (<10 mm) amended with 90g short term mix (Woodace, flowering plant special; LebanonTurf; 14% N, 6% P, 11.6% K + trace elements) and 45g dolomite for 30L of potting medium as a base start fertiliser. The pots were 250 mm in diameter and 250 mm in depth and were filled with 10.0 ± 0.05 kg of potting medium. Seeds were sown directly into pots (40 in total, 20 chicory and 20 plantain), on 7 January 2013 and pots were hand watered daily with a sprinkler. After seeds germinated, seedlings were thinned to six per pot and then to four per pot when well-established on 12 February 2013 (a total of 80 chicory plants and 80 plantain plants).

An automatic watering system, incorporating an individual dripper in each pot was established on 28 January 3013 and all pots were watered regularly to ensure plant growth was not limited. All pots were watered daily with liquid nutrients (1g/litre) (same as in experiments 1) between 27 February and 8 March 2013. Air temperatures were maintained by heating or ventilation at 12°C - 16°C at night and 20°C - 25°C during the day.

#### *4.3.2.2 Experimental design*

The experiment consisted of a 2 × 2 factorial combination of treatments in a randomised complete block design with four blocks was utilised. The two main factors were plant species (Plantain and Chicory) and water treatments (Optimum (control) and Very-Dry). The Optimum water treatment provided a set volume of water per pot (same for chicory and plantain) which was based on the level of sunlight and plant growth, to ensure soil moisture content was not limiting plant growth. The Very-Dry water treatment consisted of the plants receiving no water. The water treatment period started on 11 March (Day 1) and lasted for five days (Days 1-5).

Each treatment combination was replicated once within each of the four blocks for each measurement group, i.e. 1 set of plant species for destructive harvest on Day 1, one set of species by water treatments for destructive measurements during the water treatment period (Days 1-5) and one set of species by water treatments for non-destructive measurements (Days 1-5) and then destructive harvest on Day 6 (at the conclusion of the water treatment period).

#### *4.3.2.3 Measurements*

All physiological plant measurements were carried out during Days 1-5 of the treatment period during peak sunlight (between 12-2pm daily). Measurements were collected in a block by block manner. The photosynthetic characteristics of the plants were studied using a LI-6400 portable photosynthetic system (made by LI-COR Biosciences, USA). One measurement was taken per pot (one leaf of one plant) on the most recent fully expanded leaf of the set of non-destructive plants. The measurements recorded by the LI-6400 were: net photosynthetic rate (Pn), evapotranspiration rate (E), stomatal conductance (Cs), intercellular $CO_2$ (Ci) and leaf temperature.

Water potential ($\Psi_w$) and osmotic potential ($\Psi_o$) measurements were undertaken on the set of destructive measurement pots (one measurement per pot from one plant). $\Psi_w$ was measured using a Scholander pressure chamber as described by Turner (1988). $\Psi_w$ and $\Psi_o$ were measured via vapour pressure osmometry (model 5500, Wescor Logan, Utah) as described by Turner (1981).

Destructive harvest measurements on Day 1 and Day 6 were undertaken as per experiment 1. The volume of water that each pot contained was monitored daily using the TDR technique with moisture probes to a 20 cm depth. Probes were inserted into the middle of the pot. The relative growth rate of shoot and root between each harvest was calculated as in experiment one.

#### *4.3.2.4 Statistical Analysis*

All data were analysed using the SAS mixed data procedure. Data were analysed separately for the two destructive harvest periods (Day 1 and Day 6) and for each parameter. At Day 1 (Harvest 1), the model included the fixed effects of plant species (chicory and plantain) and the random effect of block. At Day 6 (Harvest 2) the model included the fixed effects of plant species (chicory and plantain), water treatment (Optimum and Very-Dry) and the interaction between plant species and water treatment and the random effect of block. Interactions were only presented in tables if they were significant ($P < 0.05$).

The physiological plant measurement data (Pn, E, Cs, Ci, leaf temperature, $\Psi_w$ and $\Psi_o$) and soil moisture content were also analysed using the MIXED procedure in SAS. Each model included the fixed effects of plant species (chicory and plantain) and water treatment (Optimum and Very-Dry) and the interaction between plant species and water treatment and included day within block as a repeated measure. Interactions were only presented in graphs if they were significant ($P < 0.05$).

### 4.3.3 Experiment 3: Comparison of plantain and chicory rooting depth under field conditions

An existing paddock with a sward mix based on chicory cv. 'Puna II' and plantain cv. 'Ceres Tonic' located on the Massey University Pasture and Crop Research Unit at Poultry Farm Road, Palmerston North, on a Manawatu fine sandy loam was used. The paddock had been sown in March 2011 and thus the plants were in their second growing season at the time of the experiment. The paddock had been regularly grazed with sheep.

#### *4.3.3.1 Experimental design*

On 11 February 2013, 26 pairs of plants (1 chicory and 1 plantain, n=52 in total) were excavated so that all root material was intact. Plants within each pair were within a 50 cm radius of each other. Roots were washed so that all visible dirt was removed and cut into 10 cm rooting intervals (0-10, 10-20 cm). No roots were longer than 20 cm. Roots were then oven dried at 60°C for 48 hours and dry weight was recorded.

#### *4.3.3.2 Statistical Analysis*

Data were analysed using SAS. T Tests were used to compare the mass of root within the depth interval within each species and a non-parametric ANOVA (Kruskal-Wallis Test) was used to compare the mass of roots within each depth interval between species.

## 4.4 Results

### 4.4.1 Experiment 1: Response of plantain and chicory to moisture stress under varying defoliation frequencies

#### *4.4.1.1 Water treatment- TDR measurement*

All water stress treatments were successfully established by the 31 May (approximately 2 weeks after commencing the water stress treatments). Across both species, this resulted in a greater ($P < 0.05$) volume of water in the Optimum water treatment than in the Dry water treatment, which was in turn greater ($P < 0.05$) than the Very-Dry water treatment (Table 4.1). Within the water treatments, the volume of water in each pot did not differ ($P > 0.05$) between plantain and chicory, except in the Optimum water treatment, where chicory had a greater ($P < 0.05$) volume of water than plantain.

**Table 4.1 Effect of plant species (chicory and plantain) and water treatment (Optimum and Dry and Very-Dry) on the average volume of water ($m^3/m^3$) in each pot between 31 May and 15 August 2012 (when all water treatments were all successfully established). Data presented as means ± s.e. Means within columns and rows having different letters are significantly different at *P* = 0.05.**

| Water treatment | Chicory | Plantain |
|---|---|---|
| Optimum | 26.7d ± 0.18 | 26.3c ± 0.18 |
| Dry | 8.6b ± 0.18 | 8.2b ± 0.18 |
| Very-Dry | 6.4a ± 0.18 | 6.2a ± 0.18 |

#### *4.4.1.2 Day 1 – Harvest 1*

Shoot mass and leaf mass did not differ ($P > 0.05$) between plantain and chicory (Table 4.2). Chicory had a greater ($P < 0.05$) taproot diameter and root mass than plantain.

Root length, number of live leaves per plant and shoot mass fraction were greater ($P < 0.05$) in plantain than in chicory.

**Table 4.2 Experiment 1: Effect of plant species (plantain and chicory) at Day 1 on absolute shoot mass, taproot diameter, root length, absolute root mass, shoot mass fraction, number of live leaves per plant and leaf mass. Prior to Day 1 plants were grown under optimum water conditions. Data presented as means ± s.e. Means within columns having different letters are significantly different at *P* = 0.05.**

| | Absolute shoot mass (g) | Taproot diameter (mm) | Root length (mm) | Absolute root mass (g) | Shoot/ (shoot + root) (%) | Number of live leaves per plant | Leaf mass (g/leaf) |
|---|---|---|---|---|---|---|---|
| Plantain | 7.3 | 4.7a | 173.7b | 1.5a | 83.1b | 44.1b | 0.04 |
| Chicory | 6.7 | 11.9b | 147.5a | 4.2b | 60.9a | 24.8a | 0.07 |
| s.e. | 0.55 | 0.4 | 16.66 | 0.45 | 2.07 | 3.11 | 0.01 |

### *4.4.1.3 Day 42 – Harvest 2*

#### 4.4.1.3.1 Above-ground herbage measures

There were no ($P > 0.05$) two-way or three-way interactions between species, water treatment and defoliation frequency for any above-ground herbage measure (absolute shoot mass, relative shoot growth rate, shoot mass fraction, number of live leaves per plant, leaf mass).

##### *4.4.1.3.1.1 Effect of species*

Plantain had a greater ($P < 0.05$) absolute shoot production, relative shoot growth rate (Days 1- 42), shoot mass fraction and number of live leaves per plant than chicory (Table 4.3). Chicory had a greater ($P < 0.05$) leaf mass than plantain.

**Table 4.3 Experiment 1: Effect of plant species (Plantain and Chicory), water treatment (Optimum and Dry and Very-Dry) and defoliation frequency (Weekly and Fortnightly and Three-Weekly) at Day 42 on absolute shoot mass, relative shoot growth rate (Day 1-42), shoot mass fraction, number of live leaves per plant and leaf mass. Data presented as means ± s.e. Means within columns and main effects having different letters are significantly different at *P* = 0.05.**

| | Absolute shoot mass (g) | Relative shoot growth rate (g/g/day) | Shoot/ (shoot + root) (%) | Number of live leaves per plant | Leaf mass (g/leaf) |
|---|---|---|---|---|---|
| ***Species*** | | | | | |
| Plantain | 14.7b | 0.016b | 57.4b | 35.2b | 0.032a |
| Chicory | 11.8a | 0.013a | 40.3a | 20.1a | 0.037b |
| s.e. | 0.63 | 0.001 | 1.8 | 1.28 | 0.002 |
| ***Water*** | | | | | |
| Optimum | 13.9 | 0.015 | 45.6a | 30.1b | 0.031a |
| Dry | 13.6 | 0.015 | 52.4b | 28.8b | 0.036b |
| Very-Dry | 12.3 | 0.013 | 48.6ab | 24.0a | 0.037b |
| s.e. | 0.7 | 0.001 | 2.08 | 1.42 | 0.002 |
| ***Defoliation*** | | | | | |
| Weekly | 10.8a | 0.010a | 53.7b | 28.2 | 0.026a |
| Fortnightly | 13.2b | 0.014b | 51.6b | 27 | 0.041c |
| Three-Weekly | 15.7c | 0.019c | 41.4a | 27.8 | 0.037b |
| s.e. | 0.7 | 0.001 | 2.08 | 1.42 | 0.002 |

#### *4.4.1.3.1.2 Effect of water treatment*

Water treatment had no effect ($P > 0.05$) on absolute shoot production or relative shoot growth rate (Table 4.3). However, pairwise analysis showed Very-Dry plants had a lower ($P < 0.05$) absolute shoot production than Optimum plants, with Dry plants not differing ($P > 0.05$) from either water treatment. Very-Dry plants had a lower ($P < 0.05$) number of live leaves per plant than Dry and Optimum plants, which did not differ ($P > 0.05$). Dry plants had a greater ($P < 0.05$) shoot mass fraction than Optimum plants, with Very-Dry plants not differing ($P > 0.05$) from either water treatment. Optimum plants had a lower ($P < 0.05$) leaf mass than Dry and Very-Dry plants, which did not differ ($P > 0.05$).

###### *4.4.1.3.1.3 Effect of defoliation frequency*

Absolute shoot production and relative shoot growth rate were greater ($P < 0.05$) under the Three-Weekly defoliation frequency than the Fortnightly defoliation frequency, which was in turn greater ($P < 0.05$) than the Weekly defoliation frequency (Table 4.3). The number of live leaves per plant was not affected ($P > 0.05$) by defoliation frequency. The Three-Weekly defoliation frequency had a lower ($P < 0.05$) shoot mass fraction than the Weekly and Fortnightly defoliation frequencies, which did not differ ($P > 0.05$). The Fortnightly defoliation frequency had a greater ($P < 0.05$) leaf mass than the Three-Weekly defoliation frequency, which was in turn greater ($P < 0.05$) than the Weekly defoliation frequency.

#### 4.4.1.3.2 Below-ground herbage measures

There were no ($P > 0.05$) three-way interactions between species, water treatment and defoliation frequency for any below-ground herbage measure (taproot diameter, root length, absolute root mass, relative root growth rate).

There was no ($P > 0.05$) two-way interaction between water treatment and defoliation frequency for taproot diameter. However, there was a two-way interaction ($P < 0.05$) between species and water treatment for taproot diameter (Table 4.4). Plantain taproot diameter was similar ($P > 0.05$) across water treatments and lower ($P < 0.05$) than all chicory by water treatment combinations. Chicory taproot diameter was greater ($P < 0.05$) under Optimum water treatment than under Dry and Very-Dry water treatments, which did not differ ($P > 0.05$). There was also a two-way interaction ($P < 0.05$) between species and defoliation frequency for taproot diameter. Plantain taproot diameter was similar ($p>0.05$) across defoliation frequencies. Chicory taproot diameter was greater ($p<0.05$) under Three-Weekly defoliation frequency than under Weekly and Fortnightly defoliation frequencies, which did not differ ($p>0.05$).

**Table 4.4 Experiment 1: Interactions: Effect of plant species (Plantain and Chicory), water treatment (Optimum and Dry and Very-Dry) and defoliation frequency (Weekly and Fortnightly and Three-Weekly) at Day 42 on taproot diameter, root length and absolute root mass. Data presented as means ± s.e. Means within columns and treatments having different letters are significantly different at *P* = 0.05.**

| | Taproot diameter (mm) | Root length (mm) | Absolute root mass (g) |
|---|---|---|---|
| ***Species*water*** | | | |
| Plantain-Optimum | 4.1a | | |
| Plantain-Dry | 4.2a | | |
| Plantain-Very-Dry | 4.1a | | |
| Chicory-Optimum | 12.7c | | |
| Chicory-Dry | 11.6b | | |
| Chicory-Very-Dry | 11.1b | | |
| s.e. | 0.26 | | |
| | | | |
| ***Species*defoliation*** | | | |
| Plantain-Weekly | 4.2a | 239.0b | |
| Plantain-Fortnightly | 4.1a | 257.2c | |
| Plantain-Three-Weekly | 4.1a | 284.4d | |
| Chicory-Weekly | 11.4b | 135.3a | |
| Chicory-Fortnightly | 11.5b | 140.1a | |
| Chicory-Three-Weekly | 12.4c | 135.6a | |
| s.e. | 0.26 | 7.62 | |
| | | | |
| ***Water*defoliation*** | | | |
| Optimum-Weekly | | 183.2a | 2.5a |
| Dry-Weekly | | 181.1a | 2.2a |
| Very-Dry-Weekly | | 197abc | 2.8ab |
| Optimum-Fortnightly | | 187.5a | 4.6bc |
| Dry-Fortnightly | | 217.3d | 4.7c |
| Very-Dry-Fortnightly | | 191.1ab | 4abc |
| Optimum-Three-Weekly | | 213.3cd | 8.2d |
| Dry-Three-Weekly | | 209.2bcd | 4.7c |
| Very-Dry-Three-Weekly | | 207.5bcd | 5.2c |
| s.e. | | 8.54 | 0.67 |

There was no ($P > 0.05$) two-way interaction between species and water treatment for root length. However, there was a two-way interaction ($P < 0.05$) between species and defoliation frequency on root length (Table 4.4). Chicory root length was similar ($P > 0.05$) across defoliation frequencies and lower ($P < 0.05$) than all plantain defoliation treatments. Plantain root length was greater ($P < 0.05$) under Three-Weekly defoliation frequency than Fortnightly defoliation frequency, which was in turn greater ($P < 0.05$) than the Weekly defoliation frequency. There was also an interaction ($P < 0.05$) between water treatment and defoliation treatment for root length; however, this had no obvious biological relevance.

There were no ($P > 0.05$) two-way interactions between species and water treatment or between species and defoliation frequency for absolute root mass. However, there was a two-way interaction ($P < 0.05$) between water treatment and defoliation treatment for absolute root mass (Table 4.4). Defoliation frequency and water treatment had a combined effect on absolute root mass, whereby the effect of defoliation frequency was proportionally greater ($P < 0.05$) under Optimum watering than under more severe water treatment. Chicory had a greater ($P < 0.05$) absolute root mass than plantain (Table 4.5).

There were no ($P > 0.05$) two-way interactions between species, water treatment and defoliation frequency for relative root growth rate. Plantain had a greater ($P < 0.05$) relative root growth rate (Day 1- 42) than chicory (Table 4.5). Water treatment had no effect ($P > 0.05$) on the relative root growth rate (Day 1- 42). Three-Weekly defoliation plants had a greater ($P < 0.05$) relative root growth rate (Day 1- 42) than Fortnightly defoliation plants, which was in turn had a relative root growth rate greater ($P < 0.05$) than Weekly defoliation plants.

**Table 4.5 Experiment 1: Main Effects: Effect of plant species (Plantain and Chicory), water treatment (Optimum and Dry and Very-Dry) and defoliation frequency (Weekly and Fortnightly and Three-Weekly) at Day 42 on taproot diameter, root length, absolute root mass and relative root growth rate (Day 1-42). Data presented as means ± s.e. Means within columns and treatments having different letters are significantly different at *P* = 0.05.**

| | Taproot diameter (mm) | Root length (mm) | Absolute root mass (g) | Relative root growth rate (g/g/day) |
|---|---|---|---|---|
| ***Species*** | | | | |
| Plantain | 4.1a | 260.2b | 3.76a | 0.017 |
| Chicory | 11.8b | 137.0a | 4.85b | 0.000 |
| s.e. | 0.16 | 6.18 | 0.37 | 0.002 |
| ***Water*** | | | | |
| Optimum | 8.4b | 195.0 | 5.1b | 0.012 |
| Dry | 7.9ab | 202.5 | 3.85a | 0.006 |
| Very-Dry | 7.6a | 198.5 | 3.99a | 0.008 |
| s.e. | 0.19 | 6.57 | 0.42 | 0.002 |
| ***Defoliation*** | | | | |
| Weekly | 7.8 | 187.1a | 2.5a | -0.008a |
| Fortnightly | 7.8 | 198.7b | 4.4b | 0.010b |
| Three-Weekly | 8.2 | 210.0c | 6.0c | 0.019c |
| s.e. | 0.19 | 6.57 | 0.42 | 0.002 |

### *4.4.1.4 Day 98 – Harvest 3*

#### 4.4.1.4.1 Above-ground herbage measures

All plants survived the entire experimental period. There was a three-way interaction ($P < 0.05$) between species, water treatment and defoliation frequency for relative shoot growth rate (Day 42-98). Plantain had a greater ($P < 0.05$) relative shoot growth rate than chicory under all water treatment by defoliation frequency combinations (Figure 4.2). Under all water treatments, Weekly defoliation frequency resulted in a lower ($P < 0.05$) relative shoot growth rate than less frequent defoliation treatments.

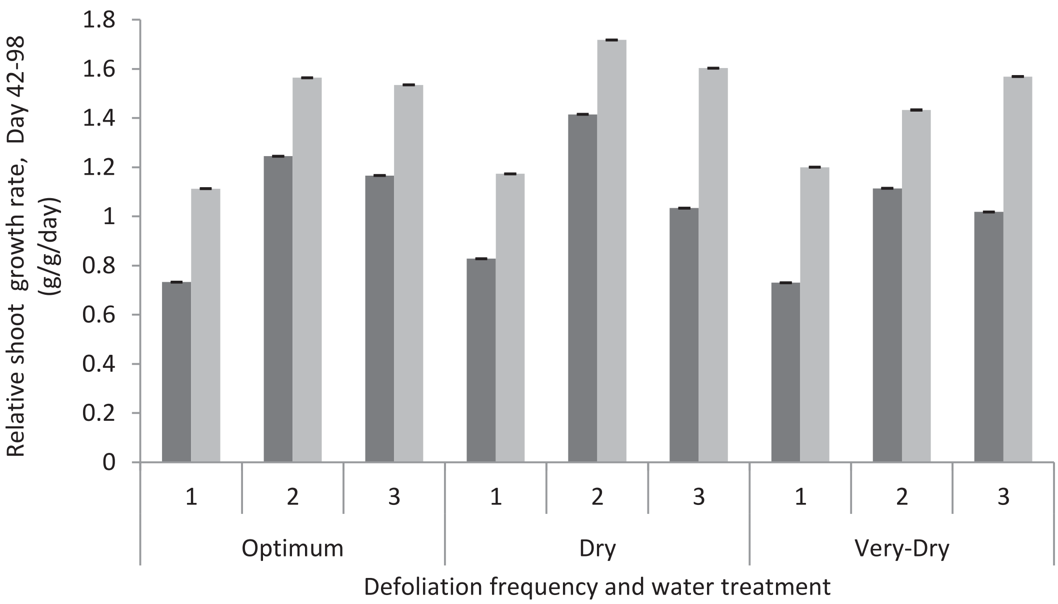



**Figure 4.2 Effect of species (Chicory (black) and Plantain (grey)), water treatment (Optimum and Dry and Very-Dry) and defoliation frequency (Weekly (1) and Fortnightly (2) and Three-Weekly (3)) on the relative shoot growth rate between Days 42-98. Vertical bars represent s.e.**

There were no ($P > 0.05$) three-way interactions between species, water treatment and defoliation frequency or two-way interactions between species and water treatment or between species and defoliation frequency for absolute shoot mass grown during the recovery period. However there was a two-way interaction ($P < 0.05$) between water treatment and defoliation frequency on shoot mass grown during the recovery period. Very-Dry plants under Fortnightly and Three-Weekly defoliation frequencies had a greater ($P < 0.05$) absolute shoot mass grown during the recovery period than Dry and Optimum plants which did not differ ($P > 0.05$) (Figure 4.3). Under Weekly defoliation frequencies Dry plants had a greater ($P < 0.05$) absolute shoot mass grown during the recovery period than Optimum plants, with Very-Dry plants not differing ($P > 0.05$) from either water treatment. Plantain had a greater ($P < 0.05$) absolute shoot mass grown during the recovery period than chicory (Table 4.6).

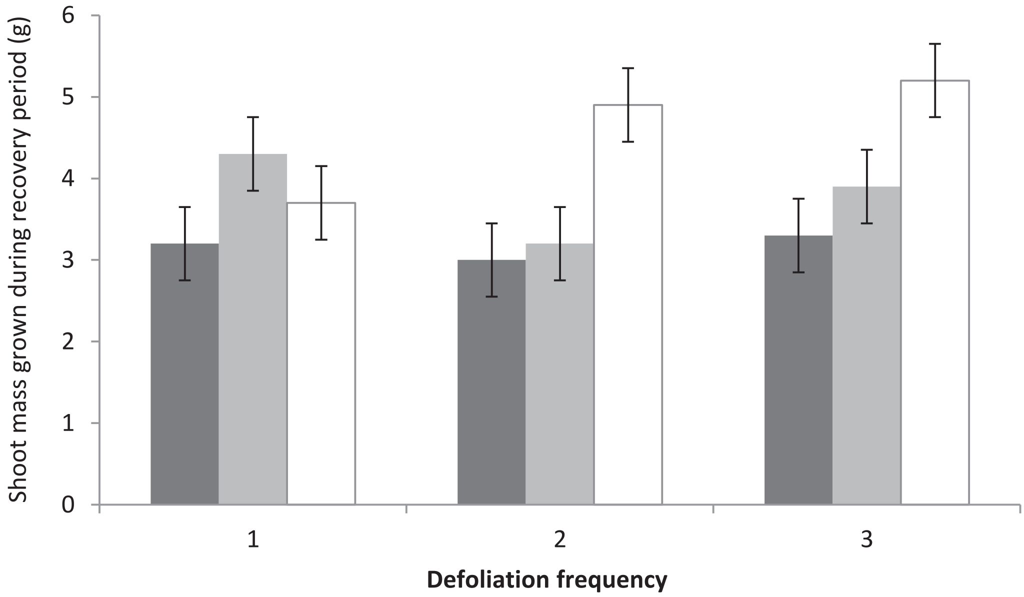



**Figure 4.3 Effect of water treatment (Optimum (dark grey) and Dry (light grey) and Very-Dry (white)) and defoliation frequency (Weekly (1) and Fortnightly (2) and Three-Weekly (3)) on absolute shoot mass grown during the recovery period (Day 85 - 98). Vertical bars represent s.e.**

**Table 4.6 Experiment 1: Effect of plant species (Plantain and Chicory), water treatment (Optimum and Dry and Very-Dry Fortnightly and Three-Weekly) at Day 98 on shoot mass grown during water treatment period and during recovery period, rela mass fraction, number of live leaves per plant and leaf mass. Data presented as means ± s.e. Means within columns and significantly different at *P* = 0.05.**

| | Absolute shoot mass grown during water treatment period (g) | Absolute shoot mass grown during recover period (g) | Relative shoot growth rate (g/g/day) | Shoot/ (shoot + root) (%) | Number o leaves pe |
|---|---|---|---|---|---|
| ***Species*** | | | | | |
| Plantain | 14.8 | 4.2b | 1.43b | 66.2b | 33.8 |
| Chicory | 13.7 | 3.5a | 1.03a | 40.2a | 24.5 |
| s.e. | 0.61 | 0.29 | 0.001 | 1.78 | 0.86 |
| ***Water*** | | | | | |
| Optimum | 15.7b | 3.2a | 1.23b | 50.3 | 32.0 |
| dry | 14.8b | 3.8b | 1.30c | 54.7 | 27.7 |
| VD | 12.2a | 4.6c | 1.18a | 54.5 | 27.8 |
| s.e. | 0.69 | 0.32 | 0.001 | 2.12 | 1.04 |
| ***Defoliation*** | | | | | |
| Weekly | 12.5a | 3.8 | 0.96a | 60.4b | 30.2 |
| Fortnightly | 13.8a | 3.7 | 1.42c | 51.9a | 29.0 |
| Three-Weekly | 16.3b | 4.1 | 1.32b | 47.3a | 28.3 |
| s.e. | 0.69 | 0.32 | 0.001 | 2.12 | 1.04 |

There were no ($P > 0.05$) two-way or three-way interactions between species, water treatment and defoliation frequency for the parameters; absolute shoot mass grown during the water treatment period, shoot mass fraction, number of live leaves per plant and leaf mass. Absolute shoot mass grown during the water treatment period did not differ ($P > 0.05$) between chicory and plantain (Table 4.6). Very-Dry plants had a lower ($P < 0.05$) absolute shoot mass grown during the water treatment period than Optimum and Dry plants, which did not differ ($P > 0.05$). Three-Weekly defoliation frequency plants had a greater ($P < 0.05$) absolute shoot mass grown during the water treatment period than Weekly and Fortnightly defoliation frequency plants, which did not differ ($P > 0.05$).

Plantain had a greater ($P < 0.05$) shoot mass fraction than chicory (Table 4.6). Water treatment had no effect ($P > 0.05$) on shoot mass fraction. Weekly defoliation frequency plants had a greater ($P < 0.05$) shoot mass fraction than Fortnightly and Three-Weekly defoliation frequency plants, which did not differ ($P > 0.05$).

Plantain had a greater ($P < 0.05$) number of live leaves per plant than chicory (Table 4.6). Optimum plants had a greater ($P < 0.05$) number of live leaves per plant than Dry and Very-Dry plants, which did not differ ($P > 0.05$). Defoliation frequency had no effect ($P > 0.05$) on the number of live leaves per plant.

Plantain had a lower ($P < 0.05$) leaf mass than chicory (Table 4.6). Very-Dry plants had a greater ($P < 0.05$) leaf mass than Dry plants, which was in turn greater ($P < 0.05$) than Optimum plants. Three-Weekly defoliation frequency plants had a greater ($P < 0.05$) leaf mass than Weekly and Fortnightly defoliation frequency plants, which did not differ ($P > 0.05$).

#### 4.4.1.4.2 Below-ground herbage measures

There was no ($P > 0.05$) three-way interaction between species, water treatment and defoliation frequency or two-way interactions between species and water treatment or between water treatment and defoliation frequency for absolute root mass. However, there was a two-way interaction ($P < 0.05$) between species and defoliation frequency for absolute root mass. Absolute root mass was greater ($P < 0.05$) for Chicory than Plantain under all defoliation frequencies (Figure 4.4). Within Chicory, the Three-Weekly defoliation frequency had a greater ($P < 0.05$) root mass than the Weekly and Fortnightly defoliation frequencies, which did not differ ($P > 0.05$). Within plantain, the Three-Weekly defoliation frequency had a greater ($P < 0.05$) root mass than the Weekly defoliation frequency, with Fortnightly defoliation frequency not differing ($P > 0.05$) from either treatment. The water treatment had no effect ($P > 0.05$) on absolute root mass (Table 4.7).

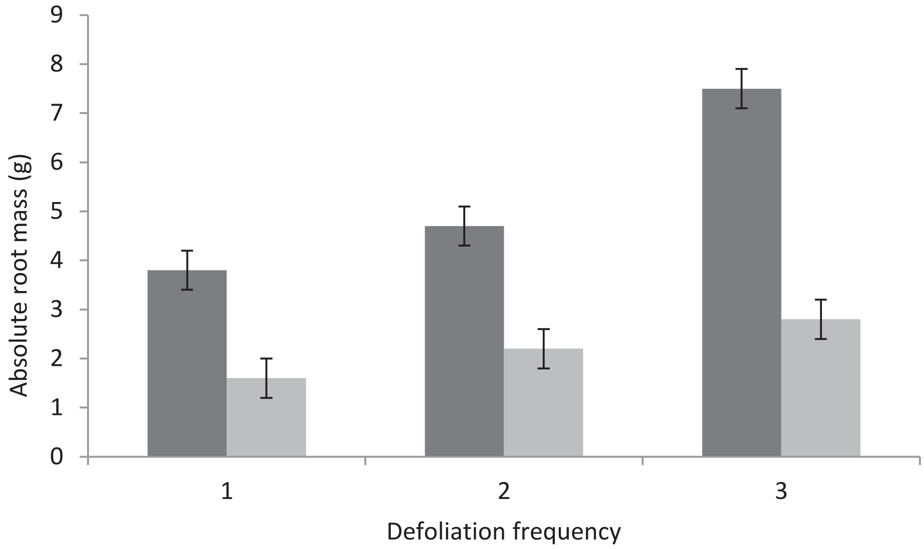



**Figure 4.4 Effect of species (Chicory (black) and Plantain (grey)) and defoliation frequency (Weekly (1) and Fortnightly (2) and Three-Weekly (3)) on absolute root mass at Day 98. Vertical bars represent s.e.**

There was a three-way interaction ($P < 0.05$) between species, water treatment and defoliation frequency for relative root growth rate (Day 42-98). Weekly defoliation frequency, under all water treatments resulted in a lower ($P < 0.05$) relative root growth rate than Fortnightly and Three-Weekly defoliation frequencies, which did not differ ($P > 0.05$) (Figure 4.5). A proportionally greater ($P < 0.05$) difference between species was observed under the Very-Dry water treatment whereby, chicory had a higher ($P < 0.05$) relative root growth rate than plantain. Under Optimum water treatment, there was a proportionally greater ($P < 0.05$) difference between Weekly and Three-Weekly defoliation frequencies on root relative growth than under Dry or Very-Dry water treatments.

**Table 4.7 Experiment 1: Effect of plant species (Plantain and Chicory), water treatment (Optimum and Dry and Very-Dry) and defoliation frequency (Weekly and Fortnightly and Three-Weekly) at Day 98 on taproot diameter, root length, absolute root mass and relative root growth rate (Day 42-98). Data presented as means ± s.e. Means within columns and main effects having different letters are significantly different at *P* = 0.05.**

| | Taproot diameter (mm) | Root length (mm) | Absolute root mass (g) | Relative root growth rate (g/g/day) |
|---|---|---|---|---|
| ***Species*** | | | | |
| Plantain | 5.0a | 238.0b | 2.2a | 1.22a |
| Chicory | 11.6b | 152.4a | 5.3b | 1.47b |
| s.e. | 0.19 | 2.76 | 0.28 | 0.001 |
| ***Water*** | | | | |
| Optimum | 8.3 | 189.2 | 3.6 | 1.48c |
| dry | 8.4 | 199.6 | 3.6 | 1.26a |
| Very-Dry | 8.2 | 196.8 | 4.2 | 1.30b |
| s.e. | 0.22 | 3.40 | 0.31 | 0.001 |
| ***Defoliation*** | | | | |
| Weekly | 8.0a | 184.4a | 2.7a | 0.87a |
| Fortnightly | 8.3ab | 194.2b | 3.5b | 1.45b |
| Three-Weekly | 8.6b | 206.9c | 5.1c | 1.72c |
| s.e. | 0.22 | 3.38 | 0.31 | 0.001 |

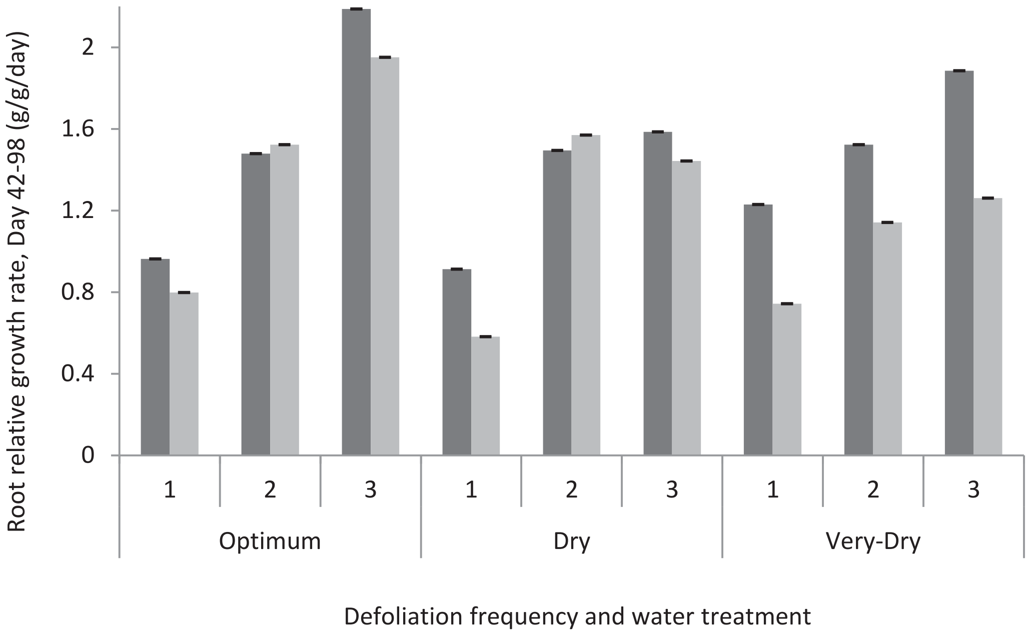



**Figure 4.5 Effect of species (Chicory (black) and Plantain (grey)), water treatment (Optimum and Dry and Very-Dry) and defoliation frequency (Weekly (1) and Fortnightly (2) and Three-Weekly (3)) on the relative root growth rate between Days 42-98. Vertical bars represent s.e.**

There was a three-way interaction ($P < 0.05$) between species, water treatment and defoliation frequency for root length. Plantain had a greater ($P < 0.05$) root length than chicory under all water treatments by defoliation frequency combinations (Figure 4.6).

There were no ($P > 0.05$) two-way or three-way interactions between species, water treatment and defoliation frequency for taproot diameter. Chicory had a greater ($P < 0.05$) taproot diameter than plantain (Table 4.7). Water treatment had no effect ($P > 0.05$) on taproot diameter or absolute root mass. Taproot diameter was greater ($P < 0.05$) under three weekly defoliation than under weekly defoliation, while fortnightly defoliation did not differ from either ($P > 0.05$).

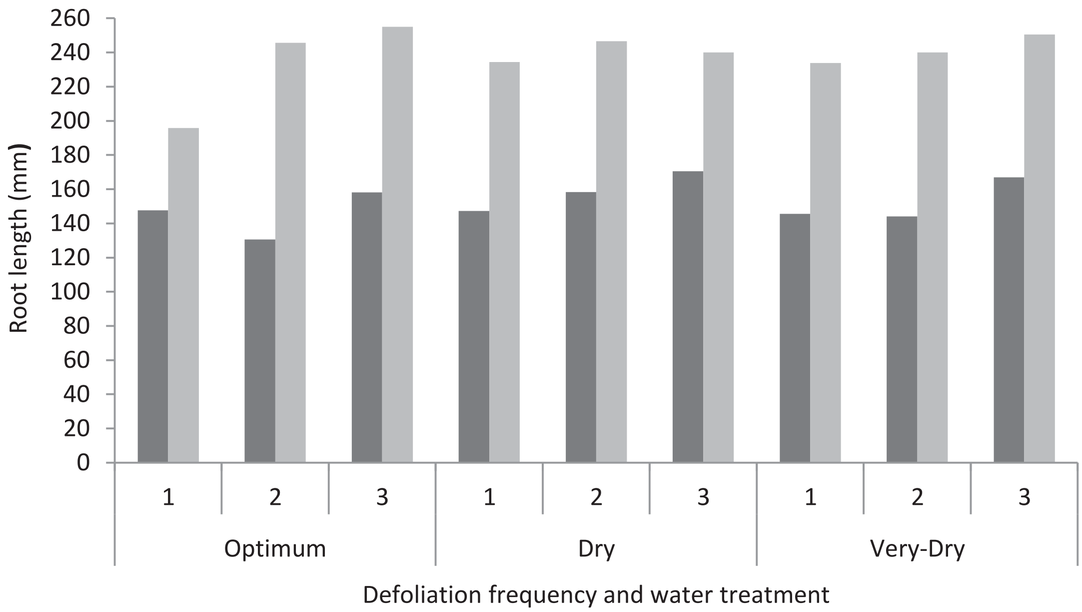



**Figure 4.6 Effect of species (Chicory (black) and Plantain (grey)), water treatment (Optimum and Dry and Very-Dry) and defoliation frequency (Weekly (1) and Fortnightly (2) and Three-Weekly (3)) on root length at Day 98. Vertical bars represent s.e.**

### 4.4.2 Experiment 2: Physiological responses of plantain and chicory to moisture stress

#### *4.4.2.1 Change in soil volumetric water content over time*

There was a three-way interaction ($P < 0.05$) between plant species, water treatment and day of measurement. On Day 1 the soil volumetric water content within each plant species did not differ ($P > 0.05$) under Optimum and Very-Dry water treatments; however plantain had a greater ($P < 0.05$) soil volumetric water content than chicory (Figure 4.7). On Day 3 the soil volumetric water content of Chicory-Optimum was lower ($P < 0.05$) than the Plantain-Optimum but greater ($P < 0.05$) than the Very-Dry water treatments of both species which did not differ ($P > 0.05$). On Days 4 and 5 the soil volumetric water content did not differ ($P > 0.05$) between the species and was lower ($P > 0.05$) for Very-Dry treatment plants than the Optimum treatment plants.

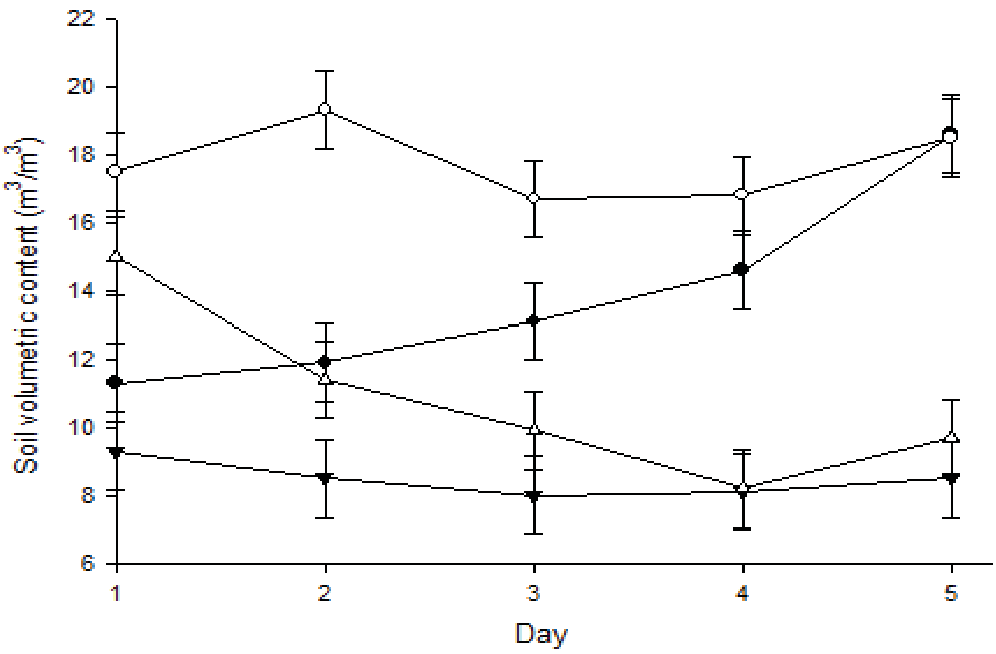



**Figure 4.7 Change in soil volumetric water content of the four species by water treatment combinations; Chicory-Optimum (●), Plantain-Optimum (○), Chicory-Very-Dry (▼) and Plantain-Very-Dry (△) over the five day treatment period. Vertical bars represent s.e.**

#### *4.4.2.2 Day 1 – Harvest 1*

##### 4.4.2.2.1 Above and below-ground herbage measures

Chicory had a greater ($P < 0.05$) absolute shoot mass, absolute root mass, taproot diameter and leaf mass than plantain (Table 4.8). Plantain had a greater ($P < 0.05$) number of live leaves per plant than chicory. There was no difference ($P > 0.05$) in the root length or shoot mass fraction between plantain and chicory.

**Table 4.8 Experiment 2: Effect of plant species (Plantain and Chicory) at Day 1 (Harvest 1) on absolute shoot mass, taproot diameter, root length, absolute root mass, shoot mass fraction, number of live leaves per plant and leaf mass. Data presented as means ± s.e. Means within columns having different letters are significantly different at *P* = 0.05.**

| | Absolute shoot mass (g) | Taproot diameter (mm) | Root length (mm) | Absolute root mass (g) | Shoot/ (shoot + root) (%) | Number of live leaves per plant | Leaf mass (g/leaf) |
|---|---|---|---|---|---|---|---|
| Plantain | 9.8a | 4.8a | 188.3 | 1.5a | 85.8 | 42.6b | 0.07a |
| Chicory | 28.5b | 13.5b | 142.7 | 4.8b | 87.1 | 20.9a | 0.34b |
| s.e. | 0.68 | 1.05 | 24.95 | 0.56 | 1.47 | 3.54 | 0.02 |

### *4.4.2.3 Day 6 – Harvest 2*

#### 4.4.2.3.1 Above-ground herbage measures

There was an interaction ($P < 0.05$) between species and water treatment on absolute shoot mass, whereby the water treatment had a proportionally greater difference for chicory than plantain. All species by water treatments differed from each other ($P < 0.05$) such that absolute shoot mass was 20.8 ± 1.8, 25.6 ± 1.8, 39.0 ± 1.8, 53.1 ± 1.8 g, for Plantain-Very-Dry, Plantain-Optimum, Chicory-Very-Dry, Chicory-Optimum, respectively. There were no other interactions ($P > 0.05$) for any other above-ground herbage measures (relative shoot growth rate, shoot mass fraction, number of live leaves per plant, leaf mass).

Chicory had a greater ($P < 0.05$) relative shoot growth rate (Days 1-6) and leaf mass than plantain (Table 4.9). Plantain had a greater ($P < 0.05$) number of live leaves per plant and shoot mass fraction than chicory.

Optimum plants had a greater ($P < 0.05$) relative shoot growth rate (Days 1-6) and number of live leaves per plant than Very-Dry plants (Table 4.9). Water treatment had no effect ($P > 0.05$) on shoot mass fraction or leaf mass.

**Table 4.9 Experiment 2: Effect of plant species (Plantain and Chicory) and water treatment (Optimum and Very-Dry) at Day 6 (end of treatment period; Harvest 2) on absolute shoot mass, relative shoot growth rate (between Days 1-6), shoot mass fraction, number of live leaves per plant and leaf mass. Data presented as means ± s.e. Means within columns and main effects are significantly different at *P* = 0.05.**

| | Absolute shoot mass (g) | Relative shoot growth rate (g/g/day) | Shoot/ (shoot + root) (%) | Number of live leaves per plant | Leaf mass (g/leaf) |
|---|---|---|---|---|---|
| ***Species*** | | | | | |
| Plantain | 23.2a | 2.66a | 85.5b | 44.5b | 0.13a |
| Chicory | 46.0b | 3.15b | 70.8a | 34.8a | 0.34b |
| s.e. | 1.27 | 0.058 | 1.30 | 3.00 | 0.02 |
| ***Water*** | | | | | |
| Optimum | 39.4b | 3.04b | 78.5 | 45.3b | 0.24 |
| Very-Dry | 29.9a | 2.77a | 77.7 | 33.9a | 0.24 |
| s.e. | 1.27 | 0.058 | 1.30 | 3.00 | 0.02 |

#### 4.4.2.3.2 Below-ground herbage measures

There were no interactions ($P > 0.05$) for any below-ground herbage measures (taproot diameter, root length, absolute root mass, relative root growth rate). Chicory had a greater ($P < 0.05$) taproot diameter, root mass and relative root growth rate (Days 1-6) than plantain (Table 4.10). Chicory had a shorter ($P < 0.05$) root length than plantain.

Optimum plants had a greater ($P < 0.05$) taproot diameter and tended ($P = 0.06$) to have a greater root length than Very-Dry plants (Table 4.10). Water treatment had no effect ($P > 0.05$) on absolute root mass or relative root growth rate (Days 1-6).

**Table 4.10 Experiment 2: Effect of plant species (Plantain and Chicory) and water treatment (Optimum and Very-Dry) at Day 6 (end of treatment period; Harvest 2) on taproot diameter, root length, absolute root mass, relative root growth rate (between Days 1-6). Data presented as means ± s.e. Means within columns and main effects having different letters are significantly at *P* = 0.05.**

| | Taproot diameter (mm) | Root length (mm) | Absolute root mass (g) | Relative root growth rate (g/g/day) |
|---|---|---|---|---|
| ***Species*** | | | | |
| Plantain | 5.7a | 217.3b | 3.9a | 1.25a |
| Chicory | 14.3b | 180.8a | 19.2b | 2.61b |
| s.e. | 0.57 | 8.55 | 1.08 | 0.098 |
| | | | | |
| ***Water*** | | | | |
| Optimum | 10.8b | 210.8 | 13.1 | 2.03 |
| Very-Dry | 9.1a | 187.3 | 10.0 | 1.83 |
| s.e. | 0.57 | 8.55 | 1.08 | 0.098 |

### *4.4.2.4 Physiological measurements*

There were three-way interactions ($P < 0.05$) between plant species, water treatment and day of measurement for photosynthetic rate, leaf temperature, evapotranspiration rate, leaf water potential and osmotic potential.

On Day 1, the photosynthetic rate (Pn) within each plant species did not differ ($P > 0.05$) under Optimum and Very-Dry water treatments, however plantain had a greater ($P < 0.05$) Pn than chicory (Figure 4.8A). Between Days 2 to 5 the Pn of chicory and plantain under Optimum water treatment did not differ ($P > 0.05$) and was greater ($P > 0.05$) than the Pn of plants under Very-Dry water treatment. On Days 2 and 4 Plantain-Very-Dry had a greater ($P < 0.05$) Pn than Chicory-Very-Dry. On Days 3 and 5 Plantain-Very-Dry had a similar ($P > 0.05$) Pn to Chicory-Very-Dry.

On Day 1, the leaf temperature within each plant species did not differ ($P > 0.05$) under Optimum and Very-Dry water treatments, however chicory had a greater ($P < 0.05$) leaf temperature than plantain (Figure 4.8B). On Day 2 the leaf temperature within each water treatment was similar ($P > 0.05$) between chicory and plantain and greater ($P < 0.05$) under Very-Dry water treatment compared to Optimum water treatment. Between Days 3 to 5 the species did not differ ($P > 0.05$) within each water treatment, with Very-Dry plants having a higher ($P < 0.05$) leaf temperature than Optimum plants.

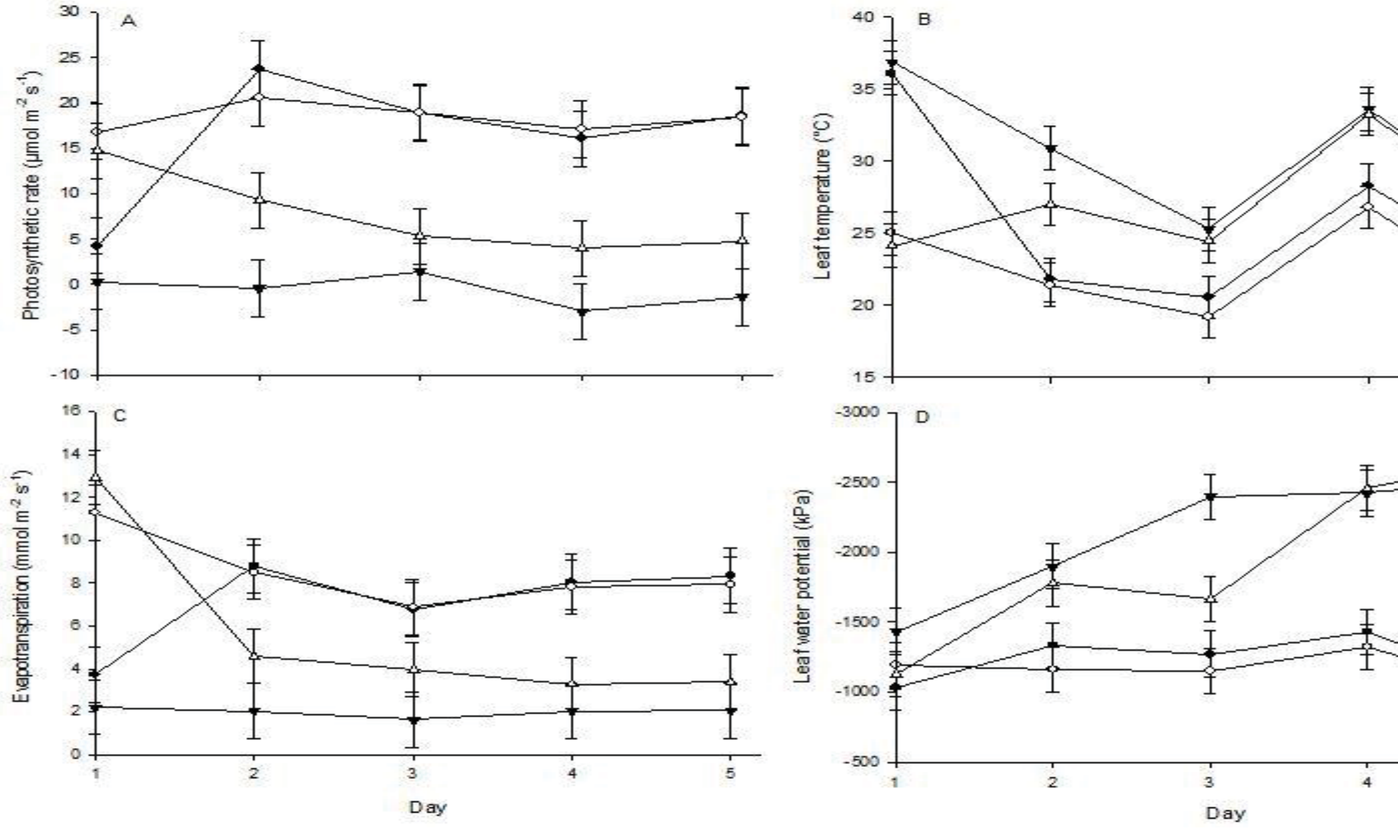



**Figure 4.8 Effect of species (chicory (black) and plantain (white)) and water treatment (Optimum (circle) and Very-Dry (trian temperature (B), evapotranspiration rate (C) and leaf water potential measured by pressure chamber (D) of plants during Vertical bars represent s.e.**

On Day 1, the evapotranspiration rate (E) within each plant species did not differ ($P > 0.05$) under Optimum and Very-Dry water treatments, however plantain had a greater ($P < 0.05$) E than chicory (Figure 4.8C). Between Days 2 to 5 the E did not differ ($P > 0.05$) between species and was greater ($P > 0.05$) for Optimum treatment plants than Very-Dry treatment plants.

On Day 1, there were no differences ($P > 0.05$) in the leaf water potential ($\Psi_w$ measured via pressure chamber) between species or water treatments (Figure 4.8D). Between Days 2 to 5 the $\Psi_w$ of Chicory-Optimum and Plantain-Optimum were similar ($P > 0.05$) and lower ($P > 0.05$) than the $\Psi_w$ of Very-Dry treatment plants. The $\Psi_w$ of Chicory-Very-Dry was greater ($P > 0.05$) than Plantain-Very-Dry on Day 3 and similar ($P > 0.05$) on Days 2, 4 and 5.

On Days 1 and 2, there were no differences ($P > 0.05$) in the leaf water potential ($\Psi_w$; measured via vapour pressure osmometry) between species or water treatments (Figure 4.9A). Between Days 2 to 5 the $\Psi_w$ of Chicory-Optimum and Plantain-Optimum were similar ($P > 0.05$) and lower ($P < 0.05$) than the $\Psi_w$ of Very-Dry treatment plants. On Days 3 and 5 there were no differences ($P > 0.05$) in the $\Psi_w$ of chicory and plantain under the Very-Dry moisture treatment. On Day 4 Chicory-Very-Dry had a greater ($P < 0.05$) negative $\Psi_w$ than Plantain-Very-Dry.

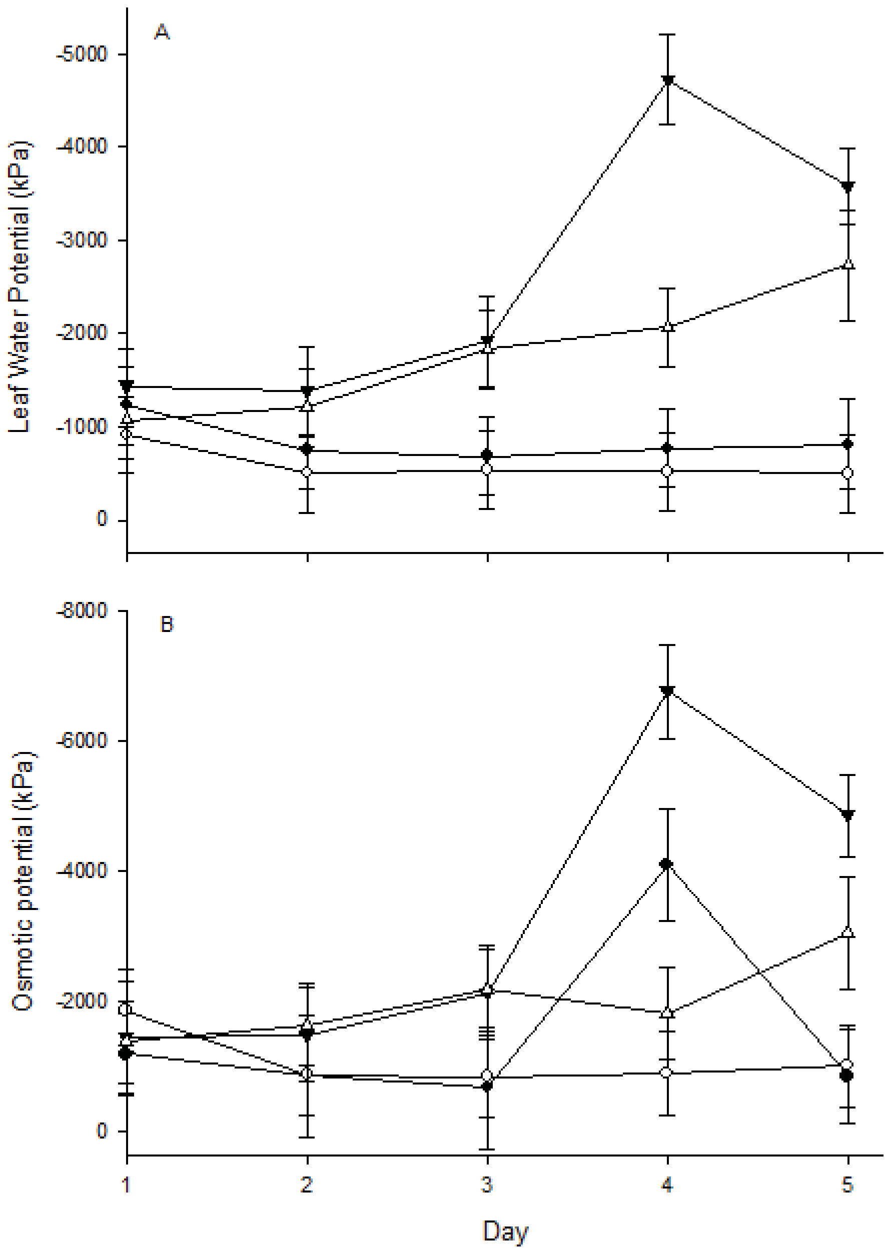



**Figure 4.9 Effect of the species (Chicory (black) and Plantain (white)) and water treatment (Optimum (circle) and Very-Dry (triangle)) on leaf water potential (A) and osmotic potential (B) of plants (measured via vapour pressure osmometry) during the water treatment period (Days 1-5). Vertical bars represent s.e.**

On days 1, 2 and 3, there were no differences ($P > 0.05$) in the osmotic potential of plants ($\Psi_o$; measured via vapour pressure osmometry) between species or water treatments (Figure 4.9B). On Day 4, the $\Psi_o$ of Plantain-Optimum and Plantain-Very-Dry were similar ($P > 0.05$) and lower ($P < 0.05$) than the $\Psi_o$ of Chicory under both water treatments. The $\Psi_o$ of Chicory-Very-Dry was greater ($P < 0.05$) than the $\Psi_o$ of Chicory-Optimum. On Day 5, the $\Psi_o$ of Chicory-Optimum and Plantain-Optimum were similar ($P > 0.05$) and lower ($P < 0.05$) than the $\Psi_o$ of Chicory-Very-Dry. The $\Psi_o$ of Plantain-Very-Dry was greater ($P < 0.05$) than that of Chicory-Optimum and similar ($P > 0.05$) to that of Plantain-Optimum and Chicory-Very-Dry.

There tended ($P = 0.07$) to be a three-way interaction between plant species and water treatment for internal $CO_2$ concentration (Ci). Chicory-Very-Dry tended ($P = 0.07$) to have a lower Ci concentration than Plantain-Optimum, with Chicory-Optimum and Plantain-Very-Dry not differing from any treatment combination, such that Ci concentration was 57 ± 705, 4062 ± 705, 1908 ± 705 and 1459 ± 705 ppm, respectively.

There were no two-way or three-way interactions ($P < 0.05$) between plant species, water treatment and day for stomatal conductance (Gs). Plantain tended ($P = 0.09$) to have greater Gs than chicory, such that Gs was 2760 ± 499 and 982 ± 499 m mol $m^{-2}$ $s^{-1}$, respectively. Very-Dry plants tended ($P = 0.05$) to have lower Gs than Optimum plants, such that Gs was 758 ± 499 and 2985 ± 499 m mol $m^{-2}$ $s^{-1}$, respectively.

### 4.4.3 Experiment 3: Comparison of plantain and chicory rooting depth under field conditions

Both plantain and chicory had a greater ($P < 0.05$) root mass in the 0-10 cm soil depth profile than in the 10-20 cm soil depth profile (Table 4.11). Chicory and plantain had a similar ($P > 0.05$) root mass in the 0-10 cm soil depth profile. Chicory had an 18% greater ($P < 0.05$) root mass in the 10-20 cm soil depth profile than plantain.

**Table 4.11 Experiment 3: Effect of species (chicory and plantain) and soil depth profile (0-10 cm and 10-20 cm) on root mass. Data presented as means ± s.e. Means within columns having different letters are significantly different at $P$ = 0.05. Means within rows having different symbols (†, ¥) are significantly different at $P$ = 0.05.**

| | root depth | Plantain | Chicory |
|---|---|---|---|
| Average dry mass of root in depth layers (g) | 0-10 cm | 0.91b ± 0.14 | 1.20b ± 0.17 |
| | 10-20 cm | 0.14a† ± 0.06 | 0.20a¥ ± 0.03 |

## 4.5 Discussion

The aim of these experiments was to provide fundamental insight into how the morphology and physiology of plantain and chicory differ under conditions of moisture stress and defoliation. Consequently, this discussion will first discuss the morphological responses of chicory and plantain and then the physiological responses of the two species with an attempt made to link these responses where appropriate.

### 4.5.1 Morphological differences between chicory and plantain

In both experiments one and two, chicory consistently had a greater root mass and taproot diameter than plantain. This was also observed in Chapter 3. Furthermore, in experiment one, under very-dry conditions the difference in the relative root growth rate of chicory compared to plantain was generally greater than under less severe moisture treatments. This suggests that chicory may have an advantage over plantain under very-dry conditions and may be more persistent in environments which experience severe droughts. Plant survival through the drought period has been shown to be related to root density and depth and to differ between species (Volaire and Thomas 1995; Bahrani *et al.* 2010; Wedderburn *et al.* 2010). Furthermore, a deep root system with a high density of roots at depth is a major trait to sustain higher yield in water-limited environments (Carrow 1996; Wasson *et al.* 2012; White and Snow 2012).

The root of chicory consists of a solid deep taproot (Kemp *et al.* 2002b). Conversely, while plantain is technically taprooted its roots make up a more fibrous system due to a greater number of adventitious roots compared to chicory (Sanderson *et al.* 2003a). In experiment three, under field conditions chicory had a greater mass of roots in the deeper soil horizons (10-20 cm) than plantain. However, under glasshouse conditions the roots of chicory were limited by pot depth and therefore possibly do not give an advantage for water extraction compared to plantain. Under field conditions, where root length is not limited Brown *et al.* (2005) found chicory was able to extract water to a maximum depth of 1.9 m. Nie *et al.* (2008) found plantain had a shorter rooting depth and a lower root density in the subsoil (10-110 cm) and deeper subsoil (110-200 cm) than kikuyu, phalaris and tall fescue, and was similar to perennial ryegrass. Consequently, in shallow soils plantain may be more productive than chicory during drought conditions. Regardless of this, under simulated drought/dry conditions it was expected that the roots of chicory would support a greater herbage yield than that of plantain, as previously reported (Nie *et al.* 2008).

In experiment one, plantain consistently had a greater relative shoot growth rate than chicory. However, this was not found to be the case in experiment two; which could be explained by the greater initial absolute shoot mass of chicory compared to plantain. Chapter 3 also showed plantain had a greater relative shoot growth rate than chicory. Van Andel and Biere (1990) suggested that relative shoot growth rate and maximum relative shoot growth rate are useful indicators of plant productivity. Previous research under field conditions has shown that plantain has a greater herbage yield than chicory under optimum growth conditions (Powell *et al.* 2007). However, when subjected to water stress, slower growth has been suggested as an adaptive feature for plant survival, because it allows plants to divert assimilates and energy, otherwise used for shoot growth, to maintain root growth, improving water acquisition (Chaves *et al.* 2003). Therefore, it could be expected that the slower shoot growth rate of chicory compared to plantain would be more pronounced under the Very-Dry water treatment, but this was not found to the case. This suggests that chicory simply has a slower shoot growth rate than plantain and it is not drastically altering its herbage growth strategy in response to moisture stress.

In experiments one and two, plantain consistently had a greater shoot mass fraction than chicory. This was also found in Chapter 3. It is well established that chicory allocates a greater energy reserve to root mass compared to plantain which focusses on shoot production (Davis 1995; Alemseged 2000; Sanderson and Elwinger 2000; Powell *et al.* 2007). Alemseged (2000) reported the shoot to root ratio in chicory steadily declined over time with growth as it allocated more to root than shoot, probably in response to localised nutrient depletion. Consequently, Alemseged (2000) concluded that this high degree of plasticity may enable chicory to sustain a high rate of resource capture by placing its root in the resource-rich zone of the soil profile. Presumably, chicory's lower shoot mass fraction would help it to survive and persist under severe moisture stress. Conversely, in plantain it was postulated that under severe moisture stress the greater partitioning of energy to above-ground growth would not be maintained, or alternatively that plant persistence may be negatively affected. Nonetheless, this was not the case as shown by both species fully recovering following the period of moisture stress, in experiment one. This was also found in Chapter 3 and illustrates that both plantain and chicory display resilience in terms of recovering from soil moisture stress. Overall, these results suggest that chicory and plantain have different growth strategies, with both species managing to maintain these growth strategies under simulated moisture stress conditions. Previous research has also shown chicory and plantain can survive periods of moisture stress (Mook *et al.* 1989; Brown *et al.* 2003; Nie *et al.* 2008).

### 4.5.2 Effect of defoliation frequency and water treatment

Carbohydrate reserves in perennial forage plants are an important source of energy necessary for winter survival and initiation of growth in spring (Kust and Smith 1961). Furthermore they can provide the necessary energy to support shoot growth following defoliation (Fankhauser *et al.* 1989). Root diameter can be used as an indicator of root carbohydrate reserves. At Day 42 of experiment one (Harvest 2), defoliation frequency had no effect on the taproot diameter of plantain, however the taproot diameter of chicory was greater under three weekly defoliation than under more frequent defoliation. By Day 98 of experiment one (Harvest 3), defoliation frequency had the same effect on both chicory and plantain with three weekly defoliation resulting in a greater taproot diameter than weekly defoliation. These results suggest that under three weekly defoliation chicory and plantain have greater root reserves and would be more likely to persist in the long term than under weekly defoliation.

Repeated severe defoliation kills plants by depleting root carbohydrate reserves in a short period because a perennial herbaceous plant, when totally defoliated, must draw on carbohydrate reserves to regenerate photosynthetic tissue (Wolf 1978). In Chapter 3, the taproot diameter of chicory was reduced under defoliation, while that of plantain was not affected. Other studies have examined the effect of defoliation height on taproot diameter. Yu *et al.* (2008) reported the taproot diameter of plantain was unaffected by defoliation height, whereas that of chicory was reduced under severe defoliation (lower residual), thus showing plantain is less sensitive to defoliation height than chicory. Furthermore, in this study, chicory showed a response to defoliation frequency at both the mid and end point of the experiment while plantain only showed a response at the end point, suggesting plantain, in the short term at least, might be more resilient to defoliation frequency than chicory.

Experiment one showed the relative shoot and root growth rates of both chicory and plantain were reduced under more frequent defoliation. This has previously been found in chicory under field conditions (Li *et al.* 1997b; Belesky *et al.* 1999; Alemseged *et al.* 2003; Sanderson *et al.* 2003a). In plantain a five week defoliation interval has been shown to produce greater shoot dry matter than a three week defoliation interval (Sanderson *et al.* 2003a; Labreveux *et al.* 2004). While Ayala *et al.* (2011b) found a three week defoliation interval produced more dry matter than a six week defoliation interval. Yu *et al.* (2008) found the shoot dry matter of chicory and plantain increased with decreasing mowing frequency, between one and three weeks. The net herbage growth rates of chicory and plantain are further investigated under a range of defoliation frequencies under field conditions in Chapter 7. In experiment one, three-weekly defoliation consistently results in a greater herbage yield as well as root mass compared to more frequent defoliation. These results suggest for both chicory and plantain three-weekly defoliation is likely to produce a greater herbage yield and a more persistent sward than a more frequent defoliation regime.

In experiment one, both chicory and plantain, under Fortnightly and Three-Weekly defoliation plants subjected to the Very-Dry water treatment produced a greater shoot mass during the recovery period (compensatory growth) than Dry and Optimum water treatments plants. While, under Weekly defoliation, Dry plants grew greater shoot mass during the recovery period (compensatory growth) than Very-Dry and Optimum water treatments plants. It is likely that under severe drought and weekly defoliation, root reserves are diminished and when the moisture stress is removed the plant does not have sufficient root reserves to display compensatory growth. While, under mild drought (dry) defoliation the plant maintains sufficient root reserves to display compensatory growth. Similarly, under less frequent defoliation the plant should be able to display compensatory growth following severe drought. Thus both plantain and chicory have the potential to produce high nutritive value dry matter, in the short term at least, following a period of severe drought when the availability of forage is limited, provided that the grazing interval has been a minimum of two weeks. Compensatory growth has also been reported in other pasture species (Horst and Nelson 1979; Volaire *et al.* 1998).

### 4.5.3 Physiological differences between chicory and plantain

Plantain and chicory generally displayed similar rates of photosynthesis and evapotranspiration per unit of leaf area under Optimum and Very-Dry conditions, with photosynthesis and evapotranspiration rates of both species being lower under Very-Dry conditions. However, experiments one and two showed plantain consistently had a greater number of live leaves per plant and a lighter mass per leaf than chicory. This difference demonstrates that chicory and plantain have different growth strategies with plantain investing its energy in many smaller leaves while chicory supports fewer larger leaves. Thus, it could be speculated that plantain may have had a greater leaf area than chicory and therefore had a higher total rate of photosynthesis per plant. A greater leaf area could explain its ability to continue to sustain its higher absolute shoot mass and relative shoot growth rate under both optimal and water stress conditions, when compared to chicory. Therefore, plantains ability to yield more than chicory under water stress conditions in the glasshouse could be as a result of morphological factors such as total leaf area rather than physiological differences between the two species. However, further research is required to confirm this. Furthermore, plantain tended to have a greater stomatal conductance than chicory under all conditions. While stomatal conductance tended to be reduced under Very-Dry conditions for both species compared to Optimum conditions. Plantain's greater stomatal conductance would allow it to sustain greater photosynthetic rates when compared to chicory under both optimal and water stress conditions. However, stomatal closure has been found to reduce water use per unit foliage cover by 20-30% (Johns 1978), and therefore chicory's drought strategy may be more sustainable in the long term than that of plantain. Moreover, in chicory, water stress that impacts on photosynthesis, may be more closely linked to non-stomatal effects, as found by Monti *et al.* (2005) in witloof chicory.

In experiment two, chicory had a greater leaf water potential than plantain under Very-Dry conditions on days three and four. In comparison to white clover, Alemseged (2000) found the leaf water potential of chicory decreased during summer as soil moisture declined, to a lesser degree, with a low of -1.0 MPa compared to -4.6 MPa. Osmotic adjustment can be defined as the lowering of osmotic potential due to net solute accumulation in response to water stress. It is considered to be a beneficial drought tolerance mechanism in some crop species as it enables the plant to maintain the physiological plant processes (Turner 1986; Thomas 1987) and delay plant dehydration (Kramer and Boyer 1995). In experiment two, osmotic adjustment was observed on Days 4 and 5 in chicory, while in plantain it was only observed on Day 5. This suggests that plantain and chicory have different growth strategies in response to moisture stress with chicory responding quicker to the moisture stress, while plantain maintained its high leaf osmotic potential for longer. The maintenance of a high leaf osmotic potential has been proposed as vital for short-term plant productivity (Passioura 1982). Therefore, plantain appears to display resistance to moderate drought, by attempting to maintain yield stability. Conversely, chicory appears to display resistance to severe drought, by partial avoidance of the stress period through summer quiescence, resulting in lower herbage yields during water stress periods (Knight 1973). In support of this, Vandoorne *et al.* (2012) concluded that witloof chicory displays a good level of water stress resistance. Drought survival (via dormancy) for perennial forage species is a valuable plant adaptation during part of the plant cycle which may enhance long term persistence and productivity under increasing drought (Lelièvre *et al.* 2011). A further adaptive advantage of chicory in the field is its deeper roots, which allow it to access sub-soil moisture and therefore potentially adjust better than plantain to water deficits in the soil surface, reducing the rate of decline in relative water content with leaf water potentials.

## 4.6 Conclusions

In conclusion, experiment one indicates that plantain performs better than chicory under simulated drought conditions in the glasshouse. Neither chicory nor plantain respond well to weekly defoliation (displaying poor shoot and root production) and performed better under three-weekly defoliation. Experiment two showed that relatively small differences in leaf water potential and osmotic potential exist between chicory and plantain, helping to explain why plantain outperforms chicory under drought conditions in the glasshouse. Experiment three shows in the field chicory has roots deeper in the soil profile than plantain, which likely explains anecdotal evidence suggesting chicory outperforms plantain under drought conditions. However, species with different root systems may be complementary by exploiting different layers of soil. Combined, these experiments indicate that both chicory and plantain are relatively tolerant of moisture stress and thus when sown in a Herb and Legume Mix, the sward is likely to perform well and persist under both a moderate and severe drought. Further experiments need to assess the performance and sustainability of the Herb and Legume Mix under grazing in field conditions (Chapter 5).

# Chapter 5 The effects of post-grazing height on sward production, plant density, taproot diameter and botanical composition of a Herb and Legume Mix

## 5.1 Abstract

Interest amongst sheep and beef farmers in Herb and Legume Mixes is increasing due to their potential as a high quality feed in drier periods of the year. There is, however, little information available on the optimal grazing management of a herb and legume sward mix. The objective of this experiment was to determine the response of a Herb and Legume Mix containing; chicory *(Cichorium intybus)*, plantain *(Plantago lanceolata)*, red clover *(Trifolium pratense)* and white clover *(T. repens)* to either Hard (post-grazing residual of 4cm) or Lax (post-grazing residual of 8cm) grazing treatments with sheep. The Herb and Legume Mix was monitored over two years (August 2011 to October 2013) across the seasons (spring, summer and autumn). The dry matter removed, botanical composition, nutritive value, plant and shoot density, number of crowns per plant, taproot diameter and water soluble carbohydrate content of roots were monitored. The dry matter removed within each season did not differ ($P > 0.05$) between the Hard and Lax grazing treatments, however the dry matter removed annually was greater ($P < 0.05$) from the Hard than the Lax grazing treatment (11.6 *vs.* 9.0 t DM/ha/year). In year one, the botanical composition of the sward was relatively stable across seasons and grazing treatments. In year two, the Hard grazing treatment contained a greater ($P < 0.05$) proportion of plantain and a lower ($P < 0.05$) proportion of red clover than the Lax grazing treatment in all seasons. All species had a lower ($P < 0.05$) plant density on 3 October 2013 (end of the experimental period) than on 25 August 2011 (start of the experimental period). The plant density of both chicory and plantain was greater ($P < 0.05$) in the Hard grazing treatment than in the Lax grazing treatment, while that of red clover and white clover did not differ ($P > 0.05$) under the grazing

treatments. The taproot diameter of chicory was greater ($P < 0.05$) at 3 October 2013 than at 25 August 2011, whereas that of plantain did not differ ($P > 0.05$) over time. The taproot diameter of chicory was greater ($P < 0.05$) under the Lax grazing treatment than the Hard grazing treatment, while that of plantain did not differ ($P > 0.05$) under the grazing treatments. The results suggest that provided the grazing interval is maintained at 3-4 weeks the herb legume sward mix is relatively resilient to a hard grazing residual of 4cm. However, hard grazing is likely to favour plantain as suggested by the changes in botanical composition and taproot diameter.

## 5.2 Introduction

New Zealand pastoral systems have historically been based on perennial ryegrass (*Lolium* perenne) with a relatively minor component of white clover (*Trifolium repens*) (Kemp *et al.* 2002b). Perennial ryegrass and white clover pastures are often perceived as permanent with replacement needed every 10 years, however, agronomic research suggests their productivity declines after three to five years (Sanderson and Webster 2009). Furthermore it is difficult to maintain a white clover percentage above 10% over the lifetime of a pasture (Brock and Hay 2001). An additional issue with ryegrass and white clover pasture is its reduction in herbage quality and quantity throughout summer and autumn resulting in reduced feed intakes and poor animal performance (Hughes *et al.* 1980; Burke *et al.* 2002; Moorhead *et al.* 2002).

Herb and Legume Mixes containing; chicory, plantain, red clover and white clover have been shown to increase ewe milk yield, support higher lamb weaning weight and greater post weaning lamb liveweight gain (Kenyon *et al.* 2010; Golding *et al.* 2011; Hutton *et al.* 2011; Sinhadipathige *et al.* 2012). Furthermore, Herb and Legume Mixes are more productive and have a longer growing season than mono-species herb swards (Kemp *et al.* 2010), although production in winter is limited. These herb mixes have the potential to offer improved persistence when compared to pure swards of chicory and plantain. All studies to date, relating to these Herb and Legume Mixes have been relatively short term in nature so little is known about the longer-term potential of a Herb and Legume Mix. If these Herb and Legume Mixes are to replace perennial ryegrass and white clover pasture in some farm scenarios they need to be persistent for a prolonged period.

Best practice grazing management has been established for pure swards of chicory, plantain and red clover and for white clover in a perennial ryegrass and white clover mix (Hyslop 1999; Sanderson *et al.* 2003a; Labreveux *et al.* 2004; Li and Kemp 2005; Lee *et al.* 2008; Ayala *et al.* 2011b). Chicory and plantain perform best under a 3-5 week rotational grazing period (Hare *et al.* 1987; Clark *et al.* 1990a; Sanderson *et al.* 2003a; Labreveux *et al.* 2004), not grazing below 5 cm regularly (Li *et al.* 1994b; Li *et al.* 1995; Li *et al.* 1997c), and avoiding grazing during late autumn and winter (Kemp 1996; Li *et al.* 1997b; Ayala *et al.* 2011b). Red clover performs best under 4-6 week rotational grazing from a pre-grazing height of 25-30 cm to a minimum post-grazing height of 8-10 cm (Barry *et al.* 1999; Hyslop 1999; Kemp *et al.* 2002b). Thus, the species have slightly different optimum grazing management practises and therefore this could cause problems in a mixed sward.

However the ability of plants to recover from grazing is not only determined by morphological and physiological characteristics, but also by competitive pressures from companion species (Briske 1996). Currently, little is known about the optimum grazing management of a Herb and Legume Mix. The purpose of this experiment is to investigate the effect of grazing intensity; hard and lax, on the plant density and root characteristics, herbage yield and botanical composition of a Herb and Legume Mix over two growing years.

## 5.3 Materials and methods

### 5.3.1 Site preparation and sowing

The experiment was undertaken between March 2011 and October 2013 on the Massey University Pasture and Crop Research Unit, 5km south of Palmerston North, on a Manawatu fine sandy loam (Dystric Fluventric Eutrochrept, Hewitt 1998). The site was prepared by spraying with Round-up Renew (360 g/l a.i of glyphosate, Monsanto) followed by mouldboard ploughing and secondary cultivation. Analysis of the soil before the experiment (15 cm sampling depth) revealed mineral concentrations of 36 mg P $kg^{-1}$, 76.8 mg N $kg^{-1}$, 86 mg K $kg^{-1}$, 27.6 mg S $kg^{-1}$, 1380 mg Ca $kg^{-1}$, 146.4 mg Mg $kg^{-1}$. The site was sown on 14 March 2011 with a sward mix of plantain (*Plantago lanceolata* cv. Ceres Tonic; 6 kg/ha), chicory (*Cichorium intybus* cv. Puna II; 6 kg/ha), red clover (*Trifolium pratense* cv. Sensation; 6kg/ha), and white clover (*T. repens* cv. Tribute; 4kg/ha) with a V-ring roller drill, in a total area of 0.24 hectares. Cropmaster 15, (Ravensdown; 15.2% N, 10% P, 10% K and 7.7% S) was applied at 250 kg/ha at sowing.

### 5.3.2 Experiment design

Two grazing treatments; Hard grazing treatment (post-grazing residual of approximately 4 cm) and Lax grazing treatment (post-grazing residual of approximately 8 cm), were arranged in a randomised complete block design, with four blocks (replicates) each with two plots giving a total of eight plots (300 $m^2$ each) (Figure 5.1). The plots were grazed with ewe lambs on a 3 to 5 week grazing cycle (Table 5.1), when pre-grazing herbage mass and sward height reached a minimum of approximately 11 cm, respectively, and grazed until the desired visual post-grazing residual was reached. Sward height was measured by taking 50 measurements in each plot using a sward stick (Jenquip) (Rhodes 1981; Hutchings 1991). The grazing periods were each defined as occurring within a season; spring (September to November), summer (December to February) and autumn (March to May), and the data from all grazing periods within each season were combined for analysis (Table 5.1).

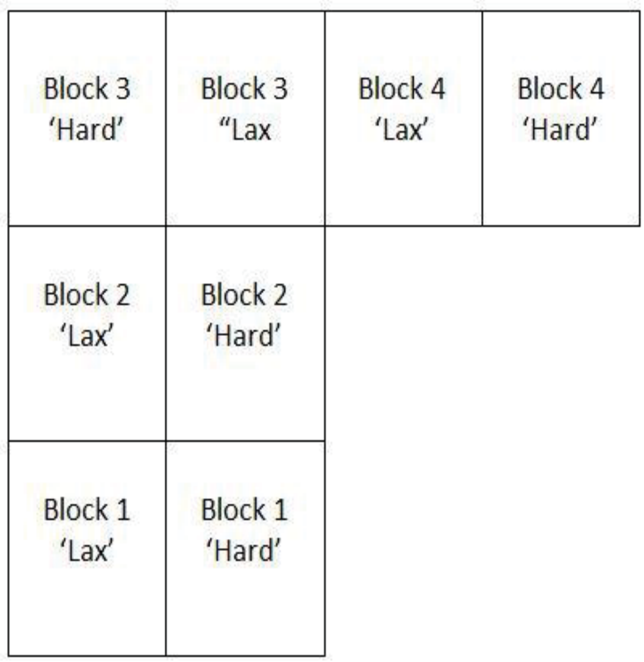



**Figure 5.1 Experimental layout displaying eight plots replicated in four blocks.**

**Table 5.1 Grazing periods within the seasons (spring, summer and autumn) over the growing years.**

| Growing years | | |
|---|---|---|
| **Year One: 2011-2012** | **Year Two: 2012-2013** | **Year Three: 2013-2014** |
| | *spring* | |
| 5 to 10 September | 3 to 9 September | 4 to 10 September |
| 26 to 29 September | 3 to 9 October | |
| 17 to 22 October | 2 to 7 November | |
| 7 to 12 November | | |
| | *summer* | |
| 28 November to 4 December | 3 to 7 December | |
| 18 to 23 December | 2 to 8 January | |
| 16 to 20 January | 6 to 10 February | |
| 5 to 8 February | | |
| | *autumn* | |
| 5 to 9 March | 22 to 27 March | |
| 16 to 22 April | 3 to 8 May | |

The same group of ewe lambs was used to graze the plots over the 2011-2012 growing season (Year One; 5 September 2011 – 22 April 2012), and these were replaced with a new group of ewe lambs during the 2012-2013 growing season (Year Two; 3 September 2012 – 9 May 2013). Finally, a new group of ewe lambs was used to graze the plots during the spring of the third growing season (final grazing period: Year Three; 4 September – 10 September 2013). The plots were never grazed during winter, as per best practise grazing management recommendations (Kemp 1996; Li *et al.* 1997b; Ayala *et al.* 2011b). The stocking rate ranged between 6-7 ewe lambs per plot, equivalent to 200-233 ewe lambs per hectare and was adjusted between grazing periods and plots depending on time of year and predicted herbage growth to ensure the post-grazing residual target was met. The residency time at each grazing event ranged between 3-8 days within each plot, and this was dictated by the time required to reach the desired post-grazing sward height. In the interims between each treatment grazing period, the ewe lambs were grazed on a perennial ryegrass, white clover pasture.

### 5.3.3 Climate and fertiliser

Climatic data, including 10 cm soil temperature (°C), 24 hour maximum and minimum air temperatures (°C), and rainfall (mm) were recorded at 0900 daily at a weather station <500 m from the experimental site; monthly summaries and 10-year averages are presented in Table 5.2. The experimental site was irrigated on 19 December 2012 and 28 February 2013 when herbage growth was severely limited as a result of lower than average monthly rainfall. Fertiliser (Urea, 46% nitrogen; Ravensdown) was applied to all plots on 17 August 2012 at a rate of 50 kg N/ha.

### 5.3.4 Pre- and post-grazing herbage mass

Herbage mass was measured both pre-grazing and post-grazing by taking three 0.1m$^2$ quadrat cuts (Frame 1993) per plot during Year One (3 × 4 plots = 12 samples per treatment) and four quadrat cuts per plot during Year Two (4 × 4 plots =16 samples per treatment). These samples were cut at ground level with an electric shearing hand-piece and then washed before oven drying for at least 24 hours at 70°C, to a constant weight.

**Table 5.2 Mean monthly 10cm soil temperature, maximum and minimum air temperatures (°C), total monthly rainfall an September 2011 and August 2013 compared with the 10-year mean.**

| | Sept | Oct | Nov | Dec | Jan | Feb | Mar | Apr |
|---|---|---|---|---|---|---|---|---|
| **Year One (Sept 2011 to Aug 2012)** | | | | | | | | |
| 10 cm soil temperature (°C) | 9.4 | 12.9 | 14.5 | 18.3 | 17.9 | 17.2 | 15.2 | 13.7 |
| Max. daily air temperature (°C) | 14.6 | 17.3 | 17.9 | 21.7 | 21.2 | 21.1 | 19.7 | 19.5 |
| Min. daily air temperature (°C) | 4.8 | 8.7 | 10.2 | 11.9 | 12.7 | 13.3 | 11.9 | 9.4 |
| rainfall (mm) | 37 | 145 | 134 | 87 | 52 | 55 | 60 | 84 |
| **Year Two (Sept 2012 to Aug 2013)** | | | | | | | | |
| 10 cm soil temperature (°C) | 9.9 | 12.0 | 14.5 | 18.4 | 18.3 | 19.3 | 17.7 | 14.3 |
| Max. daily air temperature (°C) | 15.2 | 16.1 | 17.6 | 22.3 | 23.3 | 24.1 | 23.6 | 19.2 |
| Min. daily air temperature (°C) | 7.0 | 8.5 | 9.5 | 12.1 | 11.7 | 12.2 | 11.1 | 10.8 |
| rainfall (mm) | 87 | 108 | 100 | 90 (38)* | 29 | 34 (63)* | 37.2 | 128 |
| **Mean – 10 year** | | | | | | | | |
| 10 cm soil temperature (°C) | 10.0 | 12.5 | 14.7 | 17.1 | 18.6 | 18.6 | 16.6 | 13.5 |
| Max. daily air temperature (°C) | 15.4 | 16.9 | 17.9 | 20.8 | 22.5 | 23.3 | 21.7 | 18.8 |
| Min. daily air temperature (°C) | 7.0 | 8.5 | 9.5 | 12.1 | 12.7 | 13.3 | 11.9 | 9.4 |
| rainfall (mm) | 87 | 108 | 100 | 90 | 52 | 55 | 60 | 84 |

*Irrigation applied (mm)

### 5.3.5 Apparent dry matter removal and total dry matter removal per year

Apparent DM removal in any given grazing event was calculated as the pre-grazing mass minus the post-grazing mass within the same grazing event. Total apparent DM removal per year was calculated as the sum of all apparent DM removal for each year (i.e. sum of all DM removed from all grazing events for each year).

### 5.3.6 Net herbage accumulation

Net herbage accumulation was calculated as the pre-grazing mass minus the post-grazing mass from the previous grazing, divided by the number of days between the end of the previous grazing and the start of the current grazing period.

### 5.3.7 Total number of grazing days

The total number of grazing days per year for each plot was calculated as the sum of the number of sheep grazing per day multiplied by the number of days grazed.

### 5.3.8 Botanical composition and leaf percentage

Herbage samples for botanical composition were randomly collected within plots at approximately two month intervals. Two 0.1m$^2$ randomly selected quadrats within each plot (eight samples per treatment) were cut to ground level. The samples were then separated into species (plantain, chicory, red clover, white clover, other (all other species including grasses and weeds)) and each species oven dried at 70°C to a constant weight. During Year Two, plantain and chicory was further separated into leaf and stem (including all reproductive material). The material was weighed and botanical composition percentages were calculated on a dry weight basis.

### 5.3.9 Nutritive value

One grab sample (Frame 1993) of approximately 100g wet weight was taken pre-grazing in each plot by walking in a random pattern, within that plot (four samples per treatment). However, samples from spring of Year Two were not available for analysis. All samples were freeze dried and then ground to fine granule size before analysis. Freeze dried, ground samples were analysed for digestibility (and hence predicted metabolisable energy (ME); (Roughan and Holland 1977)) and crude protein (CP) (total combustion method), neutral detergent fibre (NDF), acid detergent fibre (ADF) (Robertson and Van Soest 1981), organic matter (OM) and ash.

### 5.3.10 Plant density and sward coverage

Plant density was assessed within each plot at approximately two month intervals (Table 5.3). During Year One two spade plugs (18 × 18 cm) were dug up per plot (2 plugs × 4 plots = 8 samples per treatment), and this was increased to four spade plugs during Year Two and Year Three (4 plugs × 4 plots = 16 samples per treatment). The number of each plant species and the number of crowns on each plantain and chicory plant per sample was recorded. The taproot diameter (defined as the widest part of the root) of plantain and chicory was also measured at these times (Table 5.3; Figure 5.2).

**Table 5.3 Plant density and taproot diameter sample collection dates.**

| Growing years | | |
|---|---|---|
| **Year One: 2011-2012** | **Year Two: 2012-2013** | **Year Three: 2013-2014** |
| 25 August | 19 August | 2 September |
| 13 December | 26 November | 3 October |
| 21 February | 17 January | |
| 28 May | 27 March | |
| | 3 May | |

Shoot density was measured for chicory and plantain during Year Two and Year Three on 3 September and 27 November 2012, 18 January, 22 March, 3 May, 2 September and 3 October 2013. Four randomly selected 0.1m$^2$ quadrat samples were taken per plot (4 samples × 4 plots = 16 samples per treatment), by counting the total number of chicory and plantain shoots (clusters) within each sample area.

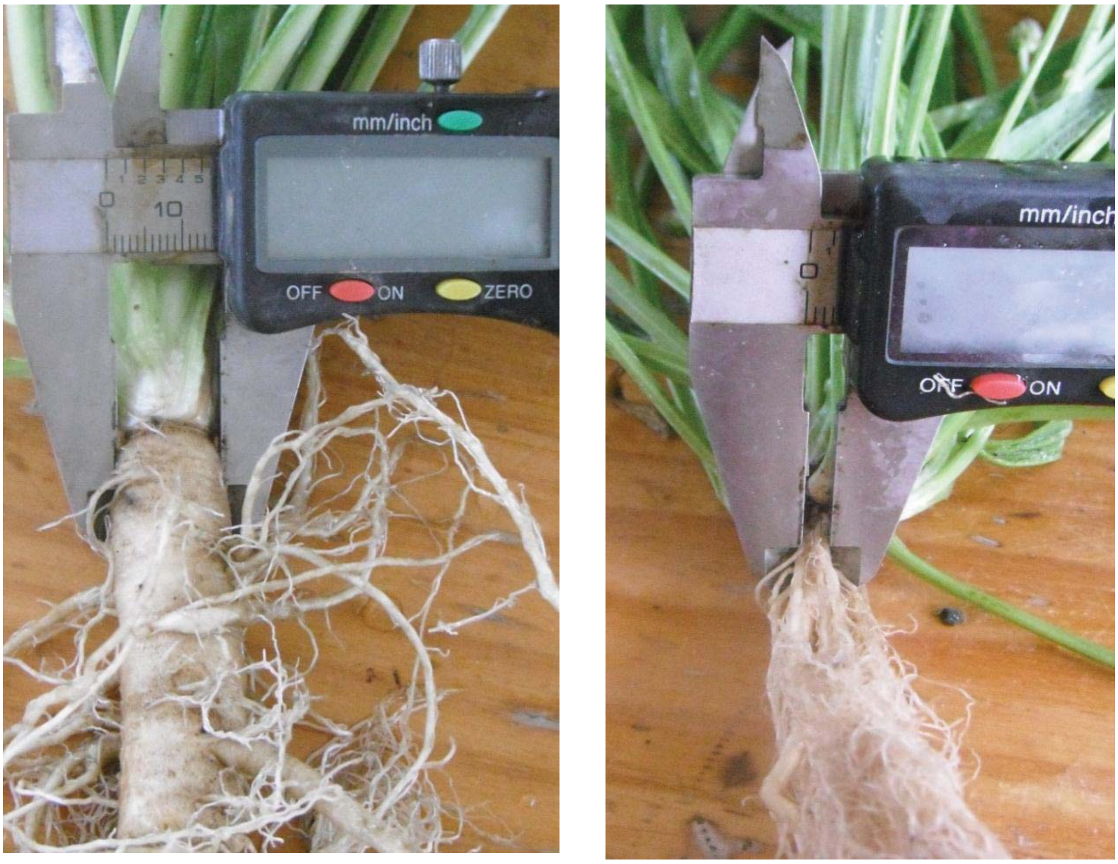



**Figure 5.2 Measuring the taproot diameter at the widest part of the root in chicory (left) and plantain (right).**

### 5.3.11 Water-soluble carbohydrate reserve content of roots

The water-soluble carbohydrate reserve content of the roots of chicory and plantain were measured during Year Two on 19 August, 26 November 2012 and 17 January, 27 March and 3 May 2013. Four spade plugs (18 × 18 cm) were harvested per plot and were bulked together, giving one chicory sample and one plantain sample per plot (1 sample × 4 plots = 4 samples for each species by treatment combination). Harvests were consistently performed at the same time each morning to limit diurnal variation in WSC contents of plants (Fulkerson and Slack 1994). After harvesting, samples were moved to a lab for processing. Roots were thoroughly washed and the top 10cm of the roots was cut before being frozen at -18°C for at least 48 hours before freeze-drying.

Once dried, samples were weighed, ground to pass through a 1-mm sieve, and analysed by enzymatic method for glucose, fructose and sucrose content (Sekin 1978) and for fructan content (McCleary and Blakeney 1999). The total water soluble carbohydrate content of the roots was calculated as the sum of the glucose, fructose, sucrose and fructan contents for each sampling date.

#### *5.3.11.1 Statistical Analysis*

All statistical analyses were performed using SAS (Statistical Analysis System, version 9.2; SAS Institute Inc., Cary, NC, US). The pre- and post-graze dry matter, apparent DM removal, net herbage accumulation data and the nutritive value data were analysed using the MIXED procedure with a model including the fixed effects of grazing treatment, season (spring, summer and autumn), year and all two-way and three-way interactions between grazing treatment, season and year and the random effect of block. Data from year two spring was not available for the nutritive value analysis.

The total apparent DM removal and the number of grazing days data were analysed using the MIXED procedure with a model including the fixed effects of grazing treatment and year and the two-way interaction between grazing treatment and year and the random effect of block.

The botanical composition data were analysed using the MIXED procedure with a model including the fixed effects of grazing treatment, season, year and species and all two-way, three-way and four-way interactions between grazing treatment, season, year and species and the random effect of block. The leaf percentage data (year two only) were analysed separately for chicory and plantain dry matter. The MIXED procedure model included the fixed effects of grazing treatment and season and the two-way interaction between grazing treatment and season and the random effect of block.

The plant density, taproot diameter, shoot density and water-soluble carbohydrate reserve parameters were analysed using the MIXED procedure with a model including the fixed effects of grazing treatment, time of harvest, species and all two-way and three-way interactions between grazing treatment, time of harvest and species and the random effect of block.

## 5.4 Results

### 5.4.1 Pre- and post-grazing herbage mass and apparent dry matter removal

There were three-way interactions ($P < 0.05$) between year, season and grazing treatment for pre- and post-grazing herbage masses and apparent dry matter removal. Within all year by seasons, the Lax grazing treatment had greater ($P < 0.05$) pre- and post-grazing masses than the Hard grazing treatment (Table 5.4). The apparent dry matter removal did not differ ($P > 0.05$) between the Hard and Lax grazing treatments within each year by season, except at Year One-Summer when the Hard grazing treatment had a greater ($P < 0.05$) apparent DM removal than the Lax grazing treatment.

**Table 5.4 Effect of grazing treatment (Hard and Lax), season (Spring and Summer and Autumn) and year (Year One; 2011-2012 and Year Two; 2012-2013) on the pre- and post-grazing herbage mass (DM basis) and apparent DM removal at each grazing event (mean ± s.e.). Means within columns having different letters are significantly different at *P* = 0.05.**

| Year | Season | Grazing Treatment | Pre-graze DM (kgDM/ha) | Post-graze DM (kgDM/ha) | Apparent DM removal (kgDM/ha) |
|---|---|---|---|---|---|
| One | Spring | Hard | 1813a ± 164 | 850a ± 125 | 963ac ± 152 |
| | | Lax | 2447bd ± 164 | 1950bc ± 125 | 605a ± 152 |
| One | Summer | Hard | 2170ab ± 164 | 990a ± 125 | 1180cd ± 152 |
| | | Lax | 2854d ± 164 | 2397d ± 125 | 723ab ± 152 |
| One | Autumn | Hard | 2165abc ± 232 | 742a ± 176 | 1422cde ± 215 |
| | | Lax | 2825d ± 232 | 1876bc ± 176 | 979acd ± 215 |
| Two | Spring | Hard | 2691cd ± 190 | 628a ± 176 | 1737e ± 176 |
| | | Lax | 3882e ± 190 | 1538b ± 176 | 1408cde ± 176 |
| Two | Summer | Hard | 2111ab ± 190 | 953a ± 44 | 1381cde ± 176 |
| | | Lax | 3591e ± 190 | 2506d ± 144 | 1436de ± 176 |
| Two | Autumn | Hard | 1872a± 232 | 730a ± 144 | 1244bcde ± 215 |
| | | Lax | 2585bd ± 232 | 2155cd ± 144 | 1046acd ± 215 |

### 5.4.2 Total apparent dry matter removal per year and total number of grazing days

There were no two-way interactions ($P > 0.05$) between year and grazing treatment for total apparent DM removal or the total number of sheep grazing days. The Hard grazing treatment had a greater ($P < 0.05$) total apparent DM removal on a per year basis and a greater total number of sheep grazing days on a per year basis than the Lax grazing treatment (Table 5.5). The total apparent DM removal did not differ ($P > 0.05$) between years one and two. Year one had a greater ($P < 0.05$) total number of sheep grazing days than year two.

**Table 5.5 The effect of grazing treatment (Hard and Lax) and year (Year One; 2011-2012 and Year Two; 2012-2013) on the total apparent DM removed and the total number of grazing days (mean ± s.e.). Means within columns and parameters having different letters are significantly different at *P* = 0.05.**

| Parameter | Total apparent DM removal (kgDM/ha/year) | Total number of grazing days (sheep by days) |
|---|---|---|
| *Grazing treatment* | | |
| Hard | 11631b ± 715 | 292b ± 2.6 |
| Lax | 8950a ± 715 | 210a ± 2.6 |
| *Year* | | |
| One | 9344 ± 715 | 266b ± 2.6 |
| Two | 11237 ± 715 | 236a ± 2.6 |

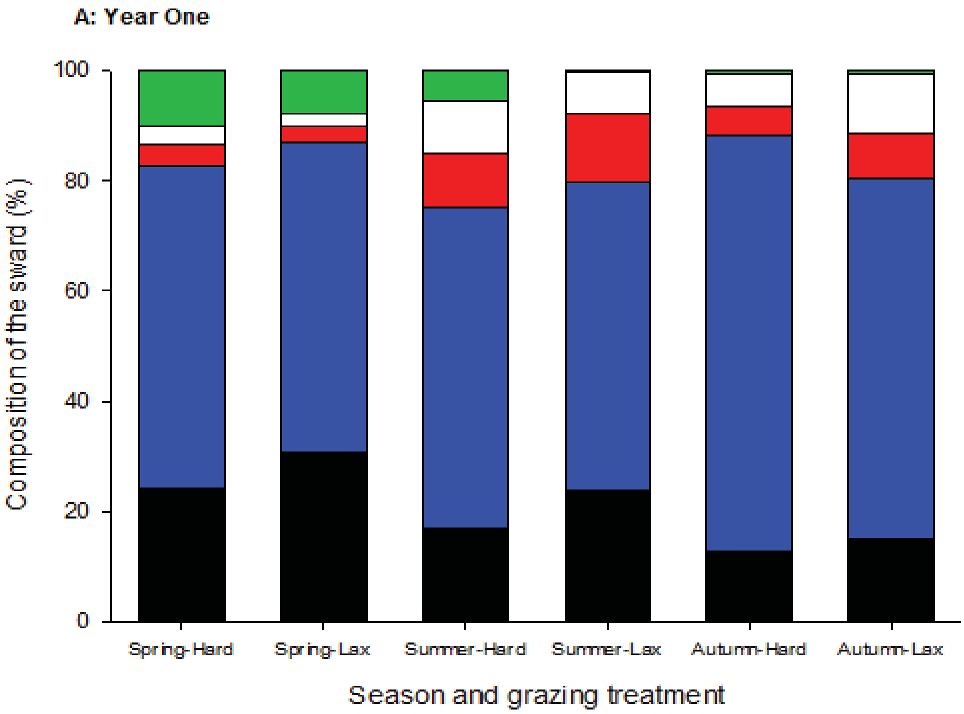
### 5.4.3 Net herbage accumulation

There were no ($P > 0.05$) three-way or two-way interactions between year, season and grazing treatment for net herbage accumulation. Net herbage accumulation was lower ($P < 0.05$) during autumn than during both spring and summer, which did not differ ($P > 0.05$), such that autumn, spring and summer were 32.5 ± 8.6, 61.2 ± 6.6 and 60.8 ± 7.9 kgDM/ha/day, respectively. Net herbage accumulation was greater ($P < 0.05$) during Year one than Year two (72 ± 6.0 and 31 ± 6.7 kgDM/ha/day, respectively). Net herbage accumulation did not differ ($P > 0.05$) between grazing treatments (52 ± 6.3 and 51 ± 6.3 kgDM/ha/day, for Hard and Lax grazing treatments respectively).

### 5.4.4 Botanical composition

There was a four-way interaction ($P < 0.05$) between year, season, grazing treatment and species for botanical composition. During year one the proportion of chicory in the sward within each season did not differ ($P > 0.05$) between the Hard and Lax grazing treatments (Figure 5.3A). The proportion of chicory in the sward in spring was greater ($P < 0.05$) than that in autumn. The proportion of plantain in the sward was greater ($P < 0.05$) under the Hard grazing treatment in Autumn than all other season by grazing treatment combinations, which did not differ ($P > 0.05$) from each other. The proportions of white clover and red clover did not differ ($P > 0.05$) between any season by grazing treatment combination.

During year two, the proportion of chicory in the sward within spring and summer did not differ ($P > 0.05$) between the Hard and Lax grazing treatments (Figure 5.3B). However, during autumn the Lax grazing treatment had a greater ($P < 0.05$) proportion of chicory than the Hard grazing treatment. The proportion of chicory in autumn was greater ($P < 0.05$) that that in summer which was in turn greater ($P < 0.05$) than that spring, under both hard and lax grazing treatments. Within each season, the proportion of plantain was greater ($P < 0.05$) under the Hard grazing treatment than the Lax grazing treatment. Conversely, within each season, the proportion of red clover was greater ($P < 0.05$) under the Lax grazing treatment than the Hard grazing treatment. The proportion of white clover in the sward did not differ ($P > 0.05$) between the Hard and Lax grazing treatments during spring and autumn. While, during summer the Lax grazing treatment had a greater ($P < 0.05$) proportion of white clover than the Hard grazing treatment.



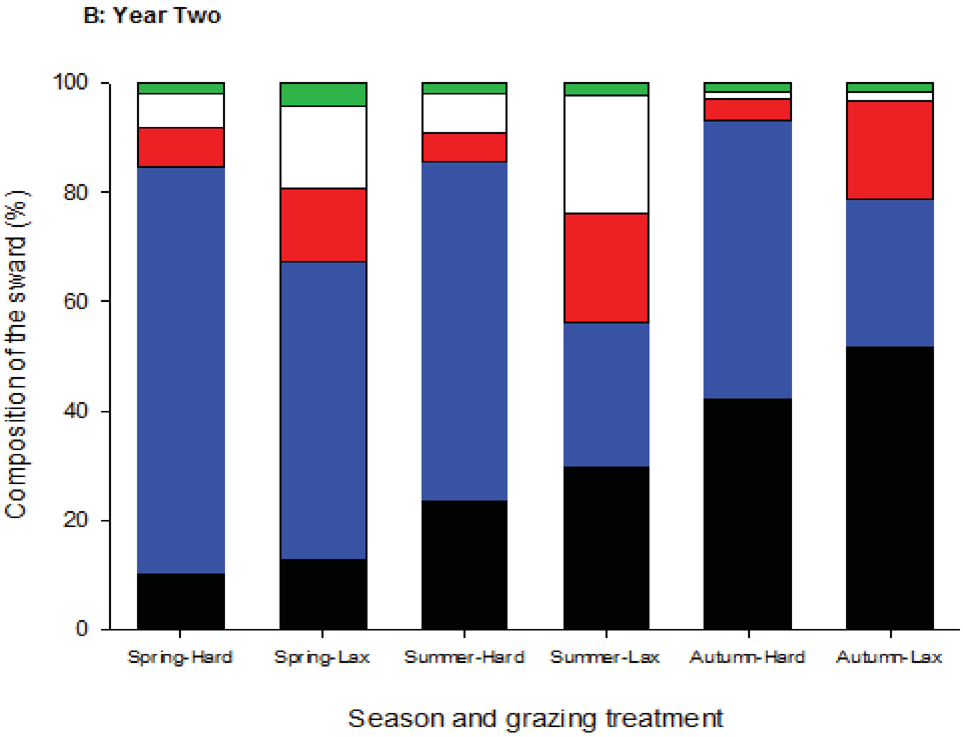




**Figure 5.3 Effect of season (Spring and Summer and Autumn) and grazing treatment (Hard and Lax) on the botanical composition (chicory (black), plantain (blue), red clover (red), white clover (white) and weed (green)) of the sward during Year One and Two. All spring and autumn values include a s.e. of 3.23% and all summer values include a s.e. of 4.57%.**

### 5.4.5 Leaf percentage in chicory and plantain during year two

In this analysis, the aim was to assess the morphology of chicory and plantain. There was no interaction ($P > 0.05$) between season and grazing treatment for the percentage of leaf in chicory dry matter. There tended ($P = 0.06$) to be an effect of season on the percentage of leaf in chicory dry matter, with pairwise analysis indicating the percentage of chicory leaf material was greater ($P < 0.05$) during spring than summer, with autumn not differing ($P > 0.05$) from either season (Table 5.6). There tended to be a greater ($P = 0.07$) percentage of chicory leaf material in the Hard grazing treatment than in the Lax grazing treatment.

There was an interaction ($P < 0.05$) between season and grazing treatment for the percentage of leaf in plantain dry matter (Table 5.6). The percentage of leaf in plantain dry matter was greater ($P < 0.05$) with each successive season, except Spring-Hard and Summer-Hard which did not differ ($P > 0.05$). During spring, the percentage of leaf in plantain dry matter was greater ($P < 0.05$) in the Hard grazing treatment than in the Lax grazing treatment. While, during summer the percentage of leaf in plantain dry matter was lower ($P < 0.05$) in the Hard grazing treatment than in the Lax grazing treatment, and during autumn, the percentage of leaf in plantain dry matter did not differ ($P > 0.05$) between the Hard and the Lax grazing treatments.

**Table 5.6 Effect of season (Spring and Summer and Autumn) and grazing treatment (Hard and Lax) on the percentage of leaf in chicory and plantain dry matter during year two. Means within columns and parameters with different letters are significantly different at *P* = 0.05.**

| Parameter | Percentage Leaf | |
|---|---|---|
| | Chicory | Plantain |
| *Season* | | |
| Spring | 93b ± 5.2 | 57a ± 3.6 |
| Summer | 75a ± 5.2 | 75b ± 3.6 |
| Autumn | 86ab ± 3.7 | 96c ± 3.1 |
| *Grazing treatment* | | |
| Hard | 90b ± 3.9 | 77 ± 3.2 |
| Lax | 80a ± 3.9 | 75 ± 3.2 |
| *Season by grazing treatment* | | |
| Spring - Hard | 99b ± 7.3 | 68b ± 4.5 |
| Spring - Lax | 86ab ± 7.3 | 47a ± 4.5 |
| Summer - Hard | 79ab ± 7.3 | 67b ± 4.5 |
| Summer - Lax | 70a ± 7.3 | 83c ± 4.5 |
| Autumn - Hard | 90b ± 5.2 | 97d ± 3.6 |
| Autumn - Lax | 82ab ± 5.2 | 96d ± 3.6 |

Note: The remaining percentage is stem material (including seed heads and flowers).

### 5.4.6 Nutritive value

There were three-way interactions ($P < 0.05$) between year, season and grazing treatment for all herbage nutritive value traits (Table 5.7). During the spring and autumn of year one, grazing treatment had no effect ($P > 0.05$) on any of the herbage nutritive values. During the summer of year one, the Hard grazing treatment had a greater ($P < 0.05$) protein percentage and a lower ($P < 0.05$) NDF percentage than the Lax grazing treatment. Contrastingly, during the summer of year two, the Hard grazing treatment had a lower ($P < 0.05$) protein percentage than the Lax grazing treatment. During the autumn of year two, the Hard grazing treatment had a greater ($P < 0.05$)

OMD percentage, ash percentage and ME content and a lower ($P < 0.05$) NDF and ADF percentage than the Lax grazing treatment.

The OMD percentage was greater ($P < 0.05$) during the spring of year one than during all other season by grazing treatment combinations, except for the autumn Lax grazing treatment in year one. The protein percentage was greater ($P < 0.05$) during the spring of year one, than during all other season by grazing treatment combinations, except for the summer Hard grazing treatment in year one. The NDF percentage was lower ($P < 0.05$) during the spring of year one, than during all other season by grazing treatment combinations. The ADF percentage was lower ($P < 0.05$) during the spring of year one, than during the summer of year one under the Lax grazing treatment and during both summer and autumn of year two, under both the Hard and Lax grazing treatments. The ME content was greater ($P < 0.05$) during the spring of year one than during the summers of both year one and two and the autumn of year two.

**Table 5.7 The effect of year (Year One; 2011-2012 and Year Two; 2012-2013), season (Spring and Summer and Autumn) nutritive value traits of the sward (mean ± s.e.): organic matter digestibility (OMD, % dry matter (DM)), ash content (ash %D detergent fibre (NDF %DM), acid detergent fibre (ADF %DM) and metabolisable energy (ME MJ/kg DM). Means within colum different at *P* = 0.05.**

| Year | Season | Treatment | OMD% | Ash % | Protein % | NDF % |
|---|---|---|---|---|---|---|
| 1 | Spring | Hard | 84.7e ± 0.68 | 12.3c ± 0.38 | 24.7e ± 0.85 | 18.5a ± 1.23 |
| | | Lax | 83.7de ± 0.68 | 12.3c ± 0.38 | 23.9de ± 0.85 | 20.2a ± 1.23 |
| 1 | Summer | Hard | 79.3bc ± 0.97 | 11.6bc ± 0.54 | 23.1cde ± 1.08 | 25.2bc ± 1.70 |
| | | Lax | 76.9b ± 0.97 | 10.4ab ± 0.54 | 19.4ab ± 1.08 | 30.7d ± 1.70 |
| 1 | Autumn | Hard | 81.0c ± 0.97 | 11.2bc ± 0.54 | 21.0bc ± 1.08 | 25.0bc ± 1.70 |
| | | Lax | 81.4cd ± 0.97 | 11.2bc ± 0.54 | 20.9bc ± 1.08 | 24.5b ± 1.70 |
| 2 | Summer | Hard | 77.7b ± 0.97 | 10.4ab ± 0.54 | 19.9ab ± 1.08 | 29.3cd ± 1.70 |
| | | Lax | 77.7b ± 0.97 | 9.6a ± 0.54 | 21.9cd ± 1.08 | 29.1bcd ± 1.70 |
| 2 | Autumn | Hard | 78.6bc ± 0.97 | 15.5d ± 0.54 | 19.9ab ± 1.08 | 28.5bcd ± 1.70 |
| | | Lax | 73.2a ± 0.97 | 10.3ab ± 0.54 | 17.9a ± 1.08 | 36.1e ± 1.70 |

Note: data from spring of Year Two was not available.

### 5.4.7 Plant density

There was no three-way interaction ($P > 0.05$) between time of harvest, grazing treatment and species or two-way interaction ($P > 0.05$) between time of harvest and grazing treatment for plant density. However, there was a two-way interaction ($P < 0.05$) between time of harvest and species for plant density (Figure 5.4). All species had a lower ($P < 0.05$) plant density on 3 October 2013 (end of the experimental period) than on 25 August 2011 (start of the experimental period). The plant density of plantain was always greater ($P < 0.05$) than that of chicory, except on 27 March 2013 and 2 September 2013, when it did not differ ($P < 0.05$) to that of chicory (Figure 5.4A). The plant density of red and white clover did not differ ($P > 0.05$) on 25 August 2011 or 3 October 2013 (start and finish of the experimental period) (Figure 5.4B). On 25 August 2011 the plant density of red and white clover was lower ($P < 0.05$) than that of both chicory and plantain. However, by 3 October 2013 the plant density of red and white clover was lower ($P < 0.05$) than that of plantain, but did not differ ($P > 0.05$) to that of chicory.

There was also a two-way interaction ($P < 0.05$) between grazing treatment and species for plant density (Table 5.8). The plant density of both chicory and plantain was greater ($P < 0.05$) in the Hard grazing treatment than in the Lax grazing treatment. While, the plant density of both red and white clover did not differ ($P > 0.05$) between the Hard and Lax grazing treatments.

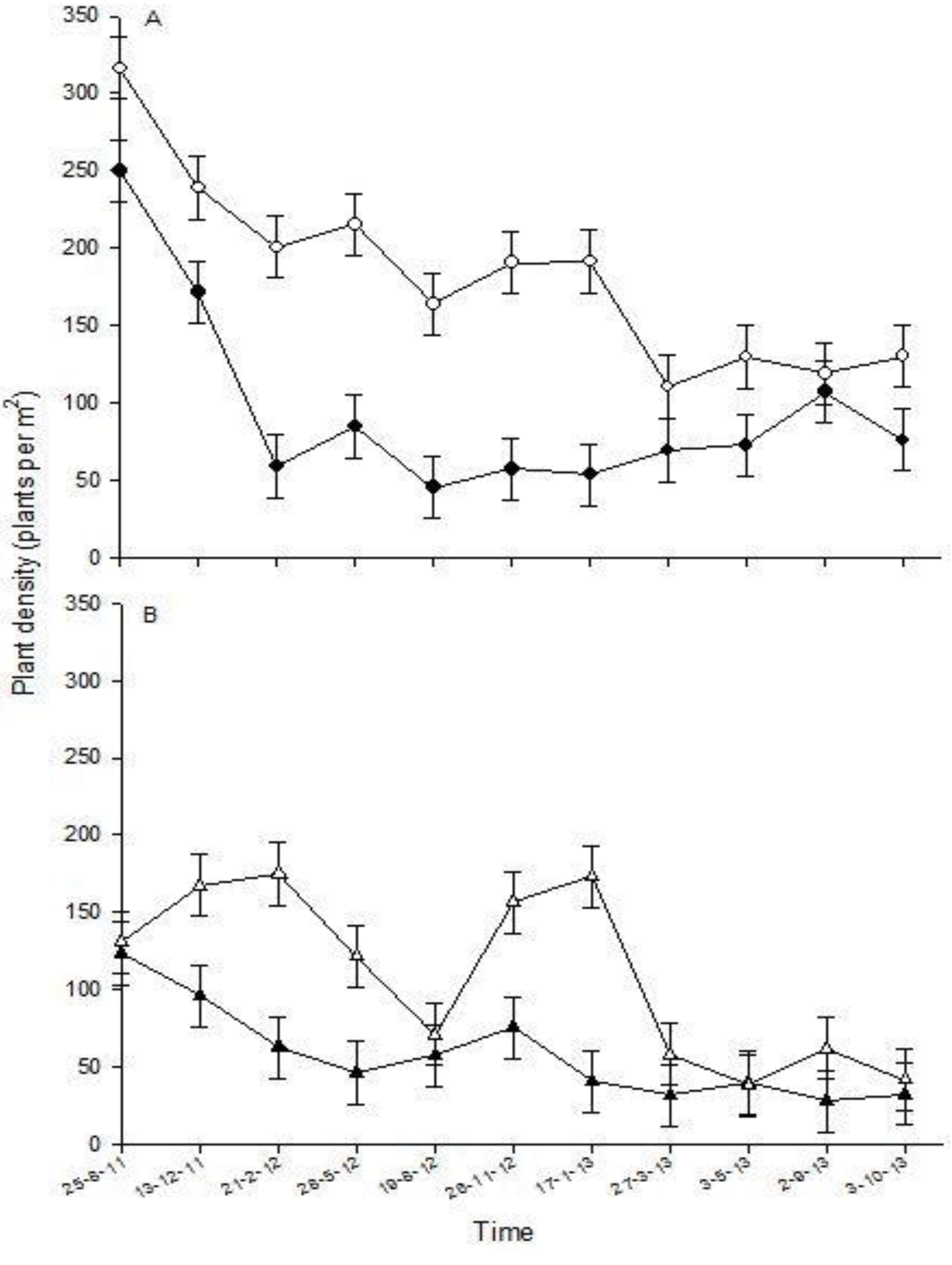



**Figure 5.4 Change in plant density of Chicory (●) and Plantain (○) (Figure A) and Red Clover (▲) and White Clover (△) (Figure B) within the sward over two years. Vertical bars represent s.e. Note species split for ease of interpretation.**

**Table 5.8 Effect of grazing treatment (Hard and Lax) on the plant density (plants per m2), number of crowns per plant and taproot diameter (mm) during year one, two and three and the shoot density (shoots per m$^2$) during year two and three. All data presented as means with standard errors. Means within parameters (within columns and rows) having different letters are significantly different at *P* = 0.05.**

| Parameter | | Grazing treatment | |
|---|---|---|---|
| | | Hard | Lax |
| *Year One, Two and Three* | | | |
| Plant density | chicory | 109.6c ± 8.6 | 80.1b ± 8.6 |
| (plants per m$^2$) | plantain | 208.3e ± 8.6 | 156.1d ± 8.6 |
| | red clover | 54.5a ± 8.6 | 60.2ab ± 8.6 |
| | white clover | 107.1c ± 8.6 | 109.7c ± 8.6 |
| Number of crowns per plant | chicory | 1.3a ± 0.07 | 1.4a ± 0.07 |
| | plantain | 1.9c ± 0.07 | 1.7b ± 0.07 |
| Taproot diameter | chicory | 8.2b ± 0.3 | 11.2c ± 0.3 |
| (mm) | plantain | 4.1a ± 0.3 | 4.6a ± 0.3 |
| *Year Two and Three only* | | | |
| Shoot density | chicory | 6.7a ± 1.9 | 5.2a ± 1.9 |
| (shoots per m$^2$) | plantain | 23.5c ± 1.9 | 15.3b ± 1.9 |

### 5.4.8 Shoot density

Shoot density was measured during Year Two and Year Three at approximately two monthly intervals. There was no ($P > 0.05$) three-way interaction between time of harvest, grazing treatment and species or two-way interactions ($P > 0.05$) between time of harvest and grazing treatment or time of harvest and species for shoot density. However, there was a two-way interaction ($P < 0.05$) between grazing treatment and species for shoot density, such that chicory shoot density did not differ ($P > 0.05$) under the Hard and Lax grazing treatments and was lower ($P < 0.05$) than that of plantain in both the Hard and Lax grazing treatments (Table 5.8). Within plantain, the Hard grazing treatment had a greater ($P < 0.05$) density than the Lax grazing treatment. Time of harvest had no ($P > 0.05$) effect on shoot density (data not shown).

### 5.4.9 Number of crowns per plant

The number of crowns per plant was only measured for the species chicory and plantain. There was no ($P > 0.05$) three-way interaction between time of harvest, grazing treatment and species or two-way interactions between time of harvest and grazing treatment or time of harvest and species for the number of crowns per plant. However, there was a two-way interaction ($P < 0.05$) between grazing treatment and species for the number of crowns per plant. The number of crowns per chicory plant did not differ ($P > 0.05$) under the Hard and Lax grazing treatments and was lower ($P < 0.05$) than that of plantain in both the Hard and Lax grazing treatments (Table 5.8). Within plantain, the number of crowns per plant was greater ($P < 0.05$) in the Hard grazing treatment than in the Lax grazing treatment. Time of harvest had no ($P > 0.05$) effect on the number of crowns per plant (data not shown).

### 5.4.10 Taproot diameter

Taproot diameter was only measured for the species chicory and plantain. There was no three-way interaction ($P > 0.05$) between time of harvest, grazing treatment and species or two-way interaction ($P > 0.05$) between time of harvest and grazing treatment for taproot diameter. However, there was a two-way interaction ($P < 0.05$) between time of harvest and species for taproot diameter. The taproot diameter of chicory was greater ($P < 0.05$) at 3 October 2013 (end of the experimental period) than at 25 August 2011 (start of the experimental period) (Figure 5.5). Whereas, the taproot diameter of plantain did not differ ($P > 0.05$) between 25 August 2011 and 3 October 2013. The taproot diameter of chicory was greater ($P < 0.05$) than that of plantain at all sampling times.

There was also a two-way interaction ($P < 0.05$) between grazing treatment and species for taproot diameter (Table 5.8). The taproot diameter of plantain did not differ ($P > 0.05$) under the Hard and Lax grazing treatments and was lower ($P < 0.05$) than that of chicory under both the Hard and Lax grazing treatments. The taproot diameter of chicory was greater ($P < 0.05$) under the Lax grazing treatment than the Hard grazing treatment.

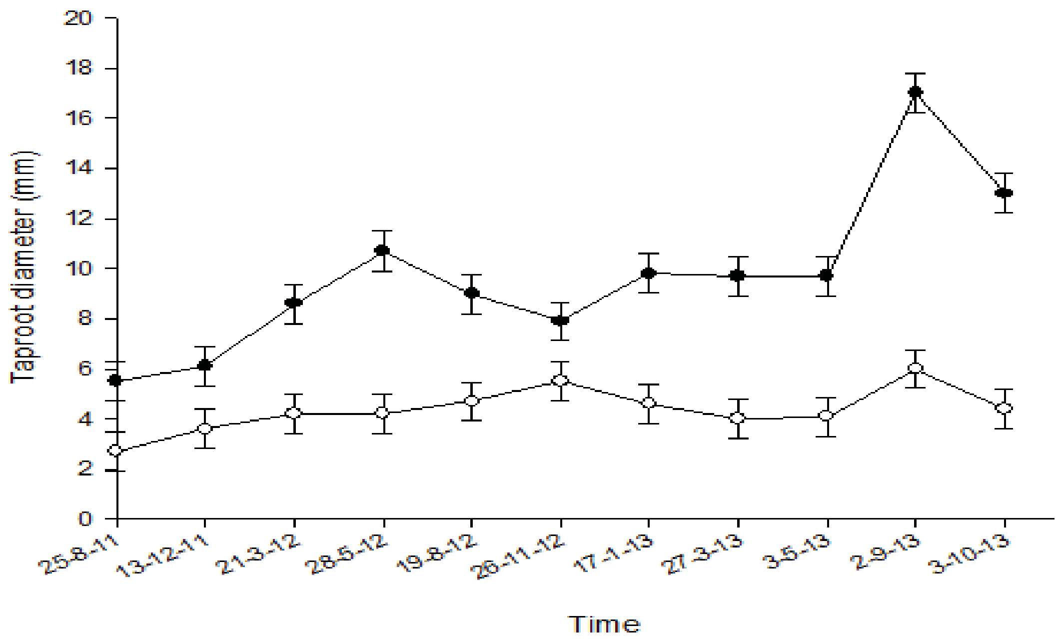



**Figure 5.5 Effect of species (Chicory (●) and Plantain (○)) on taproot diameter (mm) over time. Vertical bars represent s.e.**

### 5.4.11 Water-soluble carbohydrate reserve content of roots

The water-soluble carbohydrate reserves in the roots of chicory and plantain were monitored during Year Two only.

#### *5.4.11.1 Glucose content of roots*

There was no ($P > 0.05$) three-way interaction between time, grazing treatment and species or two-way interactions between grazing treatment and species or grazing treatment and time of harvest for glucose content. However, there was a ($P < 0.05$) two-way interaction between species and time of harvest. The glucose content of chicory was greater ($P < 0.05$) on 26 November 2012 than on 19 August 2012 (Figure 5.6A). Between 26 November 2012 and 27 March 2013 the glucose content of chicory was lower ($P < 0.05$) with each sampling date. The glucose content of chicory on 3 May 2013 was lower ($P < 0.05$) than that on 19 August 2012 and 26 November 2012 but did not differ ($P > 0.05$) from that on 17 January 2013 and 27 March 2013. The glucose content of plantain did not differ ($P > 0.05$) between all sampling dates, except being lower ($P < 0.05$) on 17 January 2013 than on 19 August 2012. Grazing treatment had no ($P > 0.05$) effect on glucose content (12.0 ± 0.7 and 10.9 ± 0.7 mg/g, for Hard and Lax grazing treatments, respectively).

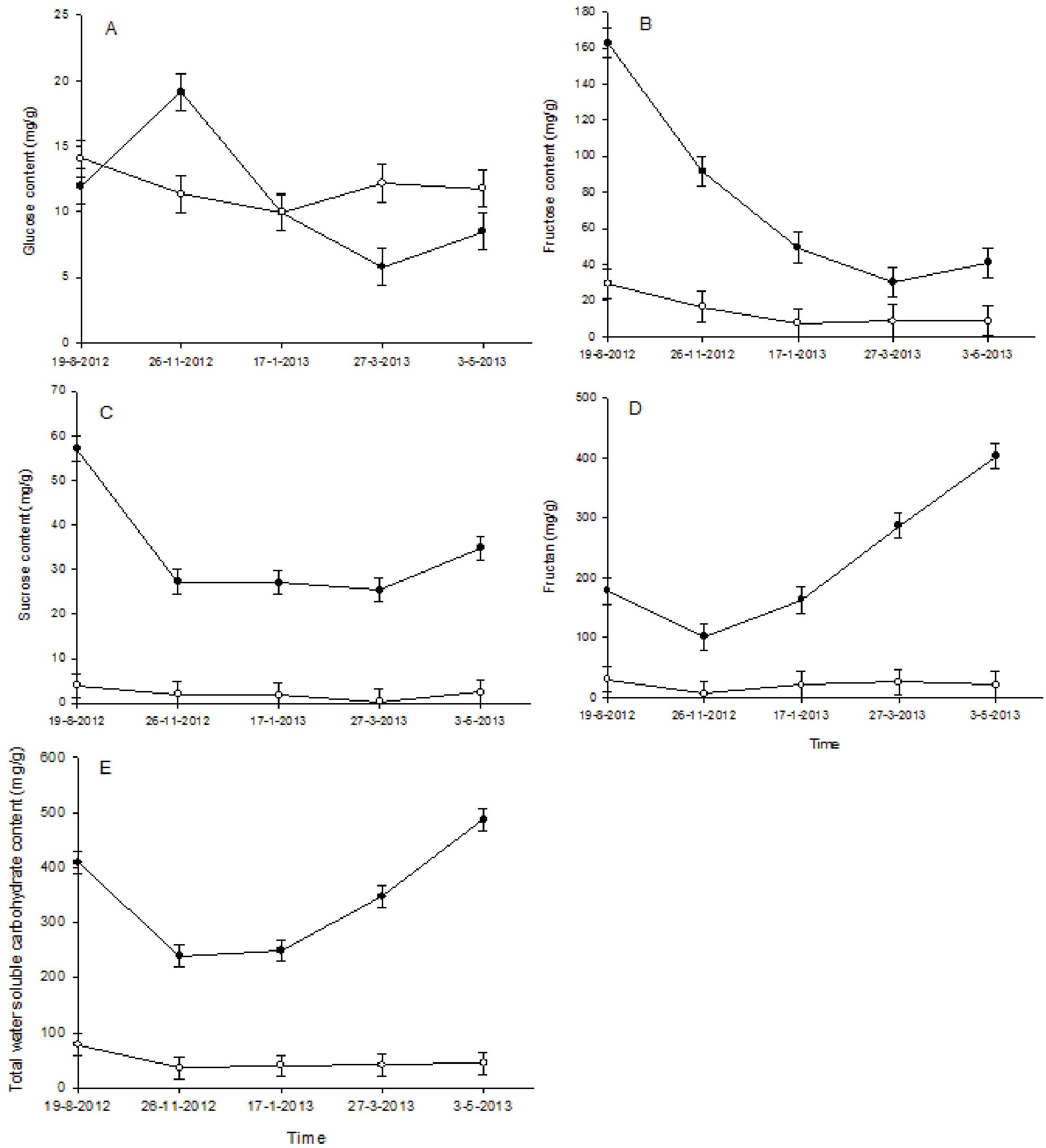



**Figure 5.6. The effect of species (Chicory (●) and Plantain (○)) on the root content of glucose (A), fructose (B), sucrose (C), fructan (D) and the total water soluble carbohydrate (E) during Year Two. Vertical bars represent s.e.**

### *5.4.11.2 Fructose content of roots*

There was no ($P > 0.05$) three-way interaction between time of harvest, grazing treatment and species or two-way interactions between grazing treatment and species or grazing treatment and time of harvest for fructose content. However, there was a ($P < 0.05$) two-way interaction between species and time of harvest. The fructose content of chicory was lower ($P < 0.05$) with each sampling date between 19 August 2012 and 17 January 2013 (Figure 5.6B). Between 17 January 2013 and 3 May 2013 chicory fructose content did not differ ($P > 0.05$). The fructose content of plantain did not differ ($P > 0.05$) over time and was always lower ($P < 0.05$) than that of chicory. Grazing treatment had no ($P > 0.05$) effect on fructose content (47.8 ± 4.4 and 41.4 ± 4.4 mg/g, for Hard and Lax grazing treatments, respectively).

#### *5.4.11.3 Sucrose content of roots*

There was no ($P > 0.05$) three-way interaction between time of harvest, grazing treatment and species or two-way interactions between grazing treatment and species or grazing treatment and time of harvest for sucrose content. However, there was a ($P < 0.05$) two-way interaction between species and time. The sucrose content of chicory was lower ($P < 0.05$) on 26 November 2012 than on 19 August 2012 (Figure 5.6C). Between 26 November 2012 and 27 March 2013 chicory sucrose content did not differ ($P > 0.05$). The sucrose content of chicory was greater ($P < 0.05$) on 3 May 2013 than on 27 March 2013. The sucrose content of plantain did not differ ($P > 0.05$) over time and was always lower ($P < 0.05$) than that of chicory. Grazing treatment had no ($P > 0.05$) effect on sucrose content (17.7 ± 1.6 and 18.6 ± 1.6 mg/g, for Hard and Lax grazing treatments, respectively).

#### 5.4.11.4 Fructan content of roots

There was no ($P > 0.05$) three-way interaction between time of harvest, grazing treatment and species or two-way interaction between grazing treatment and time of harvest for fructan content. However, there was a ($P < 0.05$) two-way interaction between species and time of harvest. The fructan content of chicory was lower ($P < 0.05$) on 26 November 2012 than on 19 August 2012 (Figure 5.6D). From 26 November 2012 onwards, the chicory fructan content was greater ($P < 0.05$) at each sampling date. The fructan content of plantain did not differ ($P > 0.05$) over time and was always lower ($P < 0.05$) than that of chicory.

There was also a two-way ($P < 0.05$) interaction between grazing treatment and species on fructan content. Chicory had a greater ($P < 0.05$) fructan content under the Lax grazing treatment than the Hard grazing treatment, while that of plantain did not differ ($P > 0.05$) under the Hard and Lax grazing treatments (Table 5.9).

**Table 5.9 Effect of grazing treatment (Hard and Lax) on the fructan content (mg/g) in the roots of Chicory and Plantain over Year Two (mean ± s.e.). Means within columns and rows having different letters are significantly different at *P* = 0.05.**

| Grazing treatment | Chicory | Plantain |
|---|---|---|
| Hard | 199.7b ± 13.5 | 22.8a ± 13.5 |
| Lax | 252.9c ± 13.5 | 19.0a ± 13.9 |

##### *5.4.11.5 Total water soluble carbohydrate content of roots (sum of glucose, fructose, sucrose and fructan)*

There was no ($P > 0.05$) three-way interaction between time of harvest, grazing treatment and species or two-way interactions between grazing treatment and species or grazing treatment and time of harvest for total water soluble carbohydrate content. However, there was a ($P < 0.05$) two-way interaction between species and time of harvest. The total water soluble carbohydrate (WSC) content of chicory did not differ ($P > 0.05$) between 26 November 2012 and 17 January 2013 and was lower ($P < 0.05$) than on 19 August 2012 (Figure 5.6E). From 17 January 2013 onwards, the WSC content of chicory was greater ($P < 0.05$) at each sampling date. The WSC content of plantain did not differ ($P > 0.05$) over time and was always lower ($P < 0.05$) than that of chicory. Grazing treatment had no ($P > 0.05$) effect on total water soluble carbohydrate content (188.9 ± 9.5 and 205.8 ± 9.5 mg/g, for Hard and Lax grazing treatments, respectively).

## 5.5 Discussion

The total dry matter harvested on a per year basis was greater from the Hard than the Lax grazing treatment (11.6 *vs.* 9.0 t DM/ha/year), however the dry matter harvested within each season did not differ between the Hard and Lax grazing treatments. This is the first experiment of its kind to report annual dry matter yields from the Herb and Legume Mix. However, this supports previous research which suggests that pure swards of chicory and plantain have a dry matter production which is equivalent to that of perennial ryegrass pastures (Rumball *et al.* 1997; Hogh-Jensen *et al.* 2006; Powell *et al.* 2007). Net herbage accumulation was lower during autumn than during spring and summer. This has been previously reported in chicory, as remobilisation of root stored assimilate supplements photosynthetic production during spring (Brown *et al.* 2000). While plantain can maintain a higher dry matter production than chicory during autumn (Yu *et al.* 2008), it is still summer active and thus accumulates most of its dry matter over the December to February period (Stewart 1996; Labreveux *et al.* 2006; Moorhead and Piggot 2009). Consequently, the Herb and Legume Mix is an ideal specialist summer-active perennial forage.

The botanical composition of the sward was relatively stable during year one under both the Hard and Lax grazing treatments across all seasons. While, during year two the effects of the grazing treatment became apparent, whereby the Hard grazing treatment contained a greater proportion of plantain in all seasons. Conversely, the Lax grazing treatment contained a greater proportion of red clover in all seasons and a greater proportion of white clover and chicory in autumn, when compared to the Hard grazing treatment. Similarly, in a glasshouse mini-ecosystem containing a mix of white clover, perennial ryegrass and plantain, Mikola *et al.* (2001) found plantain made a greater proportional contribution to total shoot mass under a more intense defoliation treatment. Conversely, in a species-rich sward (containing 8-9 sown species), Soegaard *et al.* (2013) found the proportion of chicory was not affected by grazing, while that of plantain decreased over four years grazing. It is well established that both chicory and red clover yield more and persist better under lax grazing (Clark *et al.* 1990a; Li *et al.* 1997b; Alemseged 2000; Brock *et al.* 2003), and thus it is not surprising that the diversity of the sward was better maintained under the Lax grazing treatment than the Hard grazing treatment. While, the grazing treatment did not affect ewe lamb grazing preference during year one (Chapter 6), the greater diversity of the sward in the Lax compared to the Hard grazing treatment during year two may have enabled animals to display greater grazing preference in the long-term under the Lax grazing treatment.

Both the botanical composition of the sward and the morphological fractions (composition of stem and leaf) of chicory and plantain are likely to affect the overall sward nutritive value. The Hard grazing treatment tended to have a greater proportion of chicory leaf compared to stem (90 *vs*. 80 %, respectively), while that of plantain did not differ between the Hard and Lax grazing treatments (77 *vs*. 75 %, respectively). The nutritive value of reproductive stem in both chicory and plantain is lower than that of the leaves (Clark *et al.* 1990a; Stewart 1996; Lee *et al.* 2014), with quality declining as stem matures and becomes more fibrous; as is commonly observed in forages (Chapman *et al.* 2013). Clark *et al.* (1990a) suggested the desirable proportion of leaf and stem should be 70% leaf and 30% stem. Thus, in this experiment this target was met in both the Hard and Lax grazing treatments. Within each season there were few differences in nutritive value traits between the Hard and Lax grazing treatments, except during autumn of year two when the Hard grazing treatment had a higher digestibility and metabolisable energy content and a lower fibre content (NDF and ADF) than the Lax grazing treatment. Conversely, larger differences in nutritive value traits were observed between seasons, with the spring sward having a lower NDF content and a greater digestibility and metabolisable energy content than all seasons, except autumn of year two. However, the sward had a minimum metabolisable energy content of 10.7 MJME/kg DM and an average of 11.5 MJME/kg DM. Thus, it was nearly always above the recommended 10-11 MJME/kg DM recommended to enable high lamb live weight gain and higher than that generally achievable on perennial ryegrass and white clover pasture during summer (Hodgson and Brookes 2002). This again indicates the Herb and Legume Mix is suitable as a high quality perennial forage.

The productivity (herbage yield) of a herb based sward such as the Herb and Legume Mix is dependent on both the plant density and plant size with plant size being a function of shoot density per area, number of crowns per plant and taproot diameter. However this is not a straightforward relationship, as shown by Li *et al.* (1997a) where herbage yield remained constant until year four as plant density declined but plant size increased. Thus, the overall health status of a Herb and Legume Mix should consider the plant density, shoot density, number of crowns per plant and taproot diameter of both chicory and plantain.

The plant density of all species (chicory, plantain, red clover and white clover) decreased over time. This has previously been observed in pure swards of chicory whereby swards lose approximately 30-35% of their population per year (Hume *et al.* 1995; Li *et al.* 1995; Li and Kemp 2005). The decline in plant density of plantain was slower that than seen in chicory. Previous research which has compared pure swards of chicory and plantain supports this conclusion (Labreveux *et al.* 2004; Powell *et al.* 2007; Glassey *et al.* 2012). Similarly, Nie *et al.* (2008) found plantain persisted better than chicory, whereby the frequency of chicory declined by up to 49% from year two to three, and 38% from year three to four, whereas plantain declined by 41 and 33%, respectively, over the two years. Once chicory plant density declines to about 20-30 plants/$m^2$ it has low growth rates, allows weed invasion and can be defined as an unproductive chicory crop (Li *et al.* 1994a). Whereas optimum yields of chicory can be obtained at a density of 50 – 60 plants/$m^2$ (Alemseged 2000). However, in this experiment the plant density of all species at the end of the experimental period were sufficiently high to warrant sowing a mixed sward with four productive species. Thus, it is possible that chicory and plantain are more resilient to grazing when sown in a mixed sward than when sown as a pure sward.

The plant density of both chicory and plantain was greater in the Hard grazing treatment than in the Lax grazing treatment. This was unexpected, as Li *et al.* (1995) concluded that severe grazing of chicory during spring (post-grazing residual of 50 mm or less) is likely to exacerbate the decline in plant density. Conversely, previous research in pure swards of chicory and plantain has reported post-grazing sward height does not affect plant density (Li *et al.* 1994b; Ayala *et al.* 2011b). Li *et al.* (1997c) concluded that the persistence of chicory is more sensitive to defoliation frequency than defoliation height. In support of this, Alemseged (2000) postulated that the height of stubble as a source of growing points is not important as regrowth is supported entirely through taproot reserves. Thus, as the grazing frequency in this experiment was always a minimum of three weeks, the plants may have been able to withstand the Hard grazing treatment as they had sufficient time to recover before the next grazing event. However, it is still unclear why the plant density of chicory and plantain were greater in the Hard grazing treatment than in the Lax grazing treatment. Furthermore, it is possible that if this experiment was continued for a third year the negative effects of the Hard grazing treatment in terms of changes in plant density would be more apparent.

The shoot density of chicory and plantain did not change over time. Conversely, Ayala *et al.* (2011b) found the shoot density of plantain declined over time in a 3-year-old plantain sward. Similarly, Li *et al.* (1997a) reported the shoot density of chicory remained static over three years and then declined during the fourth year. Thus, chicory and plantain may be more stable in a mixed sward than in pure swards. Alternatively the experiment may not have run long enough to observe a decline in shoot density. The shoot density of chicory did not differ under the grazing treatments, however plantain shoot density was greater in the Hard grazing treatment than in the Lax grazing treatment. Ayala *et al.* (2011b) found the shoot density of plantain was not affected by post-grazing height. Combined, these results suggest shoot density was relatively stable in the Herb and Legume Mix, with the Hard grazing treatment favouring plantain.

The number of crowns per plant did not change over time. This contrasts with previous research on chicory which has shown plants develop multiple crowns during the second growing season (Hume *et al.* 1995; Li *et al.* 1998). Furthermore, the number of crowns per chicory plant did not differ under the grazing treatments, while the number of crowns per plantain plant was greater in the Hard grazing treatment than in the Lax grazing treatment. Again, this suggests that plantain responds favourably to the Hard grazing treatment.

Tap-rooted plants utilise their carbohydrate reserves for winter survival and initiation of growth in spring (Kust and Smith 1961). Furthermore, they can provide the necessary energy to support shoot growth following defoliation (Fankhauser *et al.* 1989). Consequently, the total non-structural carbohydrates in the fleshy taproot are often used as an indicator of the physiological status of perennial forage plants such as alfalfa (*Medicago sativa* L.) (Wolf 1978). The taproot diameter provides a visual measure of carbohydrate reserves of a plant. In this experiment the taproot diameter of chicory fluctuated over time, whereas that of plantain remained stable. The taproot diameter of chicory was greater under the Lax grazing treatment than the Hard grazing treatment. This was not observed in plantain. Chapters 3 and 4 support this showing the taproot diameter of chicory was reduced under defoliation and more frequent defoliation compared to no defoliation and less frequent defoliation, while plantain taproot diameter was unaffected. Similarly, Yu *et al.* (2008) reported the taproot diameter of plantain was unaffected by cutting height, whereas that of chicory was reduced under hard cutting.

Overall, this suggests that chicory and plantain have different patterns of utilisation of their root carbohydrate reserves.

The total water soluble carbohydrate level (WSC) in chicory roots varied over sampling dates during year two, with WSC declining after the grazing began in spring and then increased steadily from mid-spring onwards to peak in late autumn. This pattern matches with legume species where root carbohydrate contents are highest during autumn (Li *et al.* 1996). This is necessary for winter survival and initiation of growth in spring (Kust and Smith 1961), and is often associated with persistence in perennial forage legumes (Grandfield 1943; Smith 1962; Smith 1964). A positive association has been observed between root starch contents and regrowth of perennial legumes following defoliation (Matches 1968; Nelson and Smith 1968). Similarly, in chicory, Li *et al.* (1997c) found under 20 mm cutting, a cultivar with the highest root carbohydrate content accumulated greater leaf mass than other cultivars. The WSC in the roots of chicory did not directly mirror the changes in the size of taproot diameter over time whereby, the taproot diameter of chicory appeared relatively stable over the WSC measurement dates. The WSC in plantain roots was substantially lower than that in chicory roots and did not vary over sampling dates. This reflected the stable size of plantains taproot diameter over time and indicates that plantain does not utilise root carbohydrate reserves as a larger tap-rooted plant does. Combined, these results suggest that changes in taproot diameter may only occur when large increases or decreases in root WSC reserves occur. Nonetheless, it is still suggested that monitoring taproot diameter is likely to be a useful measurement tool for farmers to assess the suitability of chicory and plantain for grazing.

Grazing treatment had no effect on the total water soluble carbohydrate levels in both chicory and plantain. This was unexpected, as it was assumed that Lax grazing would encourage greater root carbohydrate stores. Conversely, Alemseged (2000) found chicory has a greater allocation of assimilates to root growth under repeated defoliation compared to no defoliation and concluded that this is probably a physiological response to growth unit survival.

## 5.6 Conclusion

This experiment indicates that the Herb and Legume Mix is highly productive and persistent under sheep grazing. The sward was persistent under both Hard and Lax grazing treatments for the two year experimental period. The Hard grazing treatment favoured plantain growth and persistence whereas; the Lax grazing treatment supported the maintenance of all four species in the mix. The grazing frequency was always kept at a minimum of three weeks, thus the plants were able to recover from hard grazing. Overall, this experiment illustrates the robustness of the Herb and Legume Mix, however further experiments are required to investigate the diet selection and preference of sheep grazing the Herb and Legume Mix (Chapter 6) and to investigate the Herb and Legume Mix under a wider range of defoliation regimes and compare it to ryegrass and white clover and pure swards of chicory and plantain (Chapter 7).

# Chapter 6 Ewe lamb diet selection on plantain (*Plantago lanceolata*) and on a Herb and Legume Mix, including plantain, chicory (*Cichorium intybus*), red clover (*Trifolium pratense*) and white clover (*Trifolium repens*)

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See statement of originality (Appendix 2)

## 6.1 Abstract

The objective of Experiment 1 was to examine the diet selection and grazing preference of ewe lambs for plantain (*Plantago lanceolata*), chicory (*Cichorium intybus*), red clover (*Trifolium pratense*) and white clover (*Trifolium repens*). This was examined in a herb and legume sward mix containing plantain, chicory, red clover and white clover between seasons under both hard- and lax-grazing. During early spring, the sward predominately comprised plantain (63%) and chicory (21%) and ewe lambs grazed a greater proportion ($P < 0.05$) of these species and displayed greater ($P < 0.05$) grazing preference for plantain and chicory ahead of red and white clover. From late spring onward, ewe lambs had a greater ($P < 0.05$) defoliation rate and a greater ($P < 0.05$) grazing preference for red clover than for all other species. This greater selection of red clover was particularly evident in summer and autumn when red clover made a greater ($P < 0.05$) contribution to the sward composition (8–9%) and the overall sward nutritive value dropped. This supports previous evidence, suggesting that diet selection is intrinsically affected by availability, access and palatability. The objective of Experiment 2 was to determine whether ewe lamb grazing behaviour during late summer was affected by time since a pure plantain sward was previously grazed. Ewe lambs were observed while grazing plantain of varying weeks since previous grazing (3, 6,10 or 16 weeks). Experiment 2 found no difference ($P > 0.05$) in the average number of ewe lambs grazing each sward of varying weeks since previous grazing. However, ewe lambs grazed the 3-week-old plantain growth to a lower ($P < 0.05$) post-grazing sward height than the height of the swards of greater number of weeks since previous grazing. Organic matter digestibility and metabolisable energy content did not differ ($P > 0.05$) among all swards, regardless of weeks since previous

grazing. This suggests that the palatability of plantain is not affected by the time since it was last grazed.

## 6.2 Introduction

New Zealand pastoral systems have been based on perennial ryegrass (*Lolium perenne*), with a relatively minor component of white clover (*Trifolium repens*) (Kemp *et al.* 2002b). However, throughout summer and autumn, production of ryegrass and white clover pasture can be limited in both quality and quantity, resulting in reduced feed intake and poor animal performance (Hughes *et al.* 1980; Burke *et al.* 2002; Moorhead *et al.* 2002).

In summer, the post-weaning liveweight gains of lambs grazing perennial ryegrass pastures are typically 80–160 g/day (Fraser and Rowarth 1996; Kerr 2000; Bluett *et al.* 2001; Lindsay *et al.* 2007). In contrast, post-weaning liveweight gains of 250 g/day are achievable over summer and autumn on pure swards of chicory (*Cichorium intybus*), white clover or red clover (*Trifolium pratense*) (Scales 1995; Fraser *et al.* 2004; Marley *et al.* 2005; Lindsay *et al.* 2007), while plantain (*Plantago lanceolata*) can support post-weaning lamb liveweight gains of 220 g/day during summer (Moorhead *et al.* 2002). Combining chicory, plantain, red and white clover in a mixed sward provides a pasture that is more productive and has a longer growing season than any one of the species by itself (Kemp *et al.* 2010). In addition, this mixed sward can support higher lamb liveweight gains than does a perennial ryegrass and white clover pasture (Golding *et al.* 2011; Sinhadipathige *et al.* 2012).

When given a choice, both sheep and cattle will actively select a mixed diet, even in situations where a mono-specific diet is readily available (Parsons *et al.* 1994a; Cosgrove *et al.* 1995; Rutter *et al.* 2000). In an indoor study, Pain *et al.* (2010) observed that weaned lambs preferentially ate red clover before chicory and plantain, and this preference did not differ during both spring and summer. Likewise, in an outdoor study, Corkran (2009) observed that weaned lambs grazing a mixed sward of plantain, chicory and red clover during early autumn preferentially selected red clover before chicory and then plantain. It has been reported that lambs experience palatability problems with plantain during late summer–early autumn (Fraser and Rowarth 1996; Swainson and Hoskin 2006). Swainson and Hoskin (2006) hypothesised that this effect may be associated with reproductive development of the plant. The palatability of perennial ryegrass has been shown to differ among seasons (Hodgson 1975). No studies have examined the preference of lambs grazing herb and legume swards throughout the year.

Previous studies using perennial ryegrass and white clover have shown that within a sward, forage of a greater sward height is consistently selected, regardless of whether monocultures or mixtures are offered to sheep (Illius *et al.* 1992; Concha and Nicol 2000). Through modelling, Parsons *et al.* (1991) concluded that at high intensities of grazing, the composition of the diet is more a reflection of differences in growth rate among plant species than of the preferences of grazing animals. However, no studies have examined the effect of pre-grazing sward height (generated via hard- and lax-grazing intensities) on diet selection of lambs grazing herb and legume swards, so it is unknown whether these inferences hold for these species.

Therefore, it is probable that diet selection is affected by both grazing intensity and season. The aims of the present study were to observe the diet selection of ewe lambs grazing a herb and legume sward mix between seasons under both hard- and lax-grazing conditions and to observe the grazing behaviour of ewe lambs grazing pure swards of plantain with varying times since it was last grazed.

## 6.3 Materials and methods

### 6.3.1 Experiment 1

#### *6.3.1.1 Experimental design*

A sward mix containing plantain (Ceres Tonic; 6 kg/ha), chicory (Puna II; 6 kg/ha), red clover (Sensation; 6 kg/ha) and white clover (Tribute; 4 kg/ha) was established in a total area of 0.24 ha in March 2011. Two grazing treatments, namely hard-grazing (post-grazing residual of ~4 cm) and lax-grazing (post-grazing residual of ~8 cm), were replicated in four blocks, with individual plots of 300 $m^2$.

The same group of ewe lambs was used to graze the plots, with preference measurements being taken on four occasions over the 2011–2012 growing season, across the different seasons (6–8 September, early spring; 18–21 October, late spring; 18–23 December, summer; 6–8 March, autumn). Stocking rate ranged between six and seven ewe lambs per plot (equivalent to 200–233 ewe lambs per hectare) and was adjusted between grazing periods and plots, depending on the season and predicted herbage growth, to ensure the post-grazing residual targets were met (i.e. 4 and 8 cm), as demonstrated in Sinhadipathige *et al.* (2012). Ewe lambs remained on these plots for a maximum of 7 days. In the interim, between treatment grazing periods, the ewe lambs were grazed on a perennial ryegrass and white clover pasture.

#### *6.3.1.2 Preference measurements*

Herbage samples were collected for analysis of botanical composition on 5 September 2011 (before first grazing). These were separated into species (plantain, chicory, red clover, white clover), with weeds being excluded from analysis. Samples were oven dried at 70°C, until a constant weight was achieved and an overall sward composition was identified. This consisted of 67% plantain, 26% chicory, 4% red clover and 3% white clover.

Diet selection was measured under the two grazing treatments using 48 2-m transects (Bootsma *et al.* 1990; Betteridge *et al.* 1994; López *et al.* 2003) randomly placed within the eight plots (six per plot, 24 per treatment). Numbered wooden pegs were placed at the end of each 2-m transect to aid in relocation. A rod was placed between the pegs and tagged at 15-cm spacings. The closest plant to each 15-cm marking was identified and a coloured cable wire was placed around the base of the plant close to the ground. This resulted in 10 plants per transect, 60 plants per plot and 240 per treatment. This meant that plant species were tagged in a manner that was comparable with the botanical composition of the sward at the time of tagging (given that the botanical composition is based on DM contribution while the percentage of tagged plants is based on individual plant numbers). This resulted in tagged plants comprising 40% plantain, 35% chicory, 14% red clover and 11% white clover.

Transects and each tagged plant were checked on Days 1–3 of the grazing period, at the same time every day, with the day of ewe lamb introduction being Day 0. Whether or not a plant had been grazed was recorded. Grazing was defined to have occurred if there were signs of partial or full leaf defoliation or if reproductive material had been removed. Once identified as grazed, a plant was no longer recorded. This method allows for an accurate evaluation of grazing defoliation by observing and regularly checking individual tagged tillers and/or plants (Bootsma *et al.* 1990; Betteridge *et al.* 1994; López *et al.* 2003).

During each season (grazing period), all grazed, tagged plants were given a corresponding number (1–3) representing the day in which each was first grazed by ewe lambs. Tagged plants that were not grazed were excluded from the analysis.

Selection index (SI; utilisation relative to availability) was calculated as $SI_y = (C_y/D)/RA_y$, where $SI_y$ is the selection index for Species *y*, $C_y$ is the number of times Species *y* was consumed, *D* is the sum of all observations in the diet, and $RA_y$ is the relative abundance of Species *y* (Basha *et al.* 2009). The relative abundance of each species was calculated as the number of tagged plants of a species divided by the total number of tagged plants of all species, multiplied by 100. A SI was created for each species within each plot for each season and each day.

The SI for each species can be used as an indicator of grazing preference. A value of 1 indicates that ewe lambs are grazing a species in proportion with what is available in the sward; i.e. if the sward contains 30% chicory, then 30% of their total diet selected will be chicory. A value greater than 1 indicates that ewe lambs are actively selecting a species from the sward mix, while a value less than 1 indicates that ewe lambs are avoiding selecting that species from the sward mix.

#### *6.3.1.3 Herbage measurements*

Herbage mass was measured pre-grazing by taking three 0.1 $m^2$ quadrat cuts (Frame 1993) from each plot (12 per treatment). These samples were cut at ground level with an electric shearing hand-piece and then washed before oven drying for at least 24 h at 70°C, until a constant weight was achieved. Averages of the three dried quadrat samples were used to determine DM values for each plot. Sward height was measured pre-grazing, by taking 50 measurements in each plot with a sward stick (Rhodes 1981; Hutchings 1991).

Sward strata samples to assess the vertical distribution of herbage within the sward were collected on an undisturbed height basis on the first day of grazing preference weeks (5 September, 17 October, 18 December 2011 and 5 March 2012). Two 0.1 $m^2$ quadrat samples per plot (eight per treatment) were taken by cutting the herbage into three strata, namely above 8 cm, between 4 and 8 cm and at ground level, a method based on Milne *et al.* (1982). The samples were then separated into species (plantain, chicory, red clover, white clover, other (all other species including grasses and weeds)) and each species oven-dried at 70°C, until a constant weight was achieved. The botanical composition was calculated for each sward stratum (8+ cm, 4–8 cm, 0–4 cm) on a dry-weight basis.

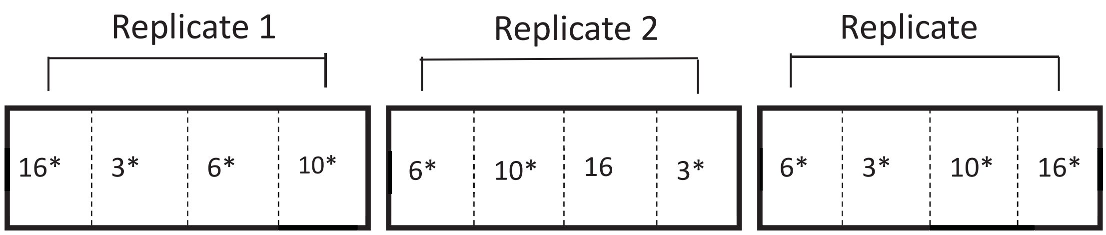
Grab herbage samples (Frame 1993) of the mixed sward (~100 g wet weight) were taken pre-grazing by walking in a random pattern, within each plot (four per treatment). All samples were freeze-dried and ground to pass a 1-mm sieve and analysed for digestibility (and hence predicted metabolisable energy; (Roughan and Holland 1977)) and crude protein (CP; total combustion method), neutral detergent fibre (NDF; (Robertson and Van Soest 1981)), organic matter (OM) and ash.

### 6.3.2 Experiment 2

#### *6.3.2.1 Experiment design*

A 2-year old sward of plantain (Ceres Tonic) was used. The sward was sprayed with 3,6-dichloro-2-methoxybenzoic acid (1.0 L/ha; Dicamba; Banvel, Orion Crop Protection, Christchurch, New Zealand) to remove other species before the experimental period. A randomised complete block design with four treatments and three replicates (blocks), making a total of 12 plots, was utilised in the study (

Figure 6.1). The aim was to observe the grazing behaviour of ewe lambs grazing pure swards of plantain herbage, with a varying number of weeks since it was previously grazed (3, 6, 10 and 16 weeks). Weeks 3 and 6 were chosen to represent younger growth, while week 10 was representative of older growth. Week 16 was used as an extreme to ensure a treatment effect would be observed if there was one. The 12 plots measured 8 × 5.8 m each, thus giving each replicate (block) a total area of 184 m$^2$. The plots were grazed to ~5 cm above ground on differing dates, to establish these swards. Before each observation event, the fences were removed from between plots (treatments), so that all four herbage treatments within the replicate (block) were accessible to the ewe lambs.




*Numbers refer to weeks since plot (treatment) was previously grazed

**Figure 6.1 Experiment design of Experiment 2, with each replicate (block) containing all four plantain treatments of varying weeks since it was previously grazed, and fences between treatments (plots) removed before sheep observation period.**

Ten Romney ewe lambs that had previously grazed a mixed sward of plantain, chicory, red and white clover at regular intervals throughout spring and summer were used in the present study. The 10 ewe lambs were divided into two groups of five. For 4 days before the study period, the two groups were grazed for a 4 hour period daily on a plantain and chicory mix that had not been grazed for 8 weeks (old) or that had not been grazed for 2 weeks (young). Grazing was managed such that one group grazed the older herbage on Days 1 and 2, while the other group grazed the young herbage. This was then reversed for Days 3 and 4. When the ewe lambs were not on the pre-study plots and between the observation periods, they were placed on ryegrass white clover pasture (herbage mass range 500–600 kgDM/ha) with water.

#### *6.3.2.2 Observation measurements*

All 10 ewe lambs were placed into each replicate (which contained all four treatments) for a 2 hour observation period. Thus, in total, three 2 hour observation sessions (one for each replicate) were undertaken between 0800 hours and 1000 hours and between 1700 hours and 1900 hours on Monday, 4 February 2013, and between 0800 hours and 1000 hours on Tuesday, 5 February 2013. Observation times were chosen to match with reported daily grazing times (Penning *et al.* 1991).

The grazing behaviour of the ewe lambs was recorded by three people. At 2-min intervals, the number of lambs grazing within each plot (treatment) was recorded. Each person was responsible for recording lambs in one or two treatment plots. Grazing was defined as head down, eating. This is a simplification of the most commonly used method of gauging grazing preference from ruminants in their natural environment (Penning and Rutter 2004; Rutter 2006).

#### *6.3.2.3 Herbage measurements*

Sward height was measured by taking 50 measurements within each plot before and after the observation period by using a sward stick. Exclusion cages (0.55 × 0.55 m) were set up within each plot before the observation period. Grab herbage samples were randomly collected from each plot and analysed for characteristics of nutritive value, as for Experiment 1. These samples were collected to represent what was observed to have been ‘eaten’ and ‘not eaten’ by the ewe lambs.

### 6.3.3 Statistical analyses

All statistical analyses were performed using SAS (Statistical Analysis System, version 9.2; SAS Institute Inc., Cary, NC, USA).

#### *6.3.3.1 Experiment 1*

##### 6.3.3.1.1 Preference data.

The cumulative proportion of plants eaten each day and the SI for each day were analysed using the MIXED procedure, with a model that included the fixed effects of day (1, 2, 3), species (plantain, chicory, red clover, white clover), season (early spring, late spring, summer, autumn) and the triple interaction among day, species and season and the random effect of block, to account for the repeated-measures through days and seasons. Alternative covariance structures were investigated on the basis of biological plausibility, including variance, compound symmetry, unstructured and autoregressive options. Using the Akaike's information criterion, a compound symmetry error structure was determined as the most appropriate residual covariance structure.

##### 6.3.3.1.2 Nutritive value, sward height and dry matter data.

The data were analysed using the MIXED procedure with a model including the fixed effects of grazing treatment (hard *v.* lax), season (early spring, late spring, summer, autumn) and the interaction between grazing treatment and season and the random effect of block to take account for the repeated-measures through seasons.

##### 6.3.3.1.3 Sward strata composition data.

The dry weight of each species was converted into a percentage of the total weight within its respective sward-height stratum. These data were analysed using the MIXED procedure, with a model including the fixed effects of grazing treatment (hard *v.* lax), season (early spring, late spring, summer, autumn), sward strata (8+ cm, 4–8 cm, 0-4 cm), species (plantain, chicory, red clover, white clover, other) and the random effect of block.

#### *6.3.3.2 Experiment 2*

##### 6.3.3.2.1 Sward-height data.

The pre- and post-grazing data were analysed using the MIXED procedure, with a model including the fixed effect of herbage treatment (3, 6, 10 and 16 weeks since plantain was last grazed) and the random effect of replicate. The post-grazing sward-height data was re-analysed with pre-grazing sward height as a covariate.

##### 6.3.3.2.2 Observation data.

The raw data gave the number of ewe lambs grazing each plot every 2 min. For analysis, every five data points (i.e. every 10 min of recording) were averaged to give the number of ewe lambs grazing each plot over 10-min intervals, which therefore gave 12 ‘time periods’ for analysis (i.e. over 120 min). Averages every 10 min were used to reduce the quantity of data involved and to reduce the error of non-independent data, since 10 ewe lambs grazing together may be expected to display some group behaviour. These data were analysed using the MIXED procedure, with a model including the fixed effects of herbage treatment (3, 6, 10 and 16 weeks since plantain was grazed), pre-experiment herbage-adjustment period (old *v.* young plantain and chicory mix), replicate and included time period as a repeated-measure.

#### 6.3.3.2.3 Nutritive-value data.

The data were analysed using the MIXED procedure, with a model including the fixed effects of herbage treatment (3, 6, 10 and 16 weeks since plantain was grazed), and grab sample type ('eaten' *v.* 'not eaten') and the random effect of replicate.

## 6.4 Results

### 6.4.1 Experiment 1

#### *6.4.1.1 Sward height and herbage mass*

There tended ($P = 0.051$) to be an interaction between grazing treatment and season for sward height, whereby the lax treatment plots had a greater ($P < 0.05$, as indicated by the pairwise analysis) sward height than the hard treatment plots in all seasons except early spring (Table 6.1). There was no ($P > 0.05$) interaction between grazing treatment and season on herbage mass. Herbage mass was lower ($P < 0.05$) during late spring than during summer and autumn, which did not differ ($P > 0.05$). Grazing treatment had no ($P > 0.05$) effect on herbage mass.

#### *6.4.1.2 Grazing treatment*

There was no ($P > 0.05$) interaction between grazing treatment (hard and lax) and season for any of the nutritive-value traits. Grazing treatment had no effect ($P > 0.05$) on sward strata composition, defoliation rate of tagged plants or SI (data not shown) and was therefore removed from all subsequent analyses.

**Table 6.1 Sward height (cm) and herbage masses (kg DM/ha) pre-grazing (mean ± s.e.) for hard- and lax-grazing treatments and for each season. Means within columns and parameters followed by the same letter are not significantly different at *P* = 0.05.**

| Parameter | Sward height (cm) | Herbage mass (kg DM/ha) |
|---|---|---|
| *Season* | | |
| Early spring | 14.6a ± 0.59 | 2264ab ± 206 |
| Late spring | 18.1b ± 0.59 | 1815a ± 169 |
| Summer | 17.9b ± 0.59 | 2486b ± 172 |
| Autumn | 18.7b ± 0.59 | 2565b ± 169 |
| *Grazing treatment* | | |
| Hard | 15.6a ± 0.18 | 2072 ± 126 |
| Lax | 19.0b ± 0.18 | 2493 ± 128 |
| *Season by grazing treatment* | | |
| Early spring – hard | 14.4a ± 0.71 | 2243a ± 292 |
| Early spring – lax | 14.9a ± 0.71 | 2285a ± 292 |
| Late spring – hard | 16.1b ± 0.71 | 1793a ± 238 |
| Late spring – lax | 20.1c ± 0.71 | 1837a ± 238 |
| Summer – hard | 15.4b ± 0.71 | 1998a ± 238 |
| Summer – lax | 20.3c ± 0.71 | 2975b ± 249 |
| Autumn – hard | 16.7b ± 0.71 | 2254a ± 238 |
| Autumn – lax | 20.6c ± 0.71 | 2877a ± 238 |

**Table 6.2 The effect of season on nutritive-value traits (mean ± s.e.): OMD (%DM), organic matter digestibility; Ash (%DM), as (%DM), neutral detergent fibre; ADF (%DM), acid detergent fibre; and ME (MJ/kg DM), metabolisable energy. Means within co significantly different at *P* = 0.05.**

| Season | OMD | Ash | CP | NDF | AD |
|---|---|---|---|---|---|
| Early spring | 84.1c ± 0.62 | 12.7c ± 0.23 | 23.8b ± 0.49 | 19.4a ± 0.82 | 13.5a ± |
| Late spring | 84.3c ± 0.62 | 11.9b ± 0.23 | 24.7b ± 0.49 | 19.3a ± 0.82 | 13.2a ± |
| Summer | 78.1a ± 0.62 | 11.0a ± 0.23 | 21.3a ± 0.49 | 27.9c ± 0.82 | 18.5c ± |
| Autumn | 81.2b ± 0.62 | 11.2a ± 0.23 | 21.0a ± 0.49 | 24.7b ± 0.82 | 15.9b ± |

#### 6.4.1.3 The early spring period

During early spring, the sward had %OMD, %CP and ME content similar ($P > 0.05$) to those found during late spring, and these were greater ($P < 0.05$) than those seen during summer and autumn (Table 6.2). The %NDF and %ADF were similar ($P > 0.05$) to those found during late spring and lower ($P < 0.05$) than those seen during summer and autumn. The %Ash during early spring was greater ($P < 0.05$) than that found in all other seasons.

The upper stratum of the sward (>8 cm) had a greater ($P < 0.05$) proportion of plantain than chicory, which was greater ($P < 0.05$) than the proportions of both red and white clover (Figure 6.2). The middle (4–8 cm) and lower (<4 cm) strata contained a greater ($P < 0.05$) proportion of chicory than did the upper stratum (>8 cm). The proportion of red and white clover in all three strata was lower ($P < 0.05$) than the proportions of plantain and chicory in each strata.

On Day 1 of grazing, there was no difference ($P > 0.05$) in the proportion of plants of each species eaten (Figure 6.3). By Days 2 and 3 of grazing, the cumulative proportions of chicory and plantain plants eaten were similar ($P > 0.05$) and greater ($P < 0.05$), respectively, than the cumulative proportions of red and white clover plants eaten (values ranged between 26% and 33% greater).

Over the first 3 days of grazing, the SI indicated that ewe lambs selected plantain and chicory more often than ($P < 0.05$) red and white clover (Figure 6.4). The SI for plantain and chicory did not differ ($P > 0.05$). Likewise, the SI for red and white clover did not differ ($P > 0.05$).

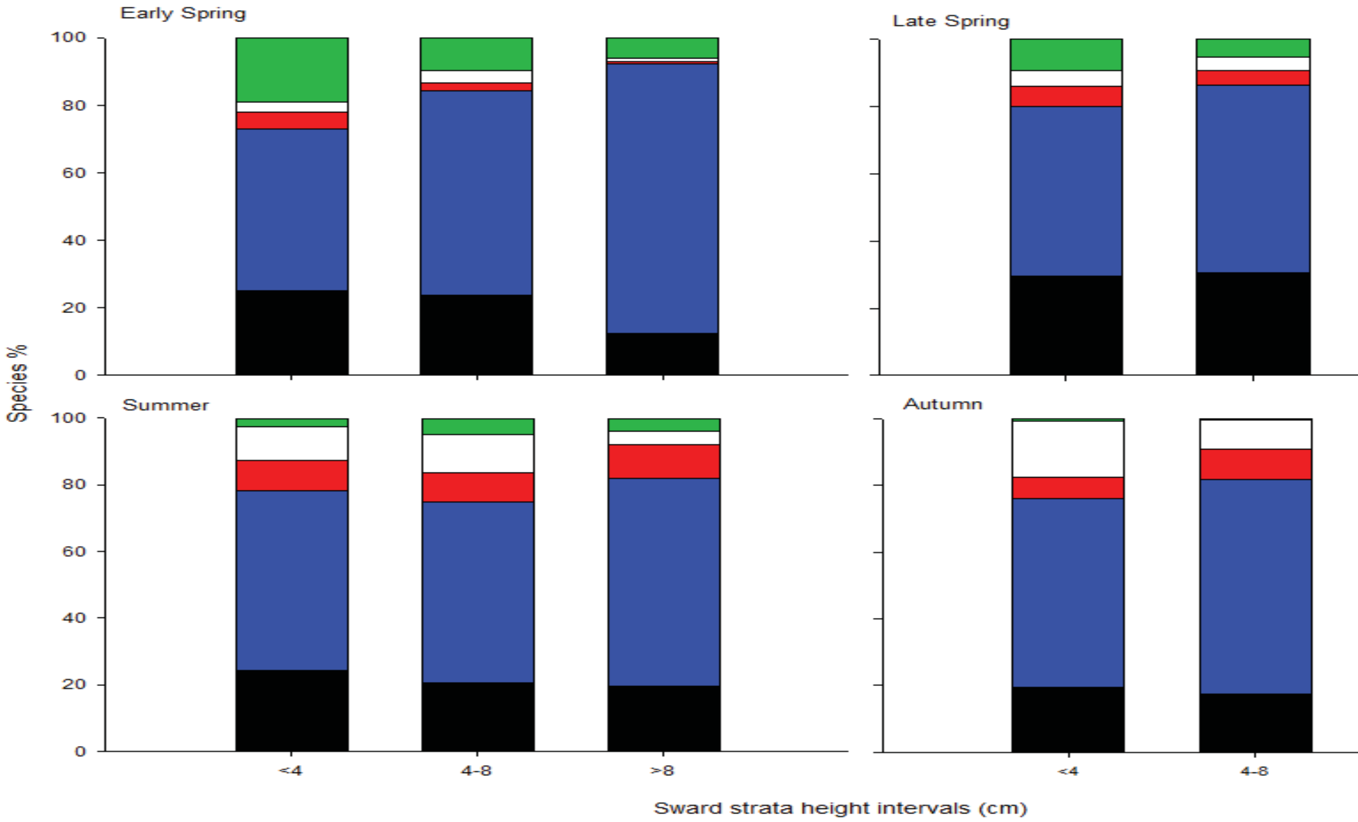



**Figure 6.2 Botanical composition of chicory (black), plantain (blue), red clover (red), white clover (white) and weed (green) w cm) during early spring (top left), late spring (bottom left), summer (top right) and autumn (bottom right). All species within 3.17%.**

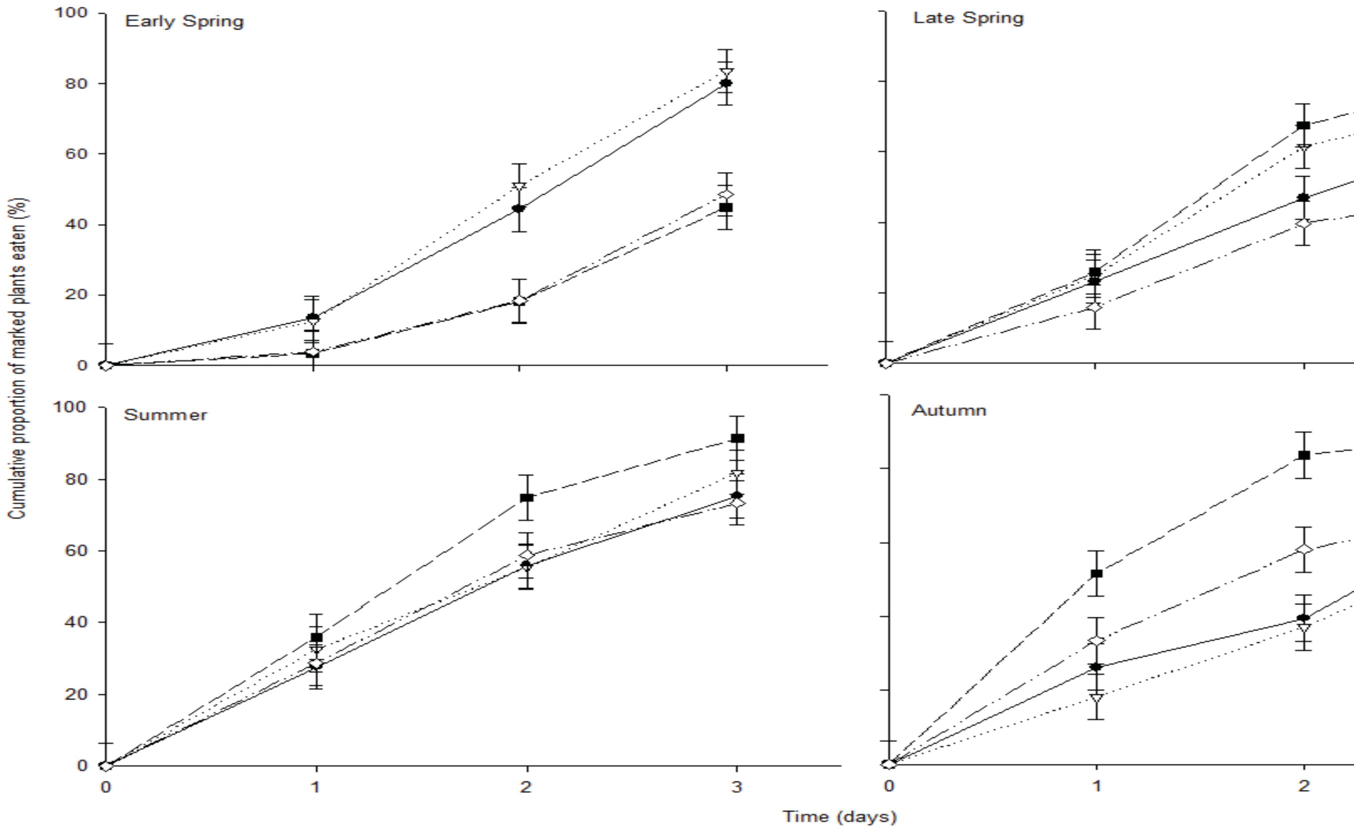



**Figure 6.3 Defoliation rate over the first 3 days of grazing of tagged plant species chicory (●), plantain (▽), red clover (■) and left), late spring (bottom left), summer (top right) and autumn (bottom right). The error bars denote the s.e of difference amo**

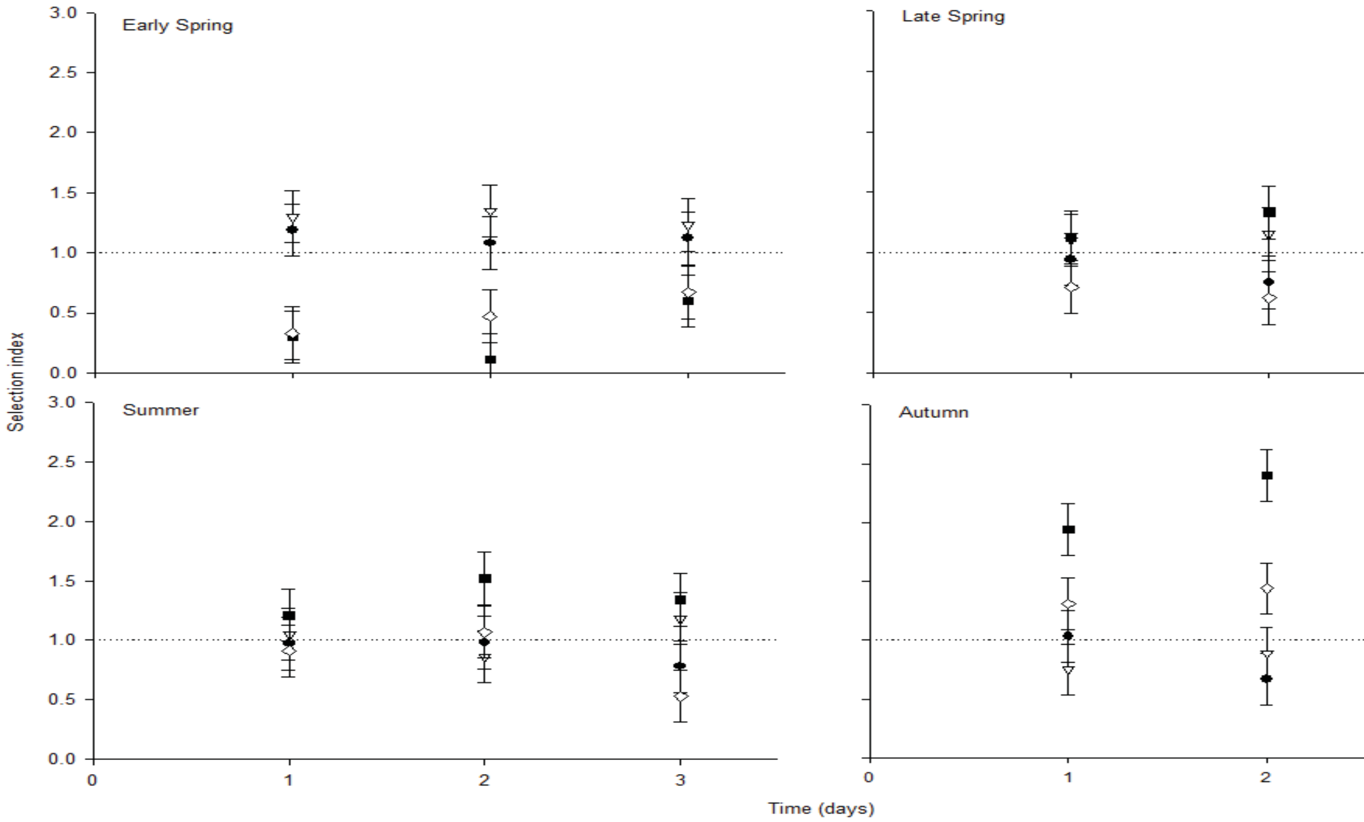



**Figure 6.4 Diet selection index over the first 3 days of grazing of tagged plant species chicory (●), plantain (▽), red clover (■**
**(top left), late spring (bottom left), summer (top right) and autumn (bottom right). The error bars denote the s.e of difference b**

#### *6.4.1.4 The late spring period*

During late spring the %OMD, %CP, %NDF, %ADF and ME content of the sward remained similar ($P > 0.05$) to that seen in early spring (Table 6.2). However, the %Ash was lower ($P < 0.05$) in late spring than in early spring.

The proportion of plantain in the upper stratum of the sward (>8 cm) was lower ($P < 0.05$) in late spring than in early spring (Figure 6.2). The middle (4–8 cm) and upper (>8 cm) strata contained a greater ($P < 0.05$) proportion of chicory in late spring than in early spring. In late spring, the proportions of red and white clover in all three strata were similar ($P > 0.05$) to that seen in early spring. Within late spring, the proportion of red and white clover in all three strata was lower ($P < 0.05$) than were the proportions of plantain and chicory in each strata.

On Day 1 of grazing, there was no difference ($P > 0.05$) in the proportion of plants of each species eaten (Figure 6.3). By Day 2 of grazing, the cumulative proportions of red clover and plantain plants eaten were similar ($P > 0.05$) and greater ($P < 0.05$), respectively, than the cumulative proportions of chicory and white clover plants eaten (values ranged between 9% and 27% greater). By Day 3 of grazing, the cumulative proportion of white clover plants eaten was 18–33% lower ($P < 0.05$) than that of all other species. The cumulative proportion of red clover plants eaten was 15% greater ($P < 0.05$) than the cumulative proportion of chicory plants eaten.

On Day 1 of grazing, the SI indicated ewe lambs displayed no grazing preference ($P > 0.05$; Figure 6.4). By Day 2 of grazing, ewe lambs displayed a grazing preference for red clover over white clover ($P < 0.05$). By Day 3 of grazing, ewe lambs displayed a negative grazing preference ($P < 0.05$) for white clover compared with all other species, which did not differ ($P > 0.05$).

#### *6.4.1.5 The summer period*

During summer the %NDF and %ADF of the sward were greater ($P < 0.05$) than those seen during early and late spring (Table 6.2). The %OMD, %CP and ME content of the sward were lower ($P < 0.05$) than those seen during early and late spring. The %Ash was lower ($P < 0.05$) in summer than in late spring.

The proportion of plantain in each stratum of the sward was similar ($P > 0.05$) to that found in late spring (Figure 6.2). The proportion of chicory in the mid- (4–8 cm) and upper (>8 cm) strata was lower ($P < 0.05$) than that found in late spring. The proportion of red clover in the upper (>8 cm) stratum was greater ($P < 0.05$) than that found in late spring.

On Day 1 of grazing, there was no difference ($P > 0.05$) in the proportion of plants of each species eaten (Figure 6.3). By Day 2 of grazing, the cumulative proportion of red clover plants eaten was greater ($P < 0.05$) than the cumulative proportion of all other plant species eaten (values ranged between 16% and 20% greater). By Day 3 of grazing, the cumulative proportion of red clover plants eaten was 18% greater ($P < 0.05$) than the cumulative proportion of white clover plants eaten, and tended to be 16% greater ($P = 0.07$) than the cumulative proportion of chicory plants eaten. The cumulative proportion of plantain plants eaten was greater ($P < 0.05$) than the cumulative proportion of white clover plants eaten, but similar ($P > 0.05$) to red clover and chicory.

The SI indicated that on the Day 1 of grazing, ewe lambs displayed no grazing preference ($P > 0.05$; Figure 6.4). By Day 2 of grazing, ewe lambs displayed a grazing preference for red clover over plantain ($P < 0.05$). By Day 3 of grazing, ewe lambs displayed a grazing preference ($P < 0.05$) for red clover and plantain over white clover.

#### *6.4.1.6 The autumn period*

During autumn the %NDF and %ADF were lower ($P < 0.05$) than those seen during summer but still higher ($P < 0.05$) that those observed during early and late spring (Table 6.2). Similarly, the %OMD and ME content were greater ($P < 0.05$) than those seen during summer but still lower ($P < 0.05$) than those observed during early and late spring.

The proportions of red clover, white clover and chicory in each stratum of the sward were similar ($P > 0.05$) to those found in summer (Figure 6.2). The proportions of plantain in the upper (>8 cm) and mid- (4–8 cm) strata of the sward were greater ($P < 0.05$) than those found during summer.

Over all 3 days of grazing, the cumulative proportion of red clover plants eaten was greater ($P < 0.05$) than that of all other species (Figure 6.3). On Day 1 of grazing, the proportion of white clover plants eaten was greater ($P < 0.05$) than the cumulative proportion of plantain plants eaten. The cumulative proportion of chicory plants eaten did not differ ($P > 0.05$) from either white clover or plantain. By Day 2 of grazing, the cumulative proportion of white clover plants eaten was greater ($P < 0.05$) than the cumulative proportion of both chicory and plantain eaten, which did not differ ($P > 0.05$) from each other. By Day 3 of grazing, there was no difference ($P > 0.05$) in the cumulative proportion of white clover, chicory and plantain plants eaten.

The SI indicated that the ewe lambs displayed a strong grazing preference ($P < 0.05$) for red clover over all other species on Days 1 and 2 of grazing (Figure 6.4). On Day 1, ewe lambs also displayed a grazing preference ($P < 0.05$) for white clover over plantain. By Day 2 of grazing, ewe lambs displayed a preference ($P < 0.05$) for white clover over both plantain and chicory, which did not differ ($P > 0.05$) from each other. By Day 3 of grazing, no differences ($P > 0.05$) in the grazing preference of each species were evident.

### 6.4.2 Experiment 2

The pre-experiment herbage-adjustment period had no effect ($P > 0.05$) on observed grazing behaviour of the ewe lambs (data not shown). During the measurement period, the ewe lambs grazed the 3-week treatment to a lower ($P < 0.05$) post-grazing sward height than for all other treatments of greater number of weeks since previous grazing (Table 6.3). There was no difference ($P > 0.05$) in post-grazing sward height of the 6, 10, 16 weeks since previous grazing treatments. When pre-grazing sward height was included as a covariate in the analysis, this relationship was still apparent (13.0 ± 1.57, 18.4 ± 1.43, 19.0 ± 1.50 and 18.6 ± 1.44 cm for treatments; 3, 6, 10 and 16 weeks since previous grazing, respectively). There was no difference ($P > 0.05$) in the average number of ewe lambs grazing any treatment of varying weeks since previous grazing at each record. The 3-week treatment had little visible stem material, whereas the other treatments appeared to have a substantial amount of visible stem.

**Table 6.3 The effect of number of weeks since plantain was previously grazed on pre- and post-grazing sward height and the average number of ewe lambs recorded as grazing at each record. Means within columns followed by the same letter are not significantly different at *P* = 0.05.**

| Weeks since plantain was grazed | Pre-graze sward height (cm) | Post-graze sward height (cm) | Average number of lambs grazing each herbage treatment at each record |
|---|---|---|---|
| 3 | 24.7a ± 0.86 | 12.6a ± 1.50 | 2.2 ± 0.24 |
| 6 | 27.7b ± 0.86 | 18.5b ± 1.50 | 1.9 ± 0.24 |
| 10 | 28.5b ± 0.86 | 19.1b ± 1.50 | 1.6 ± 0.24 |
| 16 | 27.9b ± 0.86 | 18.7b ± 1.50 | 1.8 ± 0.24 |

There were no ($P$ > 0.05) interactions between weeks since plantain was previously grazed and grab sample type (eaten or not eaten) for any of the nutritive value traits. The number of weeks since previous grazing had no effect ($P$ > 0.05) on %OMD or ME content (Table 6.4). The %CP and the %Ash were similar ($P$ > 0.05) and greater ($P$ < 0.05) for the 6- and 10-week treatments, than for the 3- and 16-week treatments, which did not differ ($P$ > 0.05) from each other. There tended to be an effect of number of weeks since previous grazing on %NDF and %ADF ($P$ = 0.09 and $P$ = 0.05, respectively), with pairwise analysis indicating a difference between the 3- and 10-week treatments ($P$ < 0.05).

In the grab samples representing 'eaten' and 'not eaten' plantain, %NDF and %ADF were lower ($P$ < 0.05) in the 'eaten' samples. The %OMD, %CP, %Ash and ME content were greater ($P$ < 0.05) in the 'eaten' samples than in the 'not eaten' samples.

**Table 6.4 The effect of number of weeks since plantain was previously grazed and 'eaten' and 'not eaten' samples of planta OMD (%DM), organic matter digestibility; Ash (%DM), ash content; CP (%DM), crude protein; NDF (%DM), neutral detergent fi ME (MJ/kg DM), metabolisable energy. Means within columns and parameters followed by the same letter are not significantly**

| Parameter | OMD | Ash | CP | NDF | ADF |
|---|---|---|---|---|---|
| | | | *Weeks since plantain was previously grazed* | | |
| 3 | 76.4 ± 0.73 | 12.8b ± 0.27 | 20.7b ± 0.83 | 32.2a ± 1.01 | 20.8a ± |
| 6 | 75.2 ± 0.73 | 10.6a ± 0.27 | 16.0a ± 0.83 | 33.7ab ± 1.01 | 22.1ab ± |
| 10 | 74.2 ± 0.73 | 10.5a ± 0.27 | 16.6a ± 0.83 | 36.5b ± 1.01 | 23.7b ± |
| 16 | 75.7 ± 0.73 | 12.1b ± 0.27 | 20.4b ± 0.83 | 34.9ab ± 1.01 | 22.1ab ± |
| | | | *Grab sample type* | | |
| Eaten | 78.1b ± 0.52 | 11.9b ± 0.19 | 21.4b ± 0.59 | 30.1a ± 0.71 | 18.9a ± |
| Not eaten | 72.6a ± 0.52 | 11.1a ± 0.19 | 15.4a ± 0.59 | 38.6b ± 0.71 | 25.4b ± |
| | | | *Weeks since plantain was previously grazed by grab sample type* | | |
| 3 – eaten | 79.7c ± 1.03 | 12.9b ± 0.38 | 24.0d ± 1.17 | 26.1a ± 1.42 | 16.6a ± |
| 3 – not eaten | 73.1a ± 1.03 | 12.6b ± 0.38 | 17.4bc ± 1.17 | 38.2d ± 1.42 | 24.9cd ± |
| 6 – eaten | 76.7bc ± 1.03 | 10.8a ± 0.38 | 19.4c ± 1.17 | 31.9bc ± 1.42 | 20.3b ± |
| 6 – not eaten | 73.7ab ± 1.03 | 10.3a ± 0.38 | 12.6a ± 1.17 | 35.6cd ± 1.42 | 24.0c ± |
| 10 – eaten | 77.0bc ± 1.03 | 10.9a ± 0.38 | 18.7c ± 1.17 | 31.8bc ± 1.42 | 20.5b ± |
| 10 – not eaten | 71.5a ± 1.03 | 10.2a ± 0.38 | 14.5ab ± 1.17 | 41.3d ± 1.42 | 26.9d ± |
| 16 – eaten | 79.1c ± 1.03 | 13.0b ± 0.38 | 23.6d ± 1.17 | 30.6ab ± 1.42 | 18.3ab ± |
| 16 – not eaten | 72.2a ± 1.03 | 11.2a ± 0.38 | 17.2bc ± 1.17 | 39.1d ± 1.42 | 25.8cd ± |

## 6.5 Discussion

Experiment 1 helped provide fundamental insight into how the defoliation intensity of each of these species changes throughout the year in an Herb and Legume Mix. Diet selection is known to be affected by three intrinsically linked factors, namely, availability (horizontal availability) (Stephens and Krebs 1986; Thornley *et al.* 1994; 1996), access (vertical availability) (Parsons *et al.* 1994b; Edwards *et al.* 1996) and palatability (Malechek and Provenza 1983; Cheeke and Shull 1985; Bryant *et al.* 1991).

During the early spring period, ewe lambs had a higher defoliation rate and selection index for chicory and plantain than did red clover and white clover. In early spring, the sward comprised predominantly plantain and chicory, particularly in the middle and upper strata, illustrating that ewe diet selection was influenced by both availability and access. Similarly, Hodgson (1990a) stated that animals will often graze apparently indiscriminately in the surface layers of a sown sward, so that the botanical composition of the herbage consumed is very similar to the composition of the surface horizons. Stephens and Krebs (1986) concluded that for an animal to display preference for a species in a mixed sward, the benefits must be sufficient to overcome the ‘lost opportunities’ to eat all the immediately available herbage. During early spring, when the nutritive value of the sward was high, the ewe lambs chose a diet of species that were most readily available.

Ewe lambs showed a strong grazing preference for red clover from the late spring period onward. The higher intake potential, palatability and high preference for red clover by grazing sheep has previously been reported (Hunt and Hay 1990; Frame *et al.* 1998a; Fraser *et al.* 2004; Speijers *et al.* 2004; Pain *et al.* 2010). Speijers *et al.* (2004) attributed the high preference of red clover to its high CP content and rapid rumen clearance compared with perennial ryegrass and many other types of forage. The decrease in the overall sward nutritive value during the summer and autumn periods could explain why the preference for red clover was greater during that period, as red clover maintains a high nutritive value throughout summer and autumn (Brown 2004).

It is well established that white clover is highly palatable (Hunt and Hay 1990; Horadagoda *et al.* 2009). Therefore, in Experiment 1, the low defoliation rate and selection index of white clover, particularly during the early and late spring periods, was unexpected. This may be as a result of the limited vertical availability of white clover compared with the other species within the Herb and Legume Mix. Edwards *et al*. (1996) showed that diet selection is sensitive to the horizontal and vertical availability of the food alternatives. Parsons *et al.* (1994b) concluded that a decrease or increase in the relative vertical availability of a species may reverse species preferences. Harvey *et al.* (2000) found that when the sward height (vertical availability) of white clover was reduced compared with ryegrass, ewes had higher intakes of ryegrass than white clover. Conversely, Milne *et al.* (1982) showed that the apparent preference for white clover by sheep grazing a ryegrass and white clover mix was explained by a higher proportion of white clover foliage in the upper sward strata, where white clover made up at least 10% of the upper strata. Milne *et al.* (1982) indicated that 83% of the variation in the white clover content of the diet could be accounted for by the white clover content of the upper horizons of the sward grazed by the sheep.

The SI values of chicory and plantain decreased during the summer and autumn periods. Previous research has shown that chicory remains highly digestible throughout summer and autumn (Jackson *et al.* 1996). Therefore, the drop in the SI of chicory during summer and autumn is likely to result from the decreased availability of chicory in the upper-sward (>8 cm) and mid-sward (4–8 cm) strata. While in plantain, the digestibility and protein content has been found to be very low in summer–autumn (Fraser and Rowarth 1996), potentially resulting in reduced palatability and, thus, a lower selection index. It is well established that diet selection is driven by the nutritive value and chemical composition of the feed and subsequent post-ingestive feedback (Hodgson 1990a; Provenza 1995; Hodgson and Brookes 2002). Hakkila *et al.* (1987) reported that the diet of steers grazing range grasslands changed with advancing season, to maximise dietary quality. Thus, mixed swards may allow livestock to display more selective grazing behaviour and preferentially select specific plants and plant parts to better meet their nutritional requirements at a given time point.

Grazing treatment (hard or lax) had no effect on diet selection or forage preference. This has been reported not to be the case in tall fescue pastures, where palatability has been shown to be poor under lax-grazing conditions, as its palatability reduces with aging plant material (Easton *et al.* 1994). Thus, it could have been expected that greater diet selection would have been observed under the lax-grazing treatment. Penning *et al.* (1994) reported that grazing behaviour can change in response to a reduction in sward surface height. Studies have reported that grazing animals are more selective for white clover in ryegrass and white clover mixtures at high than at low herbage availability (Milne *et al.* 1982; Curll *et al.* 1985). In contrast, Edwards *et al.* (1996) found no effect of total availability on the diet selection of sheep.

When in a leafy vegetative state, plantain is highly palatable (Stewart 1996; Burke *et al.* 2000),; however, palatability is reduced and low intakes are observed when reproductive development occurs and leaves age (seed head makes up 60% of DM on offer) (Ivins 1952; Fraser and Rowarth 1996). Previous research has reported palatability problems with plantain in the late summer–early autumn period (Robertson *et al.* 1995; Fraser and Rowarth 1996; Moorhead *et al.* 2002). It is possible that this response may be linked to grazing management, whereby plantain with a longer grazing rotation could be less palatable to sheep. Experiment 2 examined the effect of weeks since plantain was previously grazed on ewe lamb grazing behaviour and found that it did not affect ewe lamb grazing behaviour. This may be because all treatments of varying weeks since previous grazing had a high overall nutritive value and there was little difference among the treatments. Grab samples of plantain observed to be eaten by ewe lambs had a higher nutritive value than did plantain observed to be uneaten. This is not surprising, as previous research has shown that sheep will select a diet of higher nutritive value than is the combined nutritive value of the sward on offer (Hodgson 1990a; Hodgson and Brookes 2002). Alternatively, poor palatability of plantain during autumn may be due to high concentrations of bioactive compounds (aucubin and acteoside), which have been reported to peak during autumn in plantain (Tamura and Nishibe 2002).

## 6.6 Conclusions

Experiment 1 demonstrated that when grazing a Herb and Legume Mix, ewe lambs showed diet selection and preference. This preference changed among seasons, likely being due to changes in species availability, vertical access and palatability. Further studies should investigate whether the observed grazing preferences result in significant changes to the sward dynamics over time. Experiment 2 in the autumn period demonstrated that the number of weeks since plantain was previously grazed did not affect ewe lamb grazing behaviour. Therefore, this does not explain why previous research has reported palatability problems with plantain in the late summer–early autumn period.

# Chapter 7 The herbage production and plant density of pure swards of plantain and chicory compared with a Herb and Legume Mix and a perennial ryegrass and white clover mix in response to the frequency and height of mowing

## 7.1 Abstract

The herbs chicory (*Cichorium intybus*) and plantain (*Plantago lanceolata*) are regarded as offering greater herbage yield and higher nutritive value feed during summer than perennial ryegrass (*Lolium* perenne) and white clover (*Trifolium repens*) swards. Four distinct sward types; I) Chicory Sward, II) Plantain Sward, III) Herb and Legume Mix; containing chicory, plantain, red clover (*Trifolium pratense*) and white clover, IV) Ryegrass and White Clover Sward were compared under a combination of four defoliation frequencies (D.F; weekly (F1), fortnightly (F2), three-weekly (F3), six-weekly (F6)), and three defoliation heights (D.H; 75 mm (H75), 50 mm (H50), 25 mm (H25)) over 24 weeks (19 September 2012 to 6 March 2013) in Palmerston North, New Zealand. Herbage production, botanical composition, plant density, taproot diameter and herbage nutritive values were monitored. Within the Chicory Sward, herbage yield and plant density were not affected ($P > 0.05$) by D.F or D.H. However, from week 12 onwards, F2 D.F resulted in a lower ($P < 0.05$) proportion of chicory in the sward, except H75 at week 24 and a smaller ($P < 0.05$) taproot diameter than F6 D.F. Within the Plantain Sward, herbage yield, plant density and taproot diameter were not affected ($P > 0.05$) by D.F or D.H. However, at week 24, F2 D.F resulted in a lower ($P < 0.05$) proportion of plantain in the sward than F1 D.F. Furthermore, F1 and F.2 D.F's had a greater ($P < 0.05$) proportion of weed in the sward at week 24 than at week 0. Within the Herb and Legume Mix, herbage yield and plant density were not affected ($P > 0.05$) by D.F or D.H. The botanical composition was relatively stable over time and between treatments. The F1 D.F resulted in a greater ($P < 0.05$) proportion of

white clover and a lower ($P < 0.05$) proportion of plantain in the sward than F2, F3 and F6 D.F's at week 24. The F6 D.F resulted in a greater ($P < 0.05$) proportion of red clover in the sward than F1 D.F's from week 18 onwards. The taproot diameter of chicory was smaller ($P < 0.05$) under F1, F2, F3 D.F than F6 D.F from week 18 onwards. The taproot diameter of plantain only differed at week 24 when the taproot diameter of F1 D.F was smaller ($P < 0.05$) than under the F6 D.F. All of the herb based swards had a greater ($P < 0.05$) herbage nutritive value in terms of neutral detergent fibre %, organic matter digestibility % and metabolisable energy content than the Ryegrass and White Clover Sward. Overall, this experiment provides evidence for recommendations on the optimal grazing management of pure swards of chicory and plantain and of the Herb and Legume Mix.

## 7.2 Introduction

New Zealand pastoral systems are traditionally based on perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) swards (Kemp *et al.* 2002b), however, pasture quality and quantity can be limited throughout summer and autumn (Hughes *et al.* 1980; Burke *et al.* 2002). In comparison, the perennial herbs chicory (*Cichorium intybus*) and plantain (*Plantago lanceolata*) are summer active, high yielding (Hare *et al.* 1987; Brown *et al.* 2000; Powell *et al.* 2007) and maintain high nutritive value (Barry 1998; Burke *et al.* 2006; Hayes *et al.* 2010). These herb species have also been shown to support animal performance superior to that from grass based pasture (Fraser *et al.* 1988; Scales 1995; Moorhead *et al.* 2002; Hoskin *et al.* 2005; Judson *et al.* 2009).

Recently, the use by farmers of a Herb and Legume Mix containing chicory, plantain, red clover (*Trifolium pratense*) and white clover, has increased as has the potential to produce a greater herbage yield over a longer growing season than mono-species herb swards (Kemp *et al.* 2010). Herb and Legume Mixes also support higher animal performance in terms of ewe milk production and lamb live weight gains before and after weaning compared to a perennial ryegrass and white clover pasture (Kenyon *et al.* 2010; Golding *et al.* 2011; Hutton *et al.* 2011; Sinhadipathige *et al.* 2012).

Defoliation frequency and height have been shown to affect both herbage yield and plant density in chicory and plantain. More frequent defoliation of chicory has a negative effect on herbage yield (Li *et al.* 1997b; Belesky *et al.* 1999; Kemp *et al.* 2002a; Alemseged *et al.* 2003; Sanderson *et al.* 2003a) and plant density (Kemp *et al.* 2002a; Alemseged *et al.* 2003). Lower chicory defoliation height in grazing experiments has not negatively affected herbage yield early in the season (Li *et al.* 1994b) and over the entire growing season (Matthews *et al.* 1990; Jung *et al.* 1996), but under glasshouse conditions lower defoliation height has negatively affected herbage yield, root mass and plant density (Li *et al.* 1995; Li *et al.* 1997c). Field experiments with plantain report a four to five week D.F maximises herbage yield compared to a three week D.F (Sanderson *et al.* 2003a; Labreveux *et al.* 2004). While, Ayala *et al*. (2011b) found defoliation every three weeks during spring and summer resulted in significantly greater yields than defoliation every six weeks. In Chapter 5 grazing a Herb and Legume Mix to a lower residual height had a positive effect on herbage yield over two years under a three to four week grazing interval. However, it is apparent that no studies to date have examined the effects of a wide range of grazing managements i.e. defoliation frequency and height, on the performance of the Herb and Legume Mix under field conditions.

Therefore, the purpose of this experiment was to compare the defoliation responses of a Herb and Legume Mix, a perennial ryegrass and white clover sward, and pure swards of chicory and plantain over a wide range of defoliation frequencies and heights that are applicable to what might occur in commercial farming operations. In this experiment, mechanical mowing was utilised as the defoliation method rather than grazing to enable a wider range of defoliation treatments to be examined.

## 7.3 Materials and methods

### 7.3.1 Site preparation

A field experiment was established on the Massey University Pasture and Crop Research Unit, 5km south of Palmerston North at Poultry Farm Road, Palmerston North, on a Manawatu fine sandy loam (Dystric Fluventric Eutrochrept) (Hewitt 1988). A paddock was prepared by spraying with Round-up Renew (360 g/l a.i of glyphosate; Monsanto) followed by mouldboard ploughing and secondary cultivation. Analysis of the soil before the experiment (15 cm sampling depth) revealed mineral concentrations of 36 mg P $kg^{-1}$, 76.8 mg N $kg^{-1}$, 86 mg K $kg^{-1}$, 27.6 mg S $kg^{-1}$, 1380 mg Ca $kg^{-1}$, 146.4 mg Mg $kg^{-1}$.

The experiment site was divided into 144, 1 × 6 m plots and four distinct sward types; I) Chicory Sward, II) Plantain Sward, III) Herb and Legume Mix, IV) Ryegrass and White Clover Sward; (Table 7.1). The plots were sown in a randomised design on 26 March 2012 with a 15 drill cone seeder, and then the entire area was rolled. The Ryegrass and White Clover plots were defoliated to 50 mm on 15 June 2012 to encourage tillering and all plots were defoliated on 23 August 2012 to 62.5 mm to remove herbage growth and prevent shading of neighbouring plots. All defoliation was undertaken with a rear-bagged rotary mower. The Herb and Legume Mix, Plantain and Chicory plots were sprayed on 19 July 2012 with Haloxyfop + oil (Gallant NF; Dow; 0.5 L/ha + Uptake; 1.0 litres/ha) to remove weed grass species. The Herb and Legume Mix plots and Chicory plots were also sprayed with Flumetsulam + oil (Preside; Dow; 52 g ai/ha + Uptake; 1.0 litres/ha) to remove broadleaf weeds. Plantain plots were sprayed with 3,6-dichloro-2-methoxybenzoic acid (Dicamba; Mosanto 1.0 L/ha) to remove broadleaf weeds. Plots were fertilised with 50 kg $ha^{-1}$ of nitrogen in the form of urea on 19 August 2012 and 34.5 kg $ha^{-1}$ of nitrogen on 20 November 2012 and again on 31 January 2012 to ensure that plant growth was not limited by nitrogen.

**Table 7.1 Established swards and sowing rates.**

| Sward type | | Species | Equivalent Sowing rates (kg/ha) |
|---|---|---|---|
| I) | Chicory Sward | *Cichorium intybus* (*cv.* Puna Π) | 6.0 |
| II) | Plantain Sward | *Plantago lanceolata* (*cv.* Tonic) | 6.0 |
| III) | Herb and Legume Mix | *Plantago lanceolata* (*cv.* Tonic) | 6.0 |
| | | *Cichorium intybus* (*cv.* Puna II) | 6.0 |
| | | *Trifolium pratense* (*cv.* Sensation) | 6.0 |
| | | *Trifolium repens* (*cv.* Tribute) | 4.0 |
| IV) | Ryegrass and White Clover Sward | *Lolium perenne* (*cv.* One50) | 20.0 |
| | | *Trifolium repens* (*cv.* Tribute) | 4.0 |

### 7.3.2 Experimental design

Defoliation frequency (D.F) and defoliation height (D.H) treatments were allocated to plots prior to sowing. Treatments included four D.F's (weekly (F1), fortnightly (F2), three-weekly (F3), six-weekly (F6)), and three D.H's (75 mm (H75), 50 mm (H50), 25 mm (H25)). A complete randomised block design (four sward types, four D.F's, three D.H's; 48 treatments) was initially planned. However, due to poor establishment of chicory plots, the F1-Chicory treatment was not included, as previous research has shown chicory does not respond well under very frequent defoliation (Li *et al.* 1997b; Li *et al.* 1997c). Thus, the final design included a total of 45 treatments, with each treatment having three replicates (resulting in a total of 135 plots). All plots were defoliated to their respective treatment D.H's on 19 September 2012 (Week 0). Treatments began at their respective times after this date and the experiment was undertaken for the following 24 weeks.

### 7.3.3 Measurements

Herbage masses (pre-cuts) were measured by cutting to ground level using a 0.1 $m^2$ quadrat, randomly placed within each replicate in all appropriate D.F treatment replicates (i.e. during Week 1 they were taken in all F1 plots, while in Week 2 they were taken in all F1 and F2 plots). Herbage samples were oven dried at 60°C to a constant weight (for approximately 48 hours) to estimate individual replicate DM yield. Post-cuts were taken from all 135 plots on 1 November 2012 (Week 6), 12 December 2012 (Week 12), 24 January 2013 (Week 18) and 7 March 2013 (Week 24), in order to calibrate accumulated dry matter yields.

One herbage sample (~50g wet weight) was collected for botanical composition analysis from each replicate (all 135 plots) on 13 September 2012 (just prior to Week 0), 10 December 2012 (Week 12), 21 January 2013 (Week 18) and 4 March 2013 (Week 24) using the same method as DM yield samples. These samples were dissected into each sown treatment species (with the species plantain and chicory being further sorted into leaf and reproductive stem (including seedhead and flowers)), and into unsown species and dead material.

One root sample (0.18 × 0.18 m) was randomly collected to a depth of 15 cm from each plot on 13 September 2012 (just prior to Week 0), 10 December 2012 (Week 12), 21 January 2013 (Week 18) and 4 March 2013 (Week 24). Roots were washed and the number of plants of the treatment species was counted to estimate plant density. Root diameter was measured on all plantain and chicory plants at the widest part of the root.

Grab herbage samples (~50g wet weight) (Frame 1993) were collected for nutritive value analysis on 27 February 2013 (Week 23) by cutting herbage at ground level. Samples were collected from replicates which were defoliated fortnightly to 25 mm (F2-H25) and replicates which were defoliated every six weeks to 75 mm (F6-H75). This resulted in four sward types by two defoliation treatments by three replicates (a total of 24 samples). All samples were freeze dried and ground to pass a 1 mm sieve and analysed for digestibility (and hence predicted metabolisable energy; (Roughan and Holland 1977)) and crude protein (CP) (total combustion method), neutral detergent fibre (NDF), acid detergent fibre (ADF) (Robertson and Van Soest 1981), organic matter (OM) and ash.

### 7.3.4 Climatic measurements

Throughout the experiment, 100 mm soil temperature, 24 hour maximum and minimum air temperatures (°C), and rainfall (mm) were recorded at 0900 daily at a weather station <500 m from the experimental site. The experimental site was irrigated when the ground was dry as a result of lower than average monthly rainfall. Mean monthly 100 mm soil temperature, maximum and minimum temperature, radiation, total monthly rainfall and irrigation applied are presented in Table 7.2.

**Table 7.2 Mean monthly 100 mm soil temperature, maximum and minimum daily air temperatures (°C) and total monthly rainfall and irrigation applied (mm).**

| | 2012 | | | | 2013 | | |
|---|---|---|---|---|---|---|---|
| | Sept | Oct | Nov | Dec | Jan | Feb | Mar |
| **100 mm soil temperature (°C)** | 9.9 | 12 | 14.5 | 18.4 | 18.3 | 19.3 | 17.7 |
| **max daily air temp (°C)** | 15.2 | 16.1 | 17.6 | 22.3 | 23.3 | 24.1 | 23.6 |
| **min daily air temp (°C)** | 5.7 | 7.1 | 7.8 | 12.5 | 11.7 | 12.2 | 11.1 |
| **rainfall (mm)** | 59 | 64 | 20 (50)* | 100 (62)* | 29 (50)* | 34 (63)* | 37 |

*Irrigation applied (mm)

### 7.3.5 Statistical analysis

All statistical analyses were performed using SAS (Statistical Analysis System, version 9.2; SAS Institute Inc., Cary, NC, US).

#### *7.3.5.1 Dry matter accumulation*

Pre-defoliation herbage mass data from weeks 6, 12, 18 and 24 were used to generate net herbage growth per day for each replicate within each of the four time periods. F1 defoliation thus had net herbage growth data between weeks 5-6, 11-12, 17-18, 23-24, while the F6 defoliation had net herbage growth data between weeks 1-6, 7-12, 13-18 and 19-24. A MIXED procedure for repeated measures was used to analyse net herbage growth per day. The models included the fixed effects of sward type (Herb and Legume Mix, Plantain Sward, Chicory Sward, Ryegrass and White Clover Sward), D.F (F1, F2, F3, F6), D.H (H25, H50, H75) and all two-way, three-way and four-way interactions between sward type, D.F, D.H, time period and the random effect of block.

The Kenward Rogers adjustment for the degree of freedom for the denominator was applied for testing the fixed effects. Multiple mean square comparisons were performed with Tukey adjustment. Using the Akaike's information criterion, compound symmetry was used as the best error structure to model repeated measures across time.

Dry matter yield between each defoliation period was calculated as the pre-defoliation herbage mass minus the average of the nearest post-defoliation herbage mass. The total accumulated dry matter yield for each replicate was equal to the sum of the dry matter yields. The total accumulated dry matter yield (comparison across sward types) was analysed using a model including the fixed effects of sward type, D.F, D.H and all two-way and three-way interactions between sward type, D.F, D.H and the random effect of block. In addition to this, each sward type was analysed separately to reduce variability in order to detect differences between treatments within each sward type more accurately. Each sward type was analysed using the MIXED procedure with a model including the fixed effects of D.F, D.H and the two-way interaction between D.F and D.H and the random effect of block.

#### *7.3.5.2 Botanical composition*

The dry weight of each component of the sward (sown species, weeds, dead matter) was converted into a percentage of the total weight from each collected sample. Each sward type was analysed separately, due to the complexity of the data. As previously stated, botanical composition, leaf percentage, plant density and taproot diameter were all measured at four time points (Week 0, Week 12, Week 18, Week 24); from hereafter this time variable will be termed week. The data were analysed using the MIXED procedure with a model including the fixed effects of D.F, D.H, week, components of the sward and the four-way interaction of D.F, D.H, week and components of the sward and the random effect of block.

#### *7.3.5.3 Leaf percentage*

This was assessed within the herb sward types (Chicory Sward, Plantain Sward, Herb and Legume Mix). Each sward type was analysed separately. The percentage of leaf was calculated as the dry weight of leaf divided by the total DM (sum of stem and leaf) of the target species. i.e. in the Chicory Sward the percentage of leaf was calculated as the weight of chicory leaf divided by the weight of total chicory material. Within the Herb and Legume Mix, the chicory and plantain percentages were calculated separately, based on only that species. For the pure herb swards (Chicory Sward, Plantain Sward) the data were analysed using the MIXED procedure with a model including the fixed effects of D.F, D.H and week and all two-way and three-way interactions between D.F, D.H, week and the random effect of block. For the Herb and Legume Mix the data was analysed using the MIXED procedure with a model including the fixed effects of D.F, D.H, week, leaf species (chicory and plantain) and the four-way interaction of D.F, D.H, week and leaf species and the random effect of block.

#### *7.3.5.4 Plant density*

Each sward type was analysed separately using the MIXED procedure. The pure herb swards (Chicory Sward, Plantain Sward) model included the fixed effects of D.F, D.H and week and all two-way and three-way interactions between D.F, D.H, week and the random effect of block. The mixed swards (Herb and Legume Mix, Ryegrass and White Clover Sward) model included the fixed effects of D.F, D.H, species and week and all two-way, three-way and four-way interactions between species, D.F, D.H, week and the random effect of block.

#### *7.3.5.5 Taproot diameter*

Taproot diameter data were only collected on the species chicory and plantain, from the Herb and Legume mix and the pure swards of chicory and plantain. The data were analysed using the MIXED procedure with a model including the fixed effects of D.F, D.H, sward type by species (chicory-pure, chicory-mix, plantain-pure, plantain-mix) and week and all three-way and four-way interactions between D.F, D.H, sward type by species, week and the random effect of block.

#### *7.3.5.6 Herbage nutritive value*

The data were analysed using the MIXED procedure with a model including the fixed effects of defoliation treatment (F2-H25 vs. F6-H75) and sward type and the two-way interaction between defoliation treatment and sward type and the random effect of block.

## 7.4 Results

### 7.4.1 Herbage production

#### *7.4.1.1 Net herbage growth per day*

There were no four-way or three-way interactions ($P > 0.05$) between the parameters of sward type, D.F, D.H and time period on net herbage growth per day. There was a two-way interaction ($P < 0.05$) between sward type and D.F on net herbage growth per day (Figure 7.1). Ryegrass had a greater ($P < 0.05$) net herbage growth rate under the F1 D.F than under less frequent D.F treatments (F2, F3, F6) which did not differ ($P > 0.05$). While, net herbage growth rates did not differ ($P > 0.05$) between Chicory, Plantain and the Herb and Legume Mix under any D.F's. F1 plantain had a greater ($P < 0.05$) net herbage growth rate than F6 ryegrass. F1 and F3 Herb and Legume Mix had a greater ($P < 0.05$) net herbage growth rate than F6 ryegrass. D.H (H25, H50, H75) had no effect ($P > 0.05$) on net herbage growth per day (data not shown).

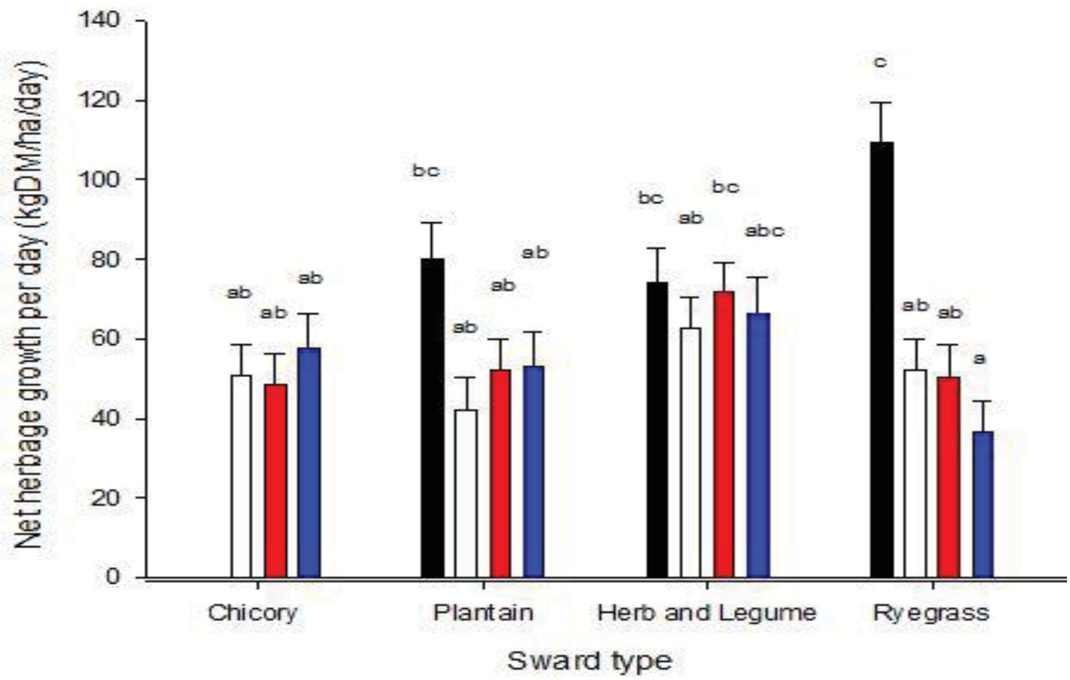



**Figure 7.1 Net herbage growth per day (kgDM/ha/day) for each sward type (Chicory Sward, Plantain Sward, Herb and Legume Mix, Ryegrass and White Clover Sward (Ryegrass)) within each defoliation frequency (F1 (black), F2 (white), F3 (red) and F6 (blue). Vertical bars represent standard errors. Means within treatments with different letters are significantly different at *P* = 0.05.**

#### *7.4.1.2 Comparison across all treatments and sward types*

There was a three-way interaction ($P < 0.05$) between sward type, D.F and D.H on total accumulated herbage yield. Plantain F2-H50 had a lower ($P < 0.05$) total accumulated herbage yield than the Herb and Legume Mix F3-H25 (Appendix 3- Figure 1). Ryegrass F6-H75 had a lower ($P < 0.05$) total accumulated herbage yield than Plantain F1-H75 and Herb and Legume Mix F3-H25 (Appendix 3- Figure 1). Ryegrass F6-H50 had a lower ($P < 0.05$) total accumulated herbage yield than Chicory F6-H25, Plantain F1-H75, F6-H50 and all F3 and F6 Herb and Legume Mix D.F treatments (Appendix 3- Figure 1).

#### 7.4.1.3 *Chicory Sward*

There was no two-way interaction (*P* > 0.05) between D.F and D.H on total accumulated herbage yield (Figure 7.2). D.F had no (*P* > 0.05) effect on total accumulated herbage yield, such that total accumulated herbage yield was 8075 ± 812, 9648 ± 812 and 10478 ± 812 kgDM/ha for F2, F3 and F6 D.F treatments, respectively. D.H had no (*P* > 0.05) effect on total accumulated herbage yield, such that total accumulated herbage yields were 9836 ± 812, 7994 ± 812 and 10371 ± 812 kgDM/ha for H25, H50 and H75 D.H treatments, respectively.

#### 7.4.1.4 *Plantain Sward*

There was a two-way interaction (*P* < 0.05) between D.F and D.H on total accumulated herbage yield (Figure 7.2). Within the F1 D.F treatment, H75 had a greater (*P* < 0.05) total accumulated herbage yield than the H50 and H25 D.H treatments, which did not differ (*P* > 0.05). Within each of F2, F3, F6 D.F treatments, total accumulated herbage yield did not differ (*P* > 0.05) between D.H's. Under H25 defoliation, total accumulated herbage yield did not differ (*P* > 0.05) between F3, F6 D.F treatments, and was greater (*P* < 0.05) than the F1 and F2 D.F treatments, which did not differ (*P* > 0.05).

#### 7.4.1.5 Herb and Legume Mix

There was no two-way interaction ($P > 0.05$) between D.F and D.H on total accumulated herbage yield (Figure 7.2). F1 and F2 D.F treatments had a total accumulated herbage yield which did not differ ($P > 0.05$) from one another but were lower ($P < 0.05$) than the F3 and F6 D.F treatments, which did not differ ($P > 0.05$) from one another. Thus total accumulated herbage yield was 8151 ± 954, 9261 ± 884, 13180 ± 884, 12878 ± 884 kgDM/ha, for F1, F2, F3 and F6 D.F treatments, respectively. D.H had no ($P > 0.05$) effect on total accumulated herbage yield, such that total accumulated herbage yield was 10090 ± 814, 11047 ± 768 and 11466 ± 768 kgDM/ha for H25, H50 and H75 D.H treatments, respectively.

#### 7.4.1.6 Ryegrass and White Clover Sward

There was a two-way interaction ($P < 0.05$) between D.F and D.H on total accumulated herbage yield (Figure 7.2). Within each of the F2, F3, F6 D.F treatments, total accumulated herbage yield did not differ ($P > 0.05$) between D.H's. Within the F1 D.F treatment, H75 had a greater ($P < 0.05$) total accumulated herbage yield than H50 and H25, which did not differ ($P > 0.05$). Furthermore, the total accumulated herbage yield of the F1-H75 treatment was greater ($P < 0.05$) than all D.F by D.H combinations, except ($P > 0.05$) for F2-H50, F3-H50. Similarly, the total accumulated herbage yield of the F3-H50 treatment was greater ($P < 0.05$) than all D.F by D.H combinations, except ($P > 0.05$) for F1-H75, F2-H50, F3-H25 of F3-H75.

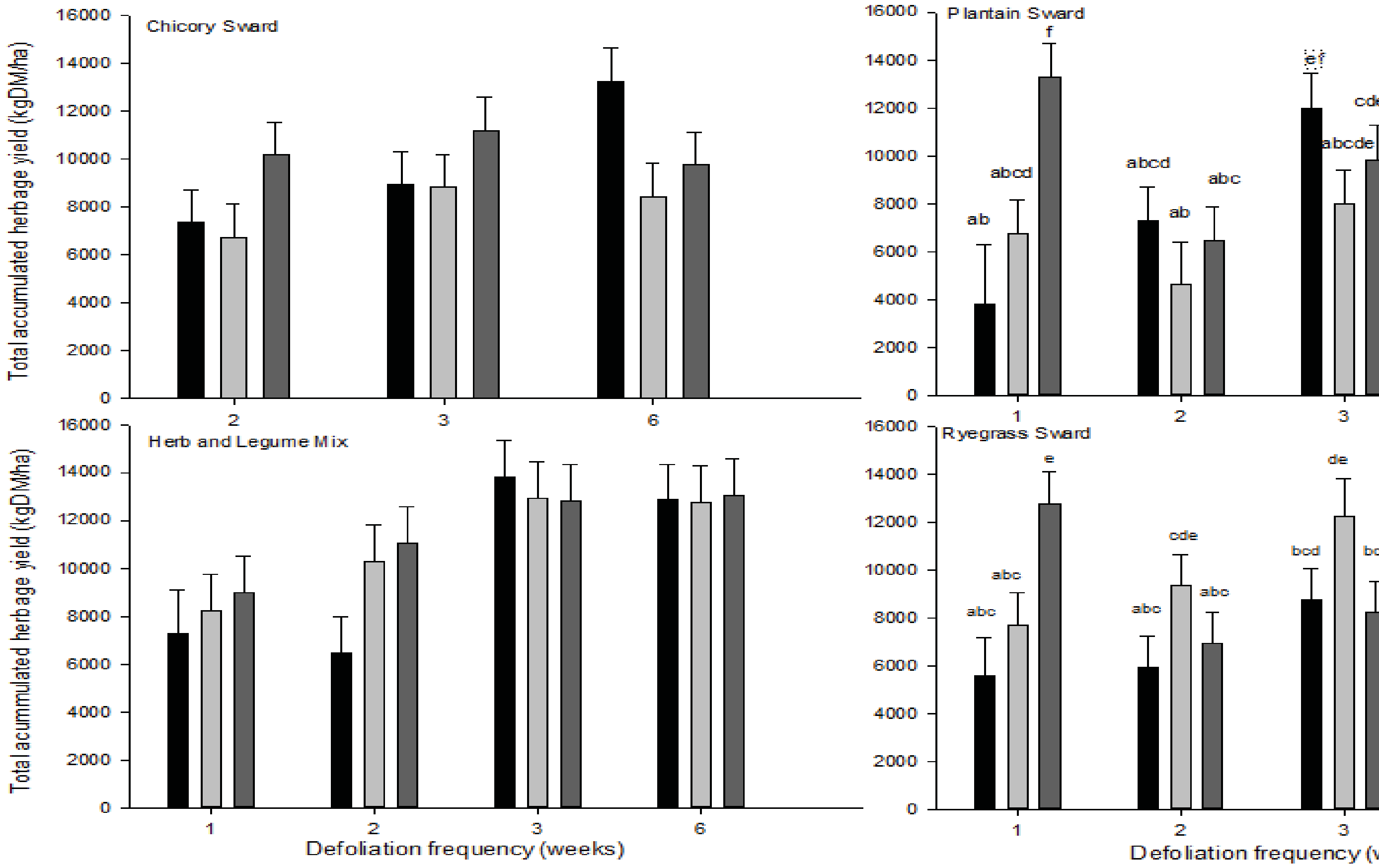



**Figure 7.2 Total accumulated herbage yield (kgDM/ha) over the 24 week experiment period for each sward type (Chicory Legume (bottom left), Ryegrass and White Clover (bottom right) with all defoliation frequencies (1, 2, 3, 6 week defoliation) a mm (light grey), 75 mm (dark grey)). Different letters (within sward types (graphs)) indicate treatments are significantly differe**

### 7.4.2 Botanical composition and leaf percentage

Within each sward type, there was a four-way interaction ($P < 0.05$) between D.F, D.H, week (0, 12, 18, 24) and components of the sward (sown species, weeds, dead matter) for botanical composition. Each sward type is presented separately. There was also a separate leaf percentage analysis (leaf/sum of stem and leaf for each sown herb species) of the herb based swards; Chicory Sward, Plantain Sward, Herb and Legume Mix.

#### *7.4.2.1 Chicory Sward*

The F6 defoliation had a greater ($P < 0.05$) proportion of chicory from week 12 onwards, than F2 defoliation under all D.H's, except H75 at week 24 (Figure 7.3). At week 24, the F6 defoliation had a lower ($P < 0.05$) proportion of weed than F2 and F3 defoliation, which did not differ ($P > 0.05$) under all D.H's, except H25 which did not differ ($P > 0.05$) between F3 and F6.

There was no ($P > 0.05$) three-way interaction between week, D.F and D.H or two-way interactions between D.F and D.H or between week and D.H for the percentage of chicory which was leaf material. However, there was a two-way ($P < 0.05$) interaction between week and D.F, such that within week 0 and week 24 there were no differences ($P > 0.05$) in the percentage of chicory leaf under all D.F's (Figure 7.4). While, at both weeks 12 and 18, the F6 defoliation had a lower ($P < 0.05$) percentage of leaf than under the F2 and F3 defoliations, which did not differ ($P > 0.05$). Furthermore, the percentage of chicory leaf at week 0 was greater ($P < 0.05$) than at all later sampling weeks, except at week 18 under the F3 defoliation.

D.H had an effect ($P < 0.05$) on the percentage of chicory which was leaf material. H25 D.H's had a greater ($P < 0.05$) percentage of leaf than H75 D.H's, while H50 D.H's did not differ ($P > 0.05$) from either D.H treatment, such that leaf percentages were 90.3 ± 1.7, 87.9 ± 1.7, 83.7 ± 1.7 for H25, H50, H75, respectively.

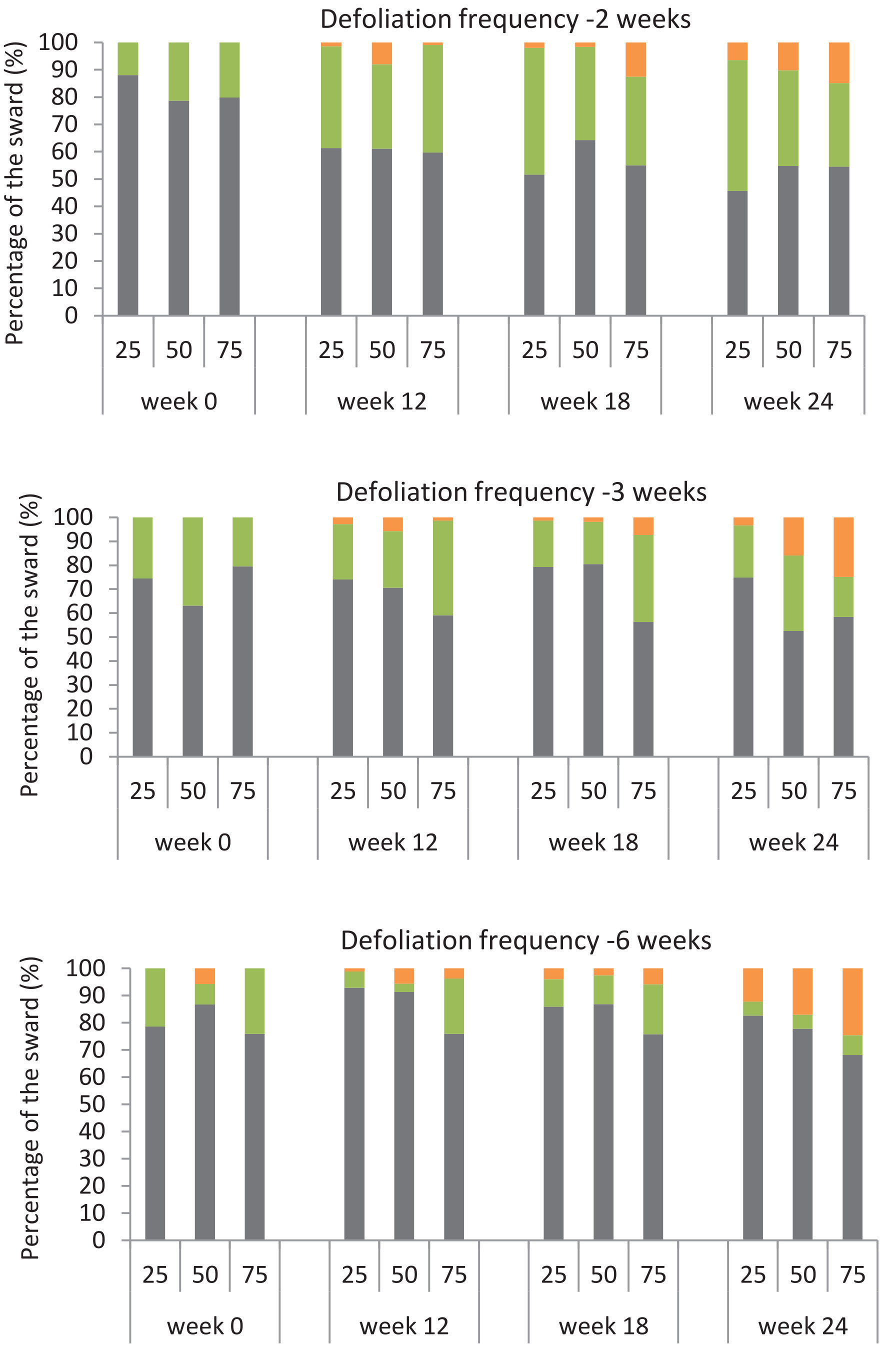



**Figure 7.3 Botanical composition of Chicory Swards (%) under the defoliation frequencies ((2 weeks (top), 3 weeks (middle), 6 weeks (bottom)) for each defoliation height (25, 50 and 75 mm) over the 24 weeks. Composition included chicory (grey), weed (green) and dead matter (orange). All components within each defoliation frequency and height include a s.e. of 6.14%.**

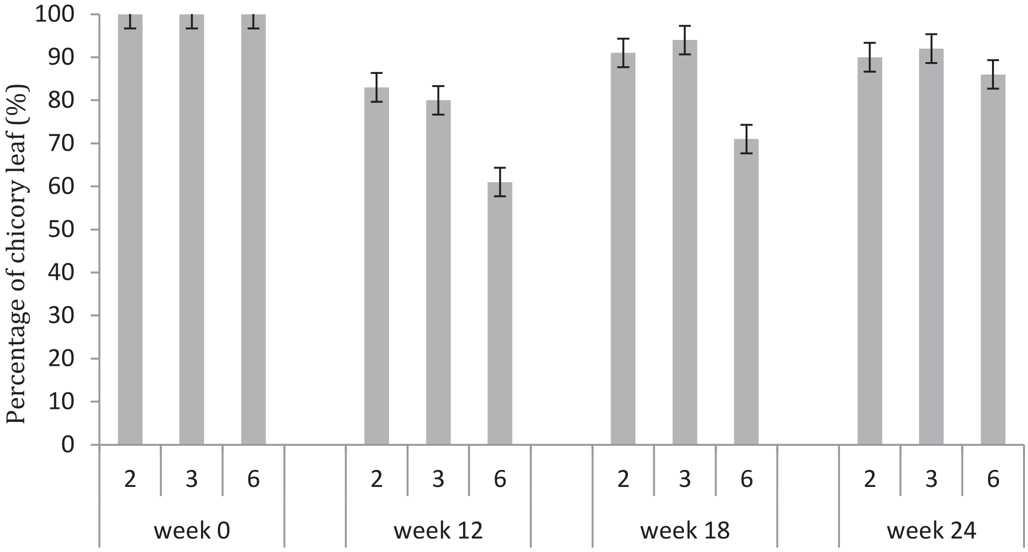



**Figure 7.4 Leaf percentage (%) of chicory in the Chicory Sward, as affected by week and defoliation frequency. Vertical bars represent standard errors.**

#### *7.4.2.2 Plantain Sward*

At week 24, the F1 and F2 defoliations had a lower ($P < 0.05$) proportion of plantain than that seen at week 0 (Figure 7.5). Conversely, the proportion of plantain under F3 and F6 defoliation did not differ ($P > 0.05$) at week 0 and week 24, under all D.H's, except for the F3-H50 treatment which was greater ($P < 0.05$) at week 0 than at week 24. While, at week 24, the F1 and F2 defoliations under all D.H's had greater ($P < 0.05$) proportions of weed and dead matter than at week 0. Furthermore, at week 24, the F6 defoliation had a greater ($P < 0.05$) proportion of plantain than the F1 defoliation, for all D.H's.

There was a significant ($P < 0.05$) three-way interaction between week, D.F and D.H for plantain leaf percentage. At weeks 12 and 18, the F6 defoliation had a lower ($P < 0.05$) percentage of leaf than F1, F2 and F3 defoliations within each D.H, except at week 18, F3-H75 (Figure 7.6). From week 12 onwards, under the F1, F2 and F3 defoliation, H75 D.H had a lower ($P < 0.05$) percentage of leaf than H25 D.H, except for F1 at week 18 and F2 at week 24. Under F6 defoliation, within all weeks, D.H had no ($P > 0.05$) effect on the percentage of leaf, except at week 18.

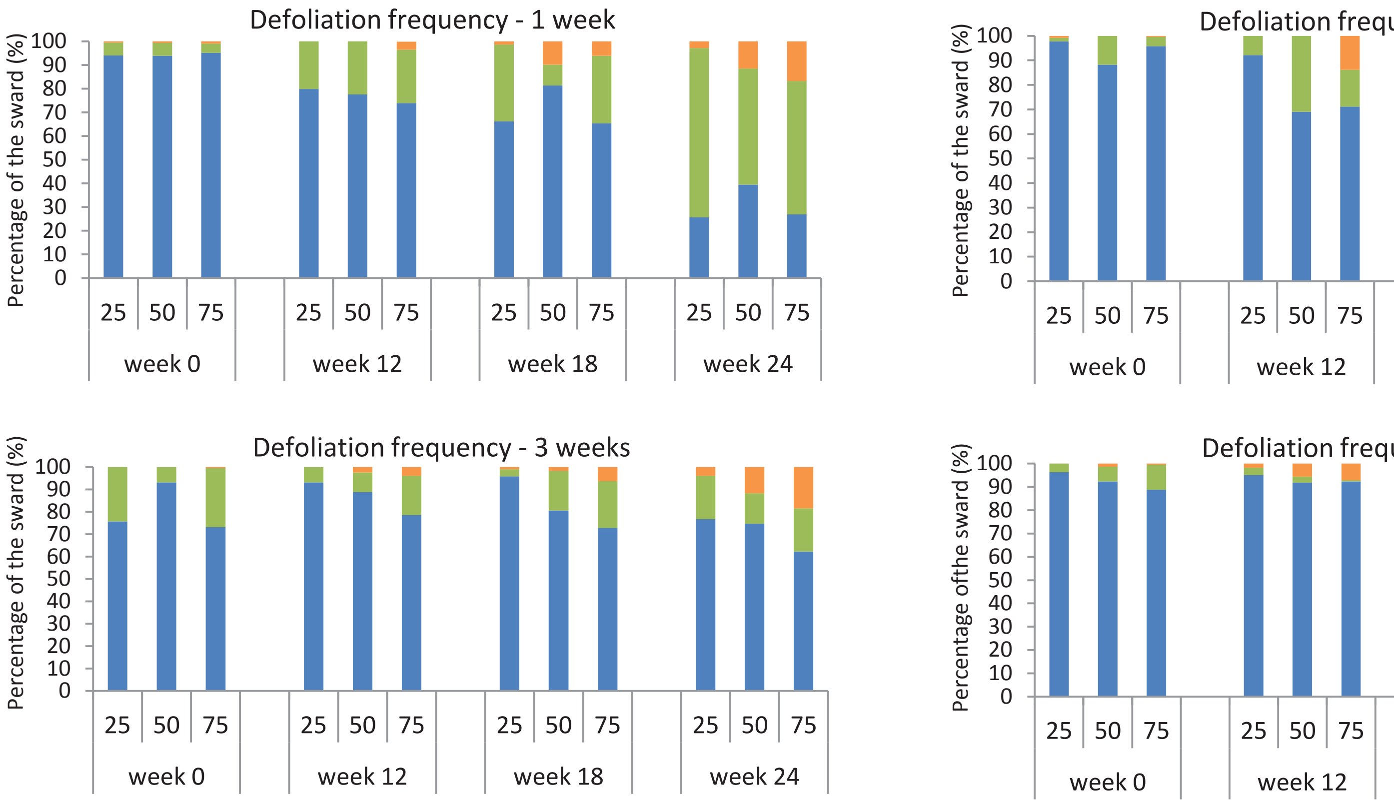



**Figure 7.5 Botanical composition of Plantain Swards (%) under the defoliation frequencies (1 week (top left), 2 weeks (top righ right)) for each defoliation height (25, 50, 75 mm) over the 24 weeks. Composition included plantain (blue), weed (green) a within each defoliation frequency and height include a s.e. of 7.06%.**

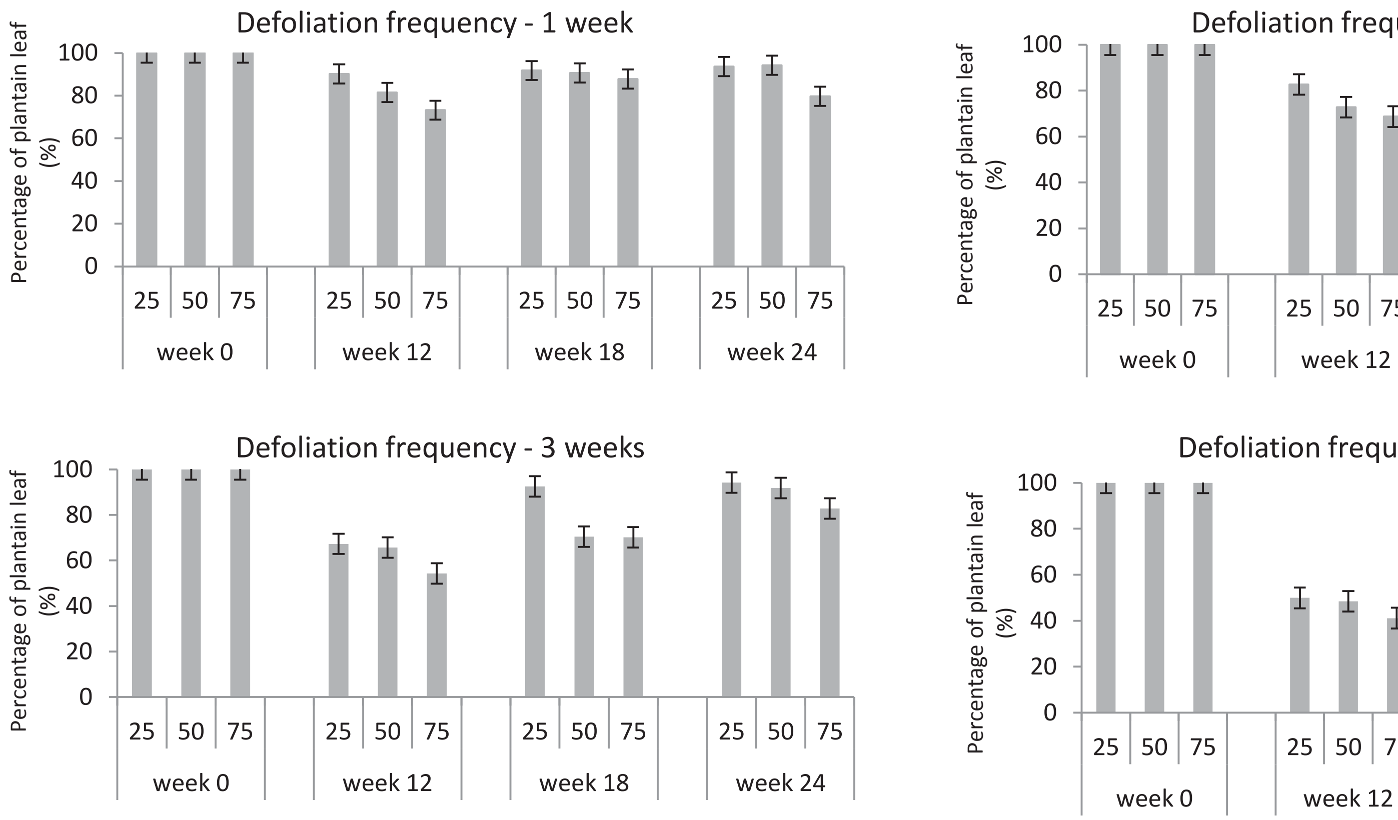



**Figure 7.6 Leaf percentage (%) of plantain in the Plantain Sward under the defoliation frequencies (1 week (top left), 2 weeks ( (bottom right)) for each defoliation height (25, 50, 75 mm) over the 24 weeks. Vertical bars represent s.e.**

#### *7.4.2.3 Herb and Legume Mix*

The proportion of chicory did not differ (*P* > 0.05) between week 0 and week 24 under any of the D.F by D.H treatments, except under F2-H25 (Figure 7.7).

At week 24, the F1 defoliation for all D.H's had a lower (*P* < 0.05) proportion of plantain than at week 0 (Figure 7.7). The F2, F3, F6 by D.H treatments did not differ (*P* > 0.05) in the proportion of plantain between week 0 and week 24, except for the F6-H75 treatment which was lower (*P* < 0.05) at week 24 than at week 0. At week 24, the F1 defoliation within all D.H's had a lower (*P* < 0.05) proportion of plantain than less frequent defoliation treatments, which did not differ from each other (*P* > 0.05).

The proportion of red clover did not differ (*P* > 0.05) between week 0 and week 24 under any of the D.F's (Figure 7.7). At weeks 18 and 24, the F6 defoliation had a greater (*P* < 0.05) proportion of red clover than the F1 defoliation for all D.H's, expect for the H75 which did not differ (*P* > 0.05) at week 24.

The proportion of white clover at week 24 under F1 defoliation for all D.H's was greater (*P* < 0.05) than at week 0 (Figure 7.7). While, under the F2, F3, F6 D.F treatments the proportion of white clover did not differ (*P* > 0.05) between week 0 and week 24. At week 24, the F1 D.F treatment within each D.H had a greater (*P* < 0.05) proportion of white clover than F2, F3, F6 D.F treatments, expect for the H75 D.H treatment.

There was a significant ($P < 0.05$) four-way interaction between week, D.F, D.H and species (chicory and plantain) for leaf percentage. The percentage of chicory leaf under the F1, F2 and F3 D.F by D.H treatments did not differ ($P > 0.05$) across all weeks, except at week 18 under F1-H50 and week 12 under F3-H75 (Figure 7.8). From week 12 onward, under F6 D.F treatment there was no differences ($P > 0.05$) in the percentage of chicory leaf from week to week within each D.H treatment.

At week 0, the percentage of plantain leaf under all D.F by D.H treatments was greater ($P < 0.05$) than at week 12, except F1-H25 (Figure 7.8). The percentage of plantain leaf within all F1, F2 and F3 D.F by D.H treatments did not differ ($P > 0.05$) between week 18 and week 24. The percentage of plantain leaf under F6 D.F treatment was lower ($P < 0.05$) than all F1, F2 and F3 D.F treatment at weeks 12 and 18, except at week 12 F6-H75 and at week 18 F6-H50. While, under F6 D.F, for all D.H treatments the percentage of plantain leaf was greater ($P < 0.05$) at week 24 than at week 18.

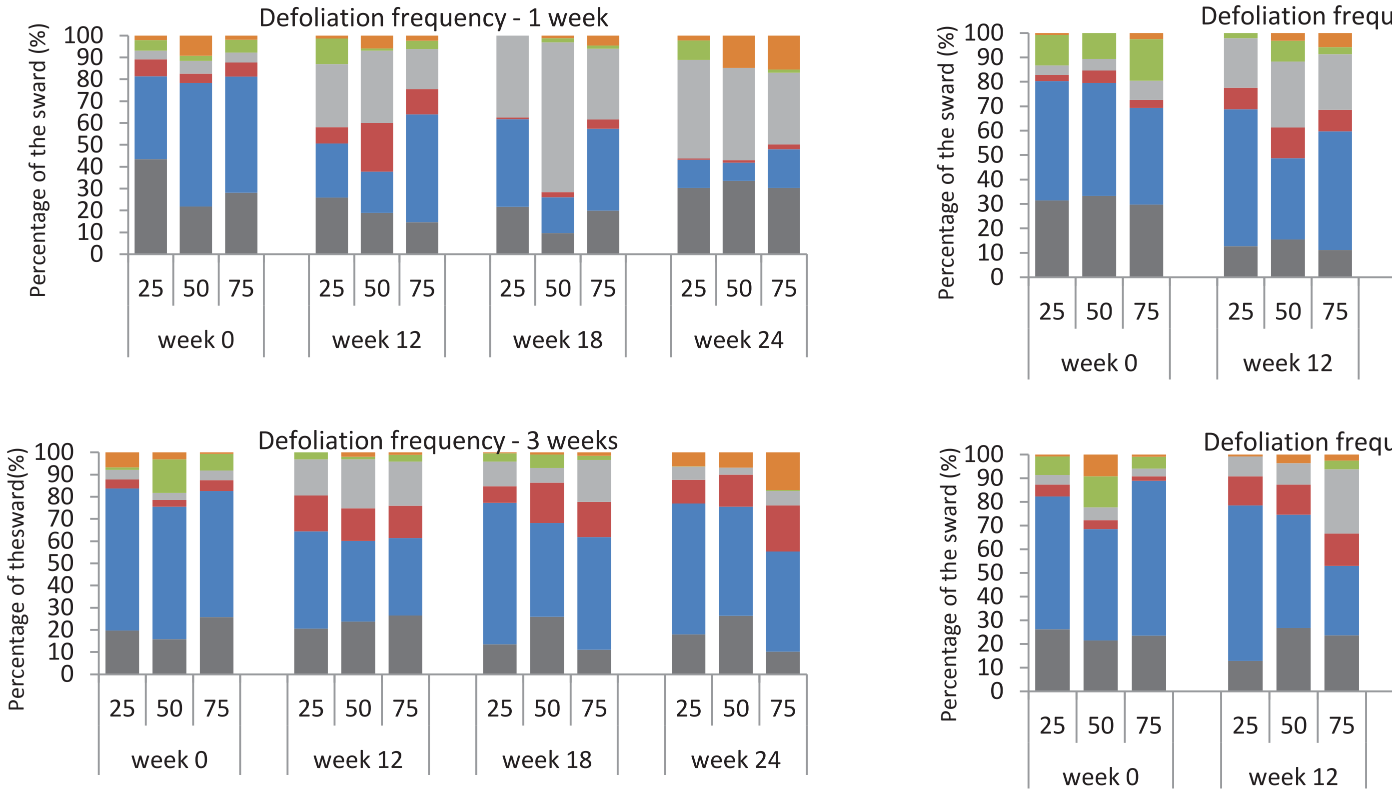



**Figure 7.7 Botanical composition of the Herb and Legume Mix (%) under the defoliation frequencies (1 week (top left), 2 w weeks (bottom right)) for each defoliation height (25, 50, 75 mm) over the 24 weeks. Composition included chicory (dark gre clover (light grey), weed (green), dead matter (orange). All components within each defoliation frequency and height include a**

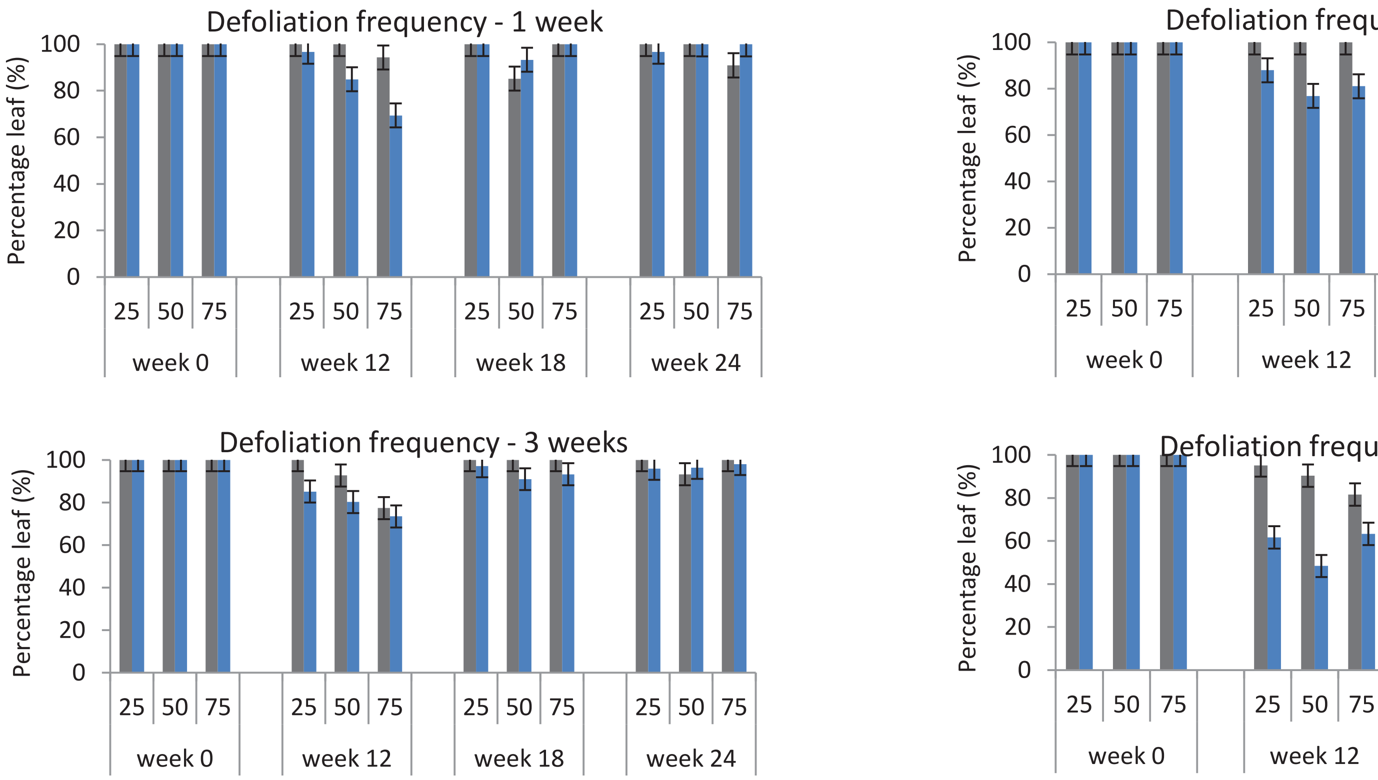



**Figure 7.8 Leaf percentage (%) of chicory (grey) and plantain (blue) in the Herb and Legume Mix under the defoliation frequenc**
**3 weeks (bottom left), 6 weeks (bottom right)) for each defoliation height (25, 50, 75 mm) over the 24 weeks. Vertical bars rep**

#### *7.4.2.4 Ryegrass and White Clover Sward*

At week 24, under all D.F by D.H treatments there was a lower ($P < 0.05$) proportion of ryegrass than at week 0 (Figure 7.9). At week 24, under all D.F by D.H treatments there was a greater ($P < 0.05$) proportion of white clover than at week 0, except under the F2-H75 treatment, which did not differ ($P > 0.05$) between weeks 0 and 24. At week 24, within each D.F, the H25 D.H treatment had a greater ($P < 0.05$) proportion of white clover than the H75 D.H treatment.

At week 24, under all D.F by D.H treatments, dead matter was greater ($P < 0.05$) than at week 0, except under the F2-H25 and F6-H25 treatments, where dead matter did not differ ($P > 0.05$) between weeks 0 and 24. At week 24, the F1 and F2 defoliations had greater ($P < 0.05$) dead matter under H75 than H25. At week 24, the F3 and F6 defoliations had greater ($P < 0.05$) dead matter under H50 than H25, while H75 did not differ ($P > 0.05$) from either D.H treatment.

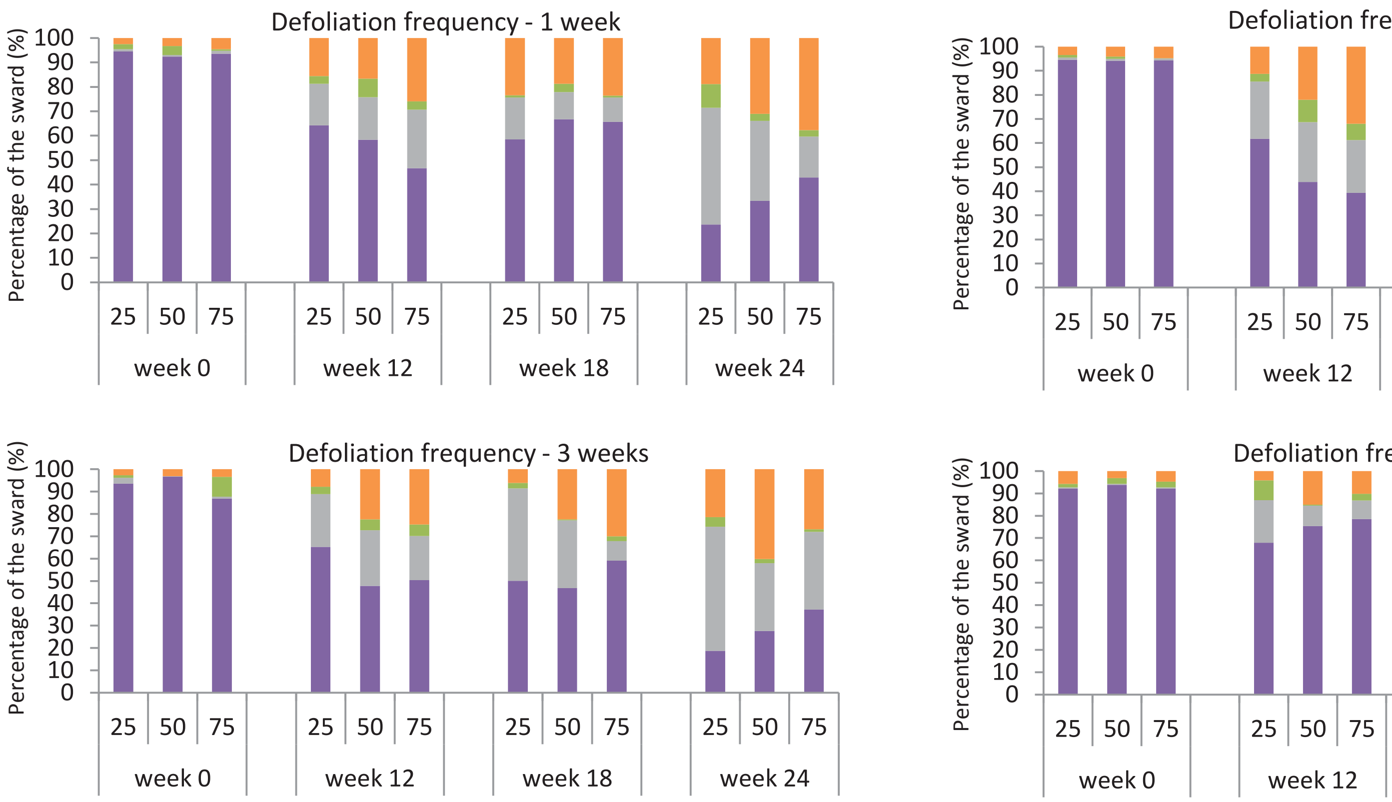



**Figure 7.9 Botanical composition of Ryegrass and White Clover Sward (%) under the defoliation frequencies (1 week (top le left), 6 weeks (bottom right)) for each defoliation height (25, 50, 75 mm) over the 24 weeks. Composition included ryegrass (green), dead matter (orange). All components within each defoliation frequency and height include a s.e. of 5.60%.**

### 7.4.3 Plant density

There were no ($P > 0.05$) two-way, three-way or four-way interactions between D.F, D.H, species and week on plant density. D.F and D.H had no effect ($P > 0.05$) on plant density (data not shown). The plant density in the Chicory Sward was lower ($P < 0.05$) at week 24 than week 0 (Table 7.3). Conversely, the plant density in the Plantain Sward did not differ ($P > 0.05$) between week 0 and week 24.

In the Herb and Legume Mix, at week 0, the plant density of chicory and plantain did not differ ($P > 0.05$) and was greater ($P < 0.05$) than that of red clover and white clover, which did not differ ($P > 0.05$) from one another (Table 7.3). The plant density of chicory and red clover was lower ($P < 0.05$) at week 24 than week 0, while that of plantain and white clover did not differ ($P > 0.05$) between week 0 and week 24. Thus at week 24, in the Herb and Legume Mix the plant density of plantain was greater ($P < 0.05$) than that of chicory, which was in turn greater ($P < 0.05$) than that of red clover and white clover, which did not differ ($P > 0.05$) from one another.

In the Ryegrass and White Clover Sward the plant density of ryegrass was greater ($P < 0.05$) than that of white clover at both week 0 and week 24 (Table 7.3). The plant density of ryegrass was lower ($P < 0.05$) at week 24 than week 0, while that of white clover did not differ ($P > 0.05$) between week 0 and week 24.

**Table 7.3 Plant density for each sward type at Week 0 and Week 24 (mean ± SE). Means within each sward type both within and between weeks with different letters are significantly different at *P* = 0.05.**

| Sward type | Plants per m$^2$ | |
|---|---|---|
| | Week 0 | Week 24 |
| *Chicory Sward* | 129b ± 10.0 | 96a ± 10.0 |
| *Plantain Sward* | 99 ± 5.9 | 91 ± 5.9 |
| *Herb and Legume Mix* | | |
| Chicory | 98d ± 5.6 | 55c ± 5.6 |
| Plantain | 82d ± 5.6 | 81d ± 5.6 |
| Red Clover | 45bc ± 5.6 | 28a ± 5.6 |
| White Clover | 49bc ± 5.6 | 33ab ± 5.6 |
| *Ryegrass and White Clover Sward* | | |
| Ryegrass | 230c ± 6.6 | 95b ± 6.6 |
| White Clover | 38a ± 6.6 | 52a ± 6.6 |

### 7.4.4 Taproot diameter of species – chicory and plantain only

There was no ($P > 0.05$) four-way interaction between D.F, D.H, sward type by species (chicory-pure, chicory-mix, plantain-pure, plantain-mix) and week on the taproot diameter of plantain and chicory. D.H had no effect ($P > 0.05$) on taproot diameter (data not shown). There was a three-way interaction ($P < 0.05$) between week, sward type by species (chicory-pure, chicory-mix, plantain-pure, plantain-mix) and D.F on the taproot diameter.

#### 7.4.4.1 *Chicory Sward*

The taproot diameter of chicory at weeks 12 and 24 was greater ($P < 0.05$) for the F6 D.F treatment than the F3 D.F treatment, which was in turn greater ($P < 0.05$) than the F2 D.F treatment (Figure 7.10). At week 18, the taproot diameter of chicory was greater ($P < 0.05$) under the F6 D.F treatment than the F3 and F2 D.F treatments, which did not differ ($P > 0.05$).

#### 7.4.4.2 *Plantain Sward*

The taproot diameter of plantain at weeks 0, 12 and 18 did not differ ($P > 0.05$) between the D.F's (Figure 7.10). At week 24, the taproot diameter of the F6 D.F treatment was greater ($P < 0.05$) than the F2 D.F treatment. The F1 and F3 D.F treatments did not differ ($P > 0.05$) from any D.F treatments.

#### 7.4.4.3 *Herb and Legume Mix*

The taproot diameter of chicory at weeks 0 and 12 did not differ ($P > 0.05$) between the D.F's (Figure 7.10). At weeks 18 and 24 the taproot diameter of the chicory F6 D.F treatment was greater ($P < 0.05$) than the chicory F1, F2 and F3 D.F treatments, which did not differ ($P > 0.05$). The taproot diameter of plantain at weeks 0, 12, 18 did not differ ($P > 0.05$) between the D.F's. At week 24 the taproot diameter of the plantain F6 D.F treatment was greater ($P < 0.05$) than the plantain F1 D.F treatment. The taproot diameter of the plantain F2 and F3 D.F treatments did not differ ($P > 0.05$) from any D.F treatment.

At week 12, the taproot diameter of chicory was greater ($P < 0.05$) than that of plantain under the F2 D.F treatment (Figure 7.10). At week 18, the taproot diameter of chicory was greater ($P < 0.05$) than that of plantain under the F1 and F6 D.F treatments. At week 24, the taproot diameter of chicory was greater ($P < 0.05$) than that of plantain under the F1, F2 and F6 D.F treatments.

#### *7.4.4.4 Comparison across sward types (Chicory Sward vs. Plantain Sward vs. Herb and Legume Mix)*

Across all weeks the taproot diameter of chicory in the Chicory Sward was greater ($P < 0.05$) than that of plantain in both the Plantain Sward and the Herb and Legume Mix under the F2, F3 and F6 D.F treatments (Figure 7.10).

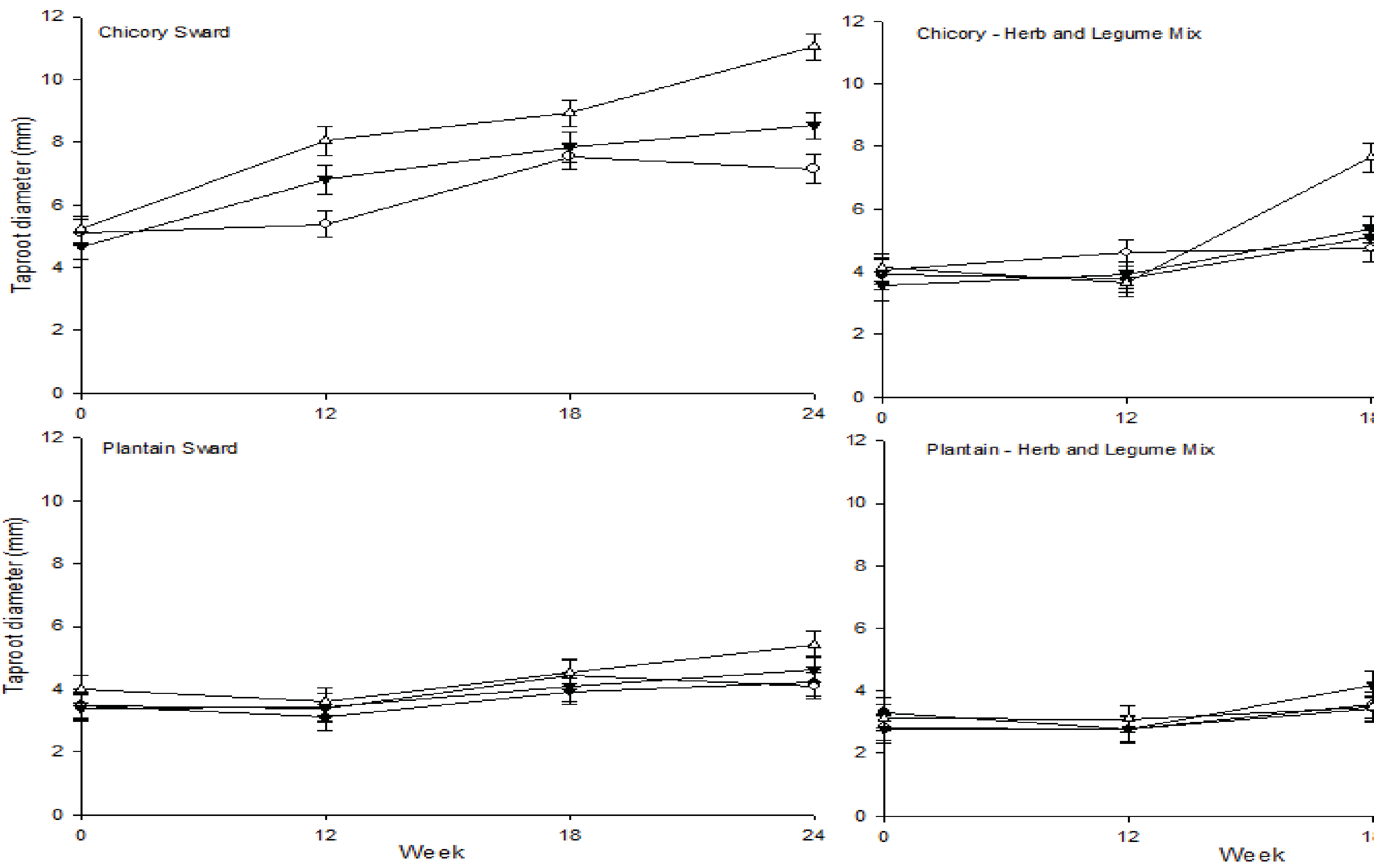



**Figure 7.10 Taproot diameter (mm) in Chicory Sward (top left), Chicory in Herb and Legume Mix (top right), Plantain Sward (b Mix (bottom right) under the defoliation frequencies F1 (●), F2 (○), F3 (▼) and F6 (△) over the 24 week experiment. Vertical ba**

### 7.4.5 Herbage nutritive value

There was no ($P > 0.05$) two-way interaction between sward type and defoliation treatment (F2-H25 vs. F6-H75) for any of the herbage nutritive value traits for the samples collected on 27 February 2013 (Week 23). F2-H25 treatment plots had a greater ($P < 0.05$) OMD and protein % and a lower ($P < 0.05$) NDF than the F6-H75 treatment plots (Table 7.4). The ash content, acid detergent fibre (ADF) and the metabolisable energy (ME) did not differ ($P > 0.05$) between the F2-H25 and F6-H75 defoliation treatments.

The Ryegrass and White Clover Sward had a lower ($P < 0.05$) protein % than both the Chicory Sward and the Herb and Legume Mix. The Ryegrass and White Clover Sward had a greater ($P < 0.05$) NDF and ADF and a lower ($P < 0.05$) OMD and ME than all other sward types. The ME of the other sward types did not differ ($P > 0.05$). The Chicory Sward had a lower ($P < 0.05$) NDF than both the Plantain Sward and the Herb and Legume Mix which did not differ ($P > 0.05$). The Plantain Sward had a lower ($P < 0.05$) OMD and a greater ($P < 0.05$) ADF than the Chicory Sward, while the Herb and Legume Mix did not differ ($P > 0.05$) from either. The ash content did not differ ($P > 0.05$) between any of the sward types.

**Table 7.4 The effect of defoliation treatment (fortnightly 25 mm defoliation (F2-H25) versus six weekly 70 m 27 February 2013 (Week 23) on nutritive value traits (mean ± s.e.): organic matter digestibility (OMD, % dry matte %DM), neutral detergent fibre (NDF %DM), acid detergent fibre (ADF %DM) and metabolisable energy (ME MJ/kg having different letters are significantly different at *P* = 0.05.**

| Parameter | OMD % | Ash % | Protein % | NDF % |
|---|---|---|---|---|
| *Defoliation treatment* | | | | |
| F2-H25 | 81.6b ± 0.45 | 15.5 ± 1.53 | 27.7b ± 0.70 | 26.1a ± 0.91 |
| F6-H75 | 78.2a ± 0.45 | 11.6 ± 1.53 | 18.4a ± 0.70 | 29.1b ± 0.91 |
| *Sward Type* | | | | |
| Chicory Sward | 83.1c ± 0.64 | 13.7 ± 2.17 | 24.5b ± 0.99 | 18.9a ± 1.28 |
| Plantain Sward | 80.8b ± 0.64 | 13.1 ± 2.17 | 22.0ab ± 0.99 | 27.6b ± 1.28 |
| Herb and Legume Mix | 81.3bc ± 0.64 | 13.3 ± 2.17 | 24.8b ± 0.99 | 24.0b ± 1.28 |
| Ryegrass and White Clover Sward | 74.4a ± 0.64 | 14.0 ± 2.17 | 20.9a ± 0.99 | 39.9c ± 1.28 |

## 7.5 Discussion

This experiment compared plant performance over a wide range of defoliation frequencies (D.F's) and defoliation heights (D.H's) which are applicable to what might occur in commercial farming operations, across four sward types (Chicory Sward, Plantain Sward, Herb and Legume Mix, Ryegrass and White Clover Sward). The aim was to assess the potential of the Herb and Legume Mix in relation to pure herb swards and the more traditional ryegrass and white clover sward. Although mowing was used rather than grazing, the herbage yields obtained in small plot mowing experiments are reliable for predicting performance of forages under grazing conditions (Matches 1968; Frame and Hunt 1971). However, it is acknowledged that plants can respond differently under mowing than grazing (Matches 1968; Frame and Hunt 1971), as livestock can selectively graze particular plant species and plant parts and treading damage can occur under grazing.

The parameters measured (herbage yield, botanical composition, plant density, taproot diameter, herbage nutritive value) are linked and thus no one particular parameter can be considered independently, in order to give conclusive recommendations as the potential of each sward type. Consequently, this discussion will firstly discuss each measurement parameter separately, and it will then integrate them and provide some practical conclusions, with a primary focus on the Herb and Legume Mix.

### 7.5.1 Herbage production

#### *7.5.1.1 Chicory Sward*

The net herbage growth rate of chicory ranged between 48 and 57 kgDM/ha/day, and the total accumulated herbage yield ranged between 6720 and 13260 kgDM/ha for the 24 week period. However, there were no differences in either the net herbage growth rate or the total accumulated herbage yield across any of the D.F's or D.H's. This was unexpected as previous research has reported that chicory grows faster and yields higher under relatively infrequent (4-6 weekly) defoliation (Li *et al.* 1997b; Belesky *et al.* 1999; Kemp *et al.* 2002a; Alemseged *et al.* 2003; Sanderson *et al.* 2003a). However, Clark *et al.* (1990a) found no difference in chicory dry matter yields over a relatively short period of eight weeks under 0 mm and 100 mm defoliation heights at 1, 2 and 4 weeks defoliation intervals. The present experiment ran for 24 weeks (almost a full growing season) so it is unclear why no differences in the net herbage growth rate or total accumulated herbage yield were observed under the wide range of the D.F's and D.H's tested. However, given the reduced proportion of chicory in the botanical composition of the sward under fortnightly defoliation compared to six-weekly defoliation it is possible that the herbage yield of chicory was reduced under fortnightly defoliation but that this was masked as weeds made a greater contribution to total herbage yield.

#### *7.5.1.2 Plantain Sward*

The net herbage growth rate of plantain ranged between 42 and 80 kgDM/ha/day, and the total accumulated herbage yield ranged between 3500 and 13280 kgDM/ha for the 24 week period. Plantain yielded less when cut to 25 mm under weekly or fortnightly defoliation than under three-weekly or six-weekly defoliation. This suggests that plantain can still yield well under a low D.H (residual of 25 mm) provided it has at least three weeks to recover in between grazing periods. Similarly, others have found plantain yields more under longer defoliation frequencies (1 *vs* 2 *vs* 4 weeks)(Yu *et al.* 2008) or (3 *vs* 5 weeks)(Sanderson *et al.* 2003a; Labreveux *et al.* 2004). Conversely, Ayala *et al.* (2011b) found three-weekly defoliation resulted in a greater yield of plantain than six-weekly defoliation. Combined these results suggest a three week D.F is more likely to produce a higher dry matter yield than a more frequent defoliation interval.

Under weekly defoliation, plantain yielded substantially more under a D.H of 75 mm than under a more severe defoliation height (50 and 25 mm). Similarly, Nelson (2010) found plantain yielded more under fortnightly defoliation to 80 mm than under more severe defoliation (20, 40, 60 mm) or less frequent defoliation (3-4 weeks). Thus, under frequent defoliation, defoliation height becomes more important. This conclusion suggests that in the short-term at least, provided the residual is at least 75 mm, plantain could be used under continuous grazing, as Hodgson (1990b) stated plants could be defoliated at 5-6 day intervals under continuous grazing. Further research of set-stocking on plantain would be needed to confirm this hypothesis.

#### *7.5.1.3 Herb and Legume Mix*

The net herbage growth rate of the Herb and Legume Mix ranged between 62 and 73 kgDM/ha/day, and the total accumulated herbage yield ranged between 6470 and 13800 kgDM/ha for the 24 week period. There was no difference in the net herbage growth rate or total accumulated herbage yield across any of the D.F's or D.H's. Similarly, Chapter 5 showed net herbage accumulation of a Herb and Legume Mix did not differ under hard and lax grazing (4 *vs* 8 cm residuals). Conversely, Navarrete *et al.* (2013) found net herbage accumulation of a herb and legume sward type over one growing season was greater under a 4-week grazing frequency than a 2-week grazing frequency. Overall, the results indicate that the Herb and Legume Mix might be robust to a wide range of D.H's, but it might be appropriate to not defoliate the sward more frequently than at a three-weekly interval in order to maximise dry matter yield.

#### *7.5.1.4 Ryegrass and White Clover Sward*

The net herbage growth rate of the Ryegrass and White Clover Sward ranged between 36 and 109 kgDM/ha/day, with the greatest rate observed under a weekly D.F. The total accumulated herbage yield ranged between 4140 and 12780 kgDM/ha over the 24 week period. Yield was not affected by D.H, except under a weekly D.F where defoliation to 75 mm resulted in a higher yield than a more severe defoliation height (50 and 25 mm). Previous research has found herbage yield from ryegrass dominant swards is maximised under 50-60 mm post-grazing residual (Wilson and Robson 1970; Fulkerson *et al.* 1994; Parsons and Chapman 2000; Lee *et al.* 2008).

There was no clear pattern in the effect of D.F on yield for the Ryegrass and White Clover Sward. Previous research in perennial ryegrass dominant swards, has shown infrequent defoliation results in higher yields than frequent defoliation under both cutting and grazing systems (Frame and Hunt 1971). Furthermore, white clover yields are highest under frequent defoliation when grown with perennial ryegrass (Brougham 1959). Recent research proposes that ryegrass-based pastures should be grazed when it reaches the 3 leaf/tiller stage, and thus the optimum defoliation frequency will vary with the ambient temperature (Fulkerson and Donaghy 2001). This experiment, illustrates the advantage of perennial ryegrass based pastures and its tolerance of a wide range of defoliation regimes (Kemp *et al.* 2002b).

### 7.5.2 Herbage nutritive value

Across all sward types, a fortnightly D.F to 25 mm resulted in a higher overall sward nutritive value in terms of OMD, Protein %, NDF and ADF compared to a six-weekly D.F to 75 mm. Previous research supports this finding, showing the nutritive value of both chicory and plantain is higher under more frequent defoliation (Jung *et al.* 1996; Sanderson *et al.* 2003b). Similarly, with ryegrass, O'Donovan and Delaby (2005) observed more severe grazing (higher stocking rate) resulted in more green leaf and less dead material and an improved sward nutritive value in comparison with swards subjected to less-severe grazing management. The higher nutritive value of the plots defoliated fortnightly to 25 mm in this experiment could result from a lesser amount of stem material, which was seen under the fortnightly D.F compared to the six-weekly D.F in the pure swards of chicory and plantain during weeks 12 and 18. The nutritive value of both chicory and plantain reproductive stem is lower than that of leaves (Clark *et al.* 1990a; Fraser and Rowarth 1996; Stewart 1996; Lee *et al.* 2014). Conversely, Chapter 5 showed grazing treatment (Hard *vs* Lax) in a Herb and Legume Mix did not affect sward nutritive value traits across the seasons. Combined, these results suggest that D.F may have a greater effect on plant morphology (leaf to stem ratio) and thus sward nutritive value than D.H.

The Chicory Sward, Plantain Sward and Herb and Legume Mix all had a higher overall sward nutritive value in terms of OMD, NDF, ADF and ME content compared to the Ryegrass and White Clover Sward. Previous research has reported Herb and Legume Mixes have lower NDF (Sinhadipathige *et al.* 2012) and higher OMD and ME content (Golding *et al.* 2011) than perennial ryegrass and white clover swards. Given the minimal differences in herbage production in the first growing season between the Herb and Legume Mix and the Ryegrass and White Clover Sward, these results suggest the main advantage in sowing a Herb and Legume Mix would be to provide forage of a greater nutritive value rather than a herbage yield advantage.

### 7.5.3 Botanical composition

#### *7.5.3.1 Chicory Sward*

The proportion of chicory at the end of the 24 week experiment period was greater in the six-weekly D.F treatment compared to the fortnightly D.F treatment (54 vs 76% chicory). Similarly, Navarrete *et al.* (2013) found the proportion of chicory in a pure sward declined over time more under fortnightly grazing than 4-weekly grazing, over one growing season. Combined, these results suggest a six-weekly D.F would ensure a greater proportion of chicory is maintained in the sward than under a more frequent D.F.

#### *7.5.3.2 Plantain Sward*

The proportion of plantain at the start of the experiment averaged 90%, while by the end of the experiment the weekly and fortnightly D.F treatments contained less than 55%. Conversely, the three-weekly and six-weekly D.F's contained an average of 71% and 75%, respectively. Moreover, under the weekly and fortnightly D.F treatments, the proportion of weed was markedly greater at week 24 than at week 0. The results here suggest that a D.F of at least three weeks will ensure a greater proportion of plantain is maintained in the sward than under a more frequent D.F.

#### *7.5.3.3 Herb and Legume Mix*

A mixed herb sward can have greater rates of legume growth and biological N fixation compared to a ryegrass and white clover sward (Goh and Bruce 2005). Li *et al.* (2012) concluded that a legume composition of 10-15% in chicory based pastures would be adequate to meet the plants nitrogen requirements. Although Dove (1989) suggested the optimum legume content of pastures should be 20-45% for maximum animal production. Thus, a herb and legume sward composition of approximately 20-30% chicory, 40-50% plantain and 30-40% clover would likely represent the ideal sward composition to maximise herbage yield, sward persistence and animal production. In this experiment from week 12 onwards across all defoliation regimes this target composition was achieved. There was little variation between the compositions of fortnightly, three-weekly and six-weekly defoliated treatments; however swards which were defoliated weekly contained a greater amount of white clover and less plantain. However, it is acknowledged that frequent defoliation via mowing can over-exaggerate the proportion of white clover in the sward compared to what would be found under grazing (Swift *et al.* 1992). Furthermore, swards which were defoliated to 25 or 50 mm, six-weekly, contained a greater amount of red clover from week 18 onwards compared to similar D.H swards defoliated weekly. The proportions of chicory and plantain in the sward remained quite stable across all D.F's and D.H's over time. These results might suggest that when sown in a mix, chicory and plantain may be more resilient to a wider range of defoliation regimes than when sown in pure swards. Further work is required to confirm this hypothesis.

#### *7.5.3.4 Ryegrass and White Clover Sward*

Across all D.F and D.H treatment combinations the proportion of ryegrass declined over time and by week 24 the sward had an average of only 33% ryegrass compared to 93% at week 0. This was very poor performance, however, ryegrass is a tillering plant (Valentine and Matthew 2002) and it should be able to recover over time (Matthew *et al.* 2013). Contrastingly, the proportion of white clover increased over time from an average of 1% at week 0 and by week 24 the sward contained an average of 35% white clover. However, as previously stated, the percentage of white clover is likely overestimated under mowing than under grazing (Swift *et al.* 1992). At the end of the experiment, the Ryegrass and White Clover Sward contained an average of 27% dead matter, whereas, the Chicory Sward and the Herb and Legume Mix only contained 14 and 10%, respectively. This high dead matter also contributed to why the Ryegrass and White Clover Swards had a lower overall nutritive value compared to the herb based swards.

### 7.5.4 Plant density and taproot diameter of herb based sward types

Plant density of chicory decreased over time in both the pure Chicory Sward and in the Herb and Legume Mix but was not differentially affected by D.F or D.H. This supports previous research that shows chicory plant density declines markedly over time losing approximately 30-35% of its population per year (Hume *et al.* 1995; Li *et al.* 1995; Li and Kemp 2005). Li *et al.* (1994a) reported that once chicory plant density declines to about 20-30 plants/m$^2$ the sward has low growth rates per area and allows weed invasion resulting in an unproductive chicory crop. Alemseged (2000) concluded that optimum yields of chicory can be obtained at a density of 50 – 60 plants/$m^2$. At the end of this 24 week experiment chicory plant density was still 96 and 55 plants per sward meter in the pure sward and the mixed sward, respectively. Thus, in this experiment production was unlikely to be limited. It may have been worthwhile to continue this experiment for a further 24 weeks to determine the long-term effects of D.F and D.H on the plant density and potential herbage production of the swards.

In contrast, the plant density of plantain remained static over time and was not affected by D.F or D.H in both the pure sward and the Herb and Legume Mix. In support of this, Labreveux *et al.* (2004) observed the decline in plant density of plantain was much slower than that seen in chicory. Similarly, Powell *et al.* (2007) reported that a year after establishment and seven grazings, plantain showed better persistence than chicory. These results suggest that plantain maybe more resilient to a wider range of defoliation regimes. Therefore, the stability over time and persistence of plantain is likely to be greater than that of chicory under commercial farming conditions.

As previously stated the plant density of all sward types was not affected by D.F or D.H. It is possible that the 24 week experimental period may have been too brief to observe effects on density. Although, previous long-term (3-4 year) grazing studies using sheep have also reported grazing frequency and post-grazing height did not affect plant density in pure swards of chicory (Li *et al.* 1997b) and plantain (Ayala *et al.* 2011b). Conversely, Navarrete (pers. comm) reported fortnightly grazing with cattle compared to monthly grazing resulted in a greater decline in the plant density of chicory in a pure sward and resulted in an unproductive crop after just one growing season, while plantain density remained stable. Alemseged (2003) found that chicory plant density did not differ between grazing management treatments in year 1, but from year 2 onward the continuous grazing treatment had a lower density than a range of rotational grazing treatments.

Taproot diameter provides a measure of the health status of a plant, as the carbohydrate reserves stored in the taproot provide the energy to support growth following defoliation (Fankhauser *et al.* 1989) and winter dormancy (Kust and Smith 1961). Consequently, the health and carbohydrate storage of taproots influences the persistence of plants (Lodge 1991). Although, plant density was not affected by D.F or D.H, the taproot diameter of chicory in both the pure sward and the mix was lower under more frequent defoliation (one, two and three weekly *vs*. six-weekly) from week 18 onward. While, the taproot diameter of plantain in both the pure sward and the mix was lower under weekly defoliation compared to six-weekly defoliation at week 24, only. Gramshaw *et al.* (1993) reported that increasing defoliation frequency from a 5-week interval to 3-week interval substantially depleted root reserves of lucerne (*Medicago sativa*). Plants with smaller taproots are possibly more susceptible to death following defoliation, and therefore likely correlated with a faster decline in plant density. Leach and Ratcliff

(1979) found that lucerne plants decreased in size before their death, with plants that eventually died notably smaller than those that survived. Chapters 3 and 4 (under glasshouse conditions) also found chicory taproot diameter was reduced under defoliation and more frequent defoliation compared to no defoliation and less frequent defoliation. Nelson (2010) found the taproot diameter of plantain in a 12 week mowing experiment was not affected by D.F. Combined, these results suggest chicory may be more vulnerable to D.F than plantain, with six-weekly defoliation favouring chicory plant health compared to more frequent defoliation.

The taproot diameter of both chicory and plantain was not affected by D.H. Yu *et al.* (2008) reported the taproot diameter of plantain was unaffected by defoliation height, whereas that of chicory was reduced under severe defoliation (lower residual). Similarly, In Chapter 5 in a Herb and Legume Mix, Hard grazed plots (4 cm residual) had a greater plant density than Lax grazed plots (8 cm residual), however the taproot diameter of chicory was greater under Lax grazing than Hard grazing, while that of plantain did not differ under the grazing treatments. As alluded to in Chapter 4, it appears that the growth strategies of plantain and chicory differ. While, plantain is technically taprooted, it does not appear to be as reliant on its taproots reserves as chicory for growth and survival. Furthermore, this supports the notion that plantain is more resilient than chicory to a wide range of D.F's and D.H's. Consequently, this reinforces the earlier statement that plantain in pure swards or mixed swards may have more potential as an alternative perennial pasture option than that of chicory in a pure sward due to its greater flexibility. These results indicate that the grazing management of a Herb and Legume Mix would need to be based on the optimal requirements of chicory in order to ensure sward species diversity is maintained.

## 7.6 Practical conclusions

The aim of this section is to generate some practical farmer management advice and an optimum grazing management regime for each sward type based on the results found in the previous section of the discussion.

### 7.6.1 Chicory Sward

The results suggest that a six-weekly D.F should result in a more productive and persistent pure sward of chicory than a more frequent defoliation interval. However, a six-weekly D.F appeared to encourage more stem growth than more frequent defoliation and consequently herbage nutritive value (OMD%, Protein%, NDF%) was lower under lax, infrequent defoliation compared to hard, frequent defoliation. Overall, D.F appeared to be more important than D.H. In conclusion, a D.F of five weeks represents the optimum grazing management of a pure sward of chicory based on this research and that of others. It represents a trade-off between maximising herbage yield and persistence versus maximising herbage nutritive value.

### 7.6.2 Plantain Sward

The results suggest that a three-weekly D.F to a residual of between 25-50 mm represents the optimum grazing management for pure swards of plantain, in order to maximise herbage yield and persistence. However, grazing to a residual of less than 50 mm is likely to have negative implications for animal intake and therefore a residual of

50 mm is advised to maximise herbage production while ensuring high levels of animal performance are achieved.

### 7.6.3 Herb and Legume Mix

The results suggest that a three-weekly D.F will produce a consistently high percentage of leaf material, a stable sward composition, a high herbage yield and a persistent sward. Furthermore, the Herb and Legume Mix is likely more robust to a wider range of D.H's than pure swards of chicory and plantain and thus has great potential under commercial farming conditions.

### 7.6.4 Ryegrass and White Clover Sward

The effect of D.F and D.H on the Ryegrass and White Clover Sward was not straightforward; however, it is possible to surmise that it responds better to more frequent defoliation than a six-weekly D.F. Overall, these results do not provide a clear message to infer the optimum grazing management of a ryegrass and white clover sward. Instead, they demonstrate the robustness of a ryegrass and white clover sward to a range of defoliation regimes, illustrating its advantage over many alternative forage types.

# Chapter 8 Overall discussion

## 8.1 Introduction

A successful perennial sward could be defined as being easy to establish, producing high annual herbage production evenly across the seasons, maintaining a high nutritive value which has a stable composition and is persistent under grazing. In New Zealand, the most predominant sown perennial sward is perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) (Kemp *et al.* 2002b). However, throughout the summer and autumn seasons production of ryegrass and white clover swards can be limited in both quality and quantity (Hughes *et al.* 1980; Burke *et al.* 2002; Moorhead *et al.* 2002). The development of a Herb and Legume Mix containing chicory, plantain, red and white clover may represent an alternative successful perennial sward. As pure swards, both chicory and plantain have been evaluated as potentially successful perennial forage crops. They both yield highly (Powell *et al.* 2007) and are very palatable to stock (Rumball *et al.* 1997; Corkran 2009). However, the persistence of chicory does not commonly exceed three years (Hume *et al.* 1995; Li *et al.* 1997b; Belesky *et al.* 2000) while the nutritive value of plantain can be poor in some periods (Fraser and Rowarth 1996; Fulkerson *et al.* 2008) and little is known about its persistence (Ayala *et al.* 2011b). Combining these two herbs in a mix with red and white clover has the potential to result in an ideal perennial forage sward, which is high yielding, with a high nutritive value and a longer growing season. Furthermore, the improved persistency of modern red clover cultivars under grazing (Ford and Barret 2011) will also be an advantage in the development and use of the Herb and Legume Mix.

A series of field and glasshouse experiments were conducted to study the potential of the Herb and Legume Mix, including the effects of drought, defoliation and animal diet selection on the sward dynamics, herbage mass production and persistence. The results were discussed in detail in Chapters 3 to 7. This section considers the findings of these chapters and discusses the implications of the Herb and Legume Mix if it was to be used as an alternative perennial forage option in New Zealand. Ultimately, the effective management for improved use of herb and legume sward mixes must involve; an understanding of the physiology of the plants, how they respond to moisture stress and defoliation and the stability of the botanical composition.

## 8.2 Morphology/Physiology

This thesis highlights the key differences between the growth characteristics of chicory and plantain. Taprooted plants utilise their root carbohydrate reserves as a source of energy for winter survival and shoot growth in spring and for shoot growth following defoliation (Kust and Smith 1961; Fankhauser *et al.* 1989). However, it appears plantain does not utilise its root reserves to the same extent as plants with a larger taproot such as chicory. The taproot diameter of plantain remained stable in response to moisture stress and different defoliation treatments (Chapters 3, 4 and 5), except in Chapter 7 where few differences were apparent between different defoliation frequencies at week 24 (i.e. only a smaller taproot under weekly compared to six-weekly defoliation). Conversely, the taproot diameter of chicory fluctuated over time and in response to different defoliation treatments (Chapters 3, 4, 5 and 7). Chicory taproot diameter followed the pattern of peaking during autumn/early spring and steadily reducing throughout spring (Chapter 5). This pattern of seasonal root carbohydrate storage has previously been reported in other taprooted plants (Li *et al.* 1996).

It was expected that the pattern of changes in taproot diameter and root WSC reserves would mirror each other such that taproot diameter would increase as root WSC reserves increase. While, this was true for plantain, with no changes in either taproot diameter or root WSC reserves over time, this was not the case for chicory. Chicory taproot diameter remained static between January and May, whereas root WSC reserves steadily increased, which suggests that changes in taproot diameter may only occur when large increases or decreases in root WSC reserves occur. However, it is still suggested that monitoring taproot diameter is likely to be a useful measurement tool for farmers to use for assessing total root reserves of chicory and plantain and thus determine their suitability for grazing.

Compared to chicory, plantain has a greater shoot mass fraction (proportion of shoot mass to total plant mass) (Chapters 3 and 4). Previous research has also found chicory allocates a greater energy reserve to root mass, whereas plantain allocates more energy to shoot production (Davis 1995; Alemseged 2000; Sanderson and Elwinger 2000; Powell *et al.* 2007). Furthermore, when included in the Herb and Legume Mix plantain has a greater shoot density than chicory (Chapter 5). Combining a greater shoot mass fraction and shoot density should result in plantain having a greater leaf area for photosynthesis and therefore a greater herbage yield than chicory supporting previous research which indicates a greater annual yield for plantain than chicory (Powell *et al.* 2007).

## 8.3 Herbage production and development under defoliation

Herbage production of the Herb and Legume Mix within seasons did not differ under the Hard and Lax grazing treatments, however on a yearly basis the Hard grazing treatment (4 cm residual) had a greater herbage production than the Lax grazing treatment (8 cm residual); 11.6 *vs* 9.0 t DM/ha/year respectively (Chapter 5). Furthermore, the plant density of both chicory and plantain was greater under the Hard grazing treatment than the Lax grazing treatment, an unexpected phenomena. In pure swards of chicory, Li *et al.* (1995) concluded that severe grazing during spring (post-grazing residual of 50 mm or less) is likely to exacerbate the decline in plant density. Conversely in pure swards of plantain, Ayala *et al.* (2011b) found post-grazing height had no effect on plant density. These results suggest that the Herb and Legume Mix is quite robust to a post-grazing height of at least 4 cm, and therefore farmers could be advised to regularly graze down to a 4 cm residual to maximise herbage production. However, when consideration is made based on plant size and density data a different conclusion can be reached. The taproot diameter of chicory was smaller under the Hard grazing treatment than the Lax grazing treatment; 8.2 *vs* 11.2 mm respectively. Contrastingly, plantain had a greater number of crowns per plant and a greater shoot density under the Hard grazing treatment than the Lax grazing treatment (1.9 *vs* 1.7 crowns per plant and 23.5 *vs* 15.3 shoot/$m^2$, respectively). Combined, these results suggest chicory and plantain respond differently to defoliation, with chicory being less able to cope with hard grazing whereas plantain was more resilient, as previously alluded to in Chapters 3 and 4. However, it is important to include chicory in the Herb and Legume Mix as it contains a higher metabolisable energy content than that of plantain (Burke *et al.* 2006; Fulkerson *et al.* 2008) and thus should support higher rates of live weight gain (Fraser and Rowarth 1996). Red clover also responds poorly to hard grazing; producing greater herbage yields and being more persistent under lax grazing (Brock *et al.* 2003; Stewart and Charlton 2006). This was evident in Chapter 5 where the proportion of red clover in the sward at the end of year two was markedly greater in the Lax grazing treatment than the Hard grazing treatment; 17.7% *vs* 3.9% respectively. Consequently, for farmers to retain a productive and persistent Herb and Legume Mix the grazing management should be determined based on the minimum requirements of chicory and red clover, which are the more vulnerable species within the Herb and Legume Mix.

There were few differences in the herbage production of the Herb and Legume Mix compared to a ryegrass and white clover sward and pure swards of chicory and plantain over a 24 week period (Chapter 7). However, the Herb and Legume Mix had a greater overall herbage nutritive value than the ryegrass and white clover sward, as previously found (Golding *et al.* 2011; Sinhadipathige *et al.* 2012). Furthermore, the proportion of chicory and plantain in the sward appeared to be more stable in the Herb and Legume Mix under a wide range of defoliation regimes than in pure swards. This suggests, that the true advantage of the Herb and Legume Mix is its superior herbage quality when compared to a ryegrass and white clover sward and that it represents a more robust sward with the potential for greater persistence than pure swards of chicory and plantain.

## 8.4 Animal factors

Ewe lambs displayed diet selection and grazing preference for species within the Herb and Legume Mix (Chapter 6). This preference varied between seasons and was affected by the species availability, vertical access and palatability, as found by (Malechek and Provenza 1983; Cheeke and Shull 1985; Stephens and Krebs 1986; Bryant *et al.* 1991; Parsons *et al.* 1994b; Thornley *et al.* 1994; Edwards *et al.* 1996). During early spring, this preference was influenced by species availability and vertical access with ewes choosing a diet of species most readily available (chicory and plantain), while from late spring onwards, preference appeared to be increasingly influenced by vertical access and palatability (choosing red clover and avoiding white clover). If the grazing preference for one species within the Herb and Legume Mix was constant, that species would be unlikely to persist over time, however this was not the case. After two years of grazing; chicory, plantain and red clover made stable contributions to the sward but white clover was variable depending on conditions; with an average of 45% chicory, 29% plantain, 11% red clover and 2% white clover (Chapter 5). Furthermore, it seems possible that the seasonal changes in the botanical composition of the sward act as a protection measure for the species as no one species was ready available and easily accessible i.e. present in the upper sward and, highly palatable in all seasons. Therefore, the observed grazing preference appeared to represent more of a trade-off decision between species in order to maximise the volume and quality of herbage consumed.

## 8.5 Further work

There is ample evidence in this thesis on the effect of defoliation on the herbage production, sward characteristics and root parameters of the Herb and Legume Mix, in the short to mid-term. However, in order for a greater number of farmers to make use of the Herb and Legume Mix as a perennial forage option, its herbage production and persistence should be studied over at least a five year term. Therefore, a large scale grazing experiment incorporating multiple farm locations with contrasting climates and soil types and hard and lax grazing would be beneficial to monitor herbage yield, botanical composition and plant density over five years. This information could be used in conjunction with that reported in this thesis to develop a comprehensive understanding of the health and sward dynamics of a Herb and Legume Mix.

Second, weed control is a potential problem associated with using the Herb and Legume Mix, as it is difficult to find a selective herbicide that is safe to use on all four species. While grass weeds can be safely controlled with the use of Haloxyfop (Gallant), controlling broad-leaved weeds is more difficult, with only one known herbicide Flumetsulum (Preside). However even Flumetsulum has been shown to cause suppression of plantain (Gawn *et al.* 2012). Therefore, further research on existing herbicides for use on the Herb and Legume mix is paramount, and development of new selective herbicides may be required.

Third, the responses of chicory and plantain to drought were examined in pure swards under glasshouse conditions in pots, removing any potential effects of rooting depth and plant/soil interactions. Therefore, future work is needed to confirm that chicory and plantain display the same morphological and physiological responses to drought under field conditions. This could be undertaken utilising a rainout shelter to generate irrigated, dry and very-dry moisture conditions and then measuring plant growth, shoot and root morphological traits and physiological traits as done in Chapter 4.

Finally, there is a need to more clearly define why diet selection and grazing preference changes between seasons. This knowledge could be used to develop more comprehensive grazing management advice for farmers across different seasons. Thus, future studies on diet selection and grazing preference in a Herb and Legume Mix should collect herbage samples of the individual plant species so that nutritive value between species within the sward can be compared.

## 8.6 Practical implications

Both chicory and plantain display a degree of drought tolerance (Chapters 3 and 4) and have the potential to be utilised successfully in drought prone regions. Under drought conditions, sward persistence will be favoured by utilising at least a three-week grazing frequency. Furthermore, following the period of drought, allowing the sward an additional period of time to recover and begin growing (approximately 2 weeks) will minimise any negative effect on root mass and taproot diameter.

Following two years of grazing the Herb and Legume Mix maintained all four species in the sward, under both hard and lax grazing management (Chapter 5). While, grazing preference was observed (Chapter 6), the sward remained stable, reflecting the resilience of the sward under grazing. Consequently, from an animal production point of view the Herb and Legume Mix consistently represents an appealing perennial sward.

Chicory was found to be more sensitive to defoliation than plantain in both the Herb and Legume Mix (Chapters 5 and 7) and in pure swards (Chapters 3, 4 and 7). Thus, grazing management decisions should be determined based on the suitability of chicory for grazing. Consequently, the sward should not be grazed more frequently than every three weeks, and should not be grazed below a 5cm residual. However, should the sward be grazed more frequently or more severely than above on an occasion, the sward should be rested for a longer period before the next grazing period in order to recover and replenish its root reserves. This will help to maximise chicory plant density and therefore maintain the sward species dynamics.

## 8.7 Main conclusions

This thesis demonstrated that:

1. Hard grazing was detrimental to chicory taproot diameter and shoot density and therefore constrains the potential management of the Herb and Legume Mix

2. Plantain was more resilient to defoliation than chicory and is therefore easier to manage in a Herb and Legume Mix

3. Ewes displayed seasonal grazing preference for various species within the Herb and Legume Mix, however, the sward composition remained relatively stable over the two year experimental period

4. Both chicory and plantain were relatively tolerant of moisture stress under glasshouse conditions, however the deeper taproot of chicory will support a greater tolerance to moisture stress in the field

This research shows the Herb and Legume Mix is resilient in the face of environmental challenges including moisture stress and preferential grazing. Provided the Herb and Legume Mix is grazed to a residual of between 4 and 8 cm using a three-weekly grazing frequency during the growing season and rested during winter (June-August) it can maintain all four plant species over at least a two year term. These results provide sufficient justification to sow and utilise the Herb and Legume Mix as a perennial forage sward in most New Zealand grazing systems.

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**Table 1: Live weight gain (g/day) of deer during spring, summer and autumn on various pasture species in New Zealand.**

| Season | Perennial ryegrass/ white clover | Chicory | Plantain | Red Clover | Stock class | Study |
|---|---|---|---|---|---|---|
| Spring | 341 | | | 354 | weaner stags | Semiadi *et* |
| | 218 | | | 242 | weaner hinds | Semiadi *et* |
| | 281a | | | 346b | weaner stags | Soetrisno *e* |
| | 188a | | | 260b | weaner hinds | Soetrisno *e* |
| | 266a | 283b | | | weaner stags | Kusmarton |
| | 217a | 303b | 204a | | mixed sex weaners | Hoskin *et a* |
| Summer | 27.2a | 6.7a | | 69.6b | hinds during lactation | Niezen *et a* |
| | 331a | 385b | | 410b | calves | Niezen *et a* |
| Autumn | 192 | | | 263 | weaner stags | Semiadi *et* |
| | 173 | | | 198 | weaner hinds | Semiadi *et* |
| | 207a | | | 237b | weaner stags | Soetrisno *e* |
| | 159a | | | 197b | weaner hinds | Soetrisno *e* |
| | 191a | 282b | | | weaner stags | Kusmarton |
| | 224 | 154 | | | stag calves | Hoskin *et a* |

Means within rows with different letters are significantly different at *P* = 0.05

# Appendix 2

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality.*

Name of Candidate: Lydia Margaret Cave

Name/Title of Principal Supervisor: Professor Paul Kenyon

Name of Published Research Output and full reference:

Cave LM, Kenyon PR, Morris ST, Lopez-Villalobos N, Kemp PO (2014) Ewe lamb diet selection on plantain (Plantago lanceolata) and on a herb and legume mix, including plantain, chicory (Cichorium intybus), red clover (Trifolium pratense) and white clover (Trifolium repens). Animal Production Science, Early Online

In which Chapter is the Published Work: Chapter 6

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate: 80%
  and / or
- Describe the contribution that the candidate has made to the Published Work:

Candidate's Signature — Date: 26/3/14

Principal Supervisor's signature — Date: 26/3/14

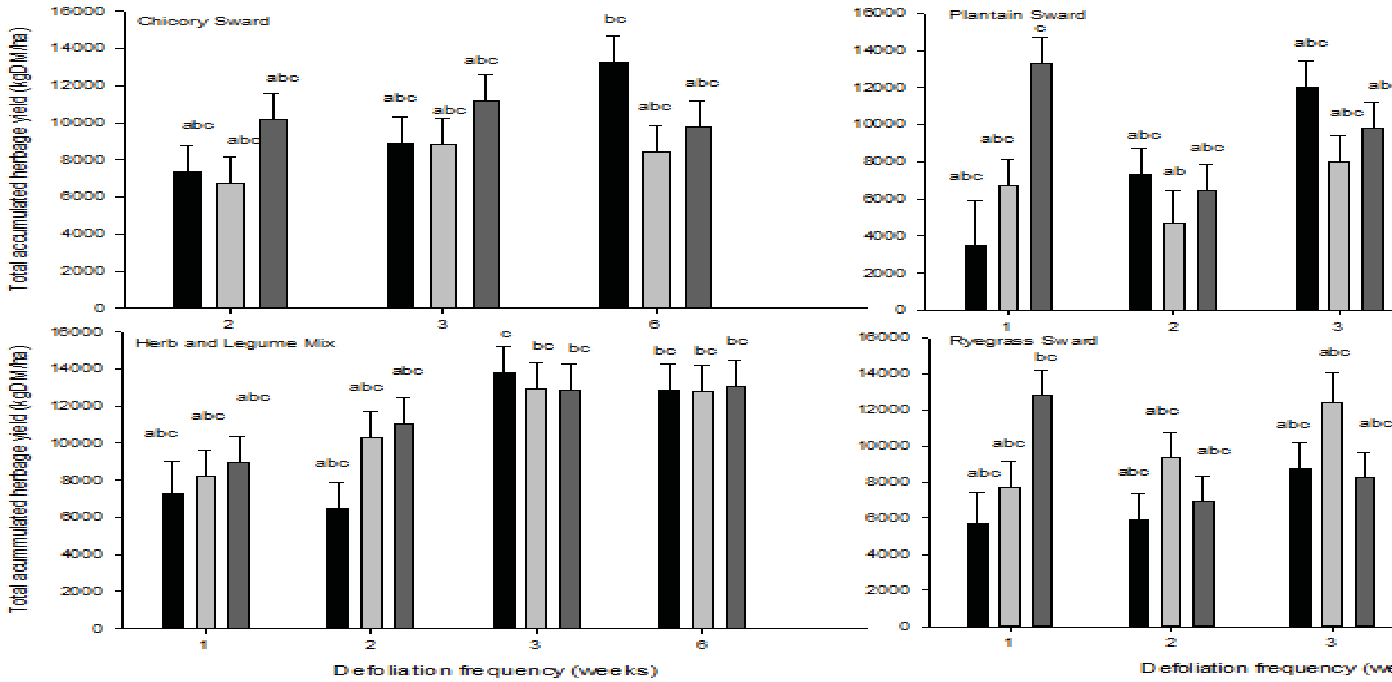


**Figure 1. Total accumulated herbage yield (kgDM/ha) over the 24 week experiment period for each sward type (Chicory (top l Mix (bottom left), Ryegrass and White Clover (bottom right) with all defoliation frequencies (1, 2, 3, 6 week defoliation) a mm (light grey), 75 mm (dark grey)). Different letters (within and across graphs) indicate treatments are significantly different**