Growth and development of three subterranean clover cultivars and their tolerance of herbicide application

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'Whatawhata' is a naturalised genotype of subterranean clover in New Zealand that has been identified as different to the parent of origin 'Mt Barker'. A field experiment was conducted from the 10th of May 2021 to the 2nd of May 2022 to characterise and quantify key aspects of growth and development of the ecotype and compare it with the cultivars 'Denmark' and 'Woogenellup'. Evaluation against 'Denmark' and 'Woogenellup' allowed recommendations to be made for the establishment and management of the cultivar for New Zealand grazing systems. 'Whatawhata' is a small leaved plant with a prostrate growth habit and exceedingly hairy petioles, trifoliate leaves, and runners. It is differentiated from 'Denmark' by the visible leaf mark and structural pubescence, while 'Woogenellup' has a large light green leaf with inconsistent hairiness on structural components. 'Whatawhata' can be classified as a 'Late' flowering cultivar with phenological development consistent with that of 'Denmark' at all stages. In comparison with 'Woogenellup', the 'Whatawhata' strain is slightly later to produce its first trifoliate leaf and reach flowering. 'Whatawhata' required 260±21.2 °Cd to produce its first trifoliate with phyllochron thereafter 64.4±7.0 °Cd/leaf. 'Woogenellup' thermal time requirement in the field (230±21.2 °Cd) and controlled experiment (188±9.29 °Cd) was consistent with published values. Thermal time requirement up to the fourth trifoliate leaf stage was between 417-489 °Cd. Runner initiation occurred with a change in photoperiod. The first runner leaf appeared at the same time for all cultivars (683±39.5 °Cd). Runner phyllochron was not different for 'Whatawhata' (83.3±9.45 °Cd) and 'Denmark' (64.5±9.45 °Cd) but was longer for 'Woogenellup' (96.1±9.45 °Cd). 'Whatawhata' (952±10.9 °Cd) and

‘Denmark’ (962±10.9 °Cd) required the same amount of thermal time after the 21st of June to produce their first flower. This was longer than for ‘Woogenellup’ (883±10.9 °Cd). Legume yield of ‘Whatawhata’ (1898±166 kg DM/ha) was consistent with that of ‘Denmark’ (1986±166 kg DM/ha) and ‘Woogenellup’ (2482±166 kg DM/ha). ‘Whatawhata’ herbicide tolerance is comparable to ‘Denmark’ and ‘Woogenellup’ with a similar response reported for pastures sprayed with flumetsulam and imazethapyr. However, the application of recommended herbicides for weed suppression did not increase total DM yield compared with unsprayed pasture. Phytotoxicity scores supported the lack of difference, with none of the pasture components reaching the commercial threshold (7) for high yield suppression. Cooler temperatures (<10 °C at application) may have lowered the efficacy of herbicides utilised. Low moisture availability and a short growing season also reduced the growth potential of subterranean clover. It is predicted that under favourable growing conditions the effects of herbicide would have been consistent with that of previous research. Based on data from this experiment, ‘Whatawhata’ can be managed as a ‘Late’ cultivar in New Zealand with establishment and grazing management consistent with that outlined for other small leafed cultivars like ‘Denmark’.

Keywords: ‘Denmark’, flumetsulam, flowering, grazing management, imazethapyr, phenology, phenotype, runners, subterranean clover, thermal time, trifoliate, *Trifolium subterraneum* L., ‘Whatawhata’, ‘Woogenellup’.

TABLE OF CONTENTS

LIST OF TABLES

`

LIST OF FIGURES

LIST OF PLATES

LIST OF EQUATIONS

LIST OF APPENDICES

1 INTRODUCTION

Subterranean clover (*Trifolium subterraneum* L.) is a winter annual legume adapted to summer dry environments. The annual life cycle of subterranean clover commences in autumn when sufficient rainfall allows seed imbibition and seedling emergence initiates canopy growth and development. The main advantage of this life cycle is that subterranean clover can provide late winter feed, earlier than perennial clovers (Olykan *et al.* 2019). Feed during this period is critical to ensure animals reach killable weights before dry conditions set in (Mills *et al.* 2015). The duration of this period of vegetative development from mid-winter to spring is cultivar dependent (Teixeira *et al.* 2021). Specifically, cultivars are classified as 'Early' or 'Late' flowering based on their response to photoperiod (Teixeira *et al.* 2020a). Flowering commences earlier in the North Island due to a higher rate of thermal time accumulation after the shortest day. 'Early' flowering subterranean clover cultivars commence flowering in August in the North Island, while the same 'Early' cultivars flower in September in the South Island (Guo *et al.* 2022). For 'Late' cultivars, flowering commences in mid-August in the North Island and mid-September or mid-October in the South Island. The plant then self-fertilizes and sets seed before it dies off with subsequent re-establishment from buried seed in autumn.

Summer dry farm systems must maximize winter and spring pasture production when moisture is non-limiting (Olykan *et al.* 2019). To do this requires nitrogen in the farm system (Mills *et al.* 2006). Subterranean clover fixes an average of 28±0.66 kg N/t of herbage grown (Lucas *et al.* 2010). This can be recycled via grazing and released from dead material when the plant dies off in summer. Subterranean clover has been identified as a suitable legume for three to four million ha of New Zealand's dryland area (Monks *et al.* 2016). This includes large areas east of the main divide and north facing slopes in many traditional summer safe regions (Guo *et al.* 2022). Hot, dry summers and erratic rainfall (Costello and Costello 2003) mean the production and persistence of perennial clover species is difficult (White and Meijer 1979; Knowles *et al.* 2003). However, the relatively open canopy generated by summer dry conditions and hard grazing allows successful establishment of subterranean clover once adequate moisture levels are reached each autumn (Grigg *et al.* 2008). The success of subterranean clover in an environment will be determined by subspecies and cultivar. The amount of feed generated is dictated by the time of the autumn break and subsequent grazing management of the sward (Moot *et al.* 2003). For New Zealand pasture conditions, where stock are often continuous grazed for long periods of the year, a cultivar that has a prostrate growth habit and multiple small leaves has been recommended (Smetham 2003a).

Subterranean clover seed is imported from Australia as there is no current commercial seed production in New Zealand (Lucas *et al.* 2015). However, sales of subterranean clover seed are limited by availability from Australia and biosecurity risks. Cultivars have been selectively bred for Australian conditions, which differ in environment and grazing systems. There is a need to develop a cultivar adapted and capable of seed production in the temperate grazing environment of New Zealand. The purpose of this dissertation is to compare the growth and development of a New Zealand selected subterranean clover ('Whatawhata') with commonly sold Australian cultivars. The aim is to describe the new genotype and therefore to define how it might be used in a New Zealand context. To do this there are four objectives.

1.1 Aims and Objectives

Primary aim: To describe the growth and development of the subterranean clover genotype 'Whatawhata' and compare it with the commercially available cultivars 'Denmark' and 'Woogenellup'.

> Objective 1: To describe the phenotype of 'Whatawhata' and compare it with known cultivars. To do this requires; photos of leaf size, marks, shape, flowers, and other structural components.
>
> Objective 2: To quantify the phenological development of 'Whatawhata' and compare it with known cultivars. This includes recording the time of; first trifoliate, runner appearance, and time of flowering. Monitoring of leaf appearance will be used to calculate the phyllochron. From these measurements 'Whatawhata' will be defined as either an early or later flowering cultivar.
>
> Objective 3: To quantify the growth of the cultivar of subterranean clover. This will be done with dry matter harvests when pasture mass has accumulated sufficiently to

be grazed. Pasture ground cover as a measure of light interception will be measured with a Trimble handheld Greenseeker that measures NVDI.

Objective 4: To describe clover cultivar tolerance to herbicide application. A recommended herbicide will be sprayed across the plots and assessment of visual damage on weeds and subterranean clover investigated.

This dissertation is set out in five chapters. The general introduction (Chapter 1) is followed by a review of the literature (Chapter 2) on growth and development of subterranean clover with the intent for potential registration of the new ‘Whatawhata’ cultivar. Chapter 3 is the materials and methods, which describes the experimental process of the field and glasshouse experiments. Chapter 4 presents the results from the field and glasshouse experiments. Chapter 5 discusses these results and Chapter 6 provides a general discussion and conclusions.

2 REVIEW OF THE LITERATURE

2.1 Seed and morphological characteristics

2.1.1 Seed characteristics

The species *Trifolium subterraneum* L. is comprised of three subspecies (ssp.) which are distinguishable through morphological differences and soil preferences (Nichols *et al.* 2013). The ssp. consists of *brachycalycinum*, *subterraneum*, and *yanninicum.* The ssp. *subterraneum* is the main genotype sown in New Zealand.

Subterranean clover seed is larger than that of the perennial clover species white (T. *repens* L.) and red (T. *pratense* L.) and so requires a higher sowing rate. Thousand seed weight (TSW) indicates the size of subterranean clover seed (Teixeira *et al.* 2018). The early growth of annual species is proportional to the seed size (Black 1956). Low seed weight enables rapid emergence of clover compared with grass species (Moot *et al.* 2000). This is due to the lower thermal time requirements of smaller seeds. However, there is a minimal difference between cultivar seed weight and emergence time. The TSW of subterranean clover is reported to be 6.7 g per thousand seeds (Tozer and Douglas 2016). White and red clover have a TSW of 0.7 and 3.4 g per thousand seeds. Therefore, the sowing rates of subterranean clover needs to be at least 10 times higher than is traditionally used for white clover.

2.1.2 Dormancy and hardseedness

The life cycle of subterranean clover ensures persistence of the species through avoidance of dry conditions as a buried seed, and re-establishment in autumn. However, continued moist conditions after autumn precipitation are not always guaranteed in summer dry areas. This can lead to premature germination and seedling death known as ‘false strike’ (Teixeira *et al.* 2022). Subterranean clover prevents ‘false strike’ from germinating its entire seed reserves through two primary mechanisms. These include embryo dormancy and impermeability of the seed coat or hardseededness (Smetham and Ying 1991). Embryo dormancy is a temporary mechanism which is broken when temperatures fall below 20 °C. Hardseededness is a physical barrier that prevents imbibition of water and subsequent germination. The seed coat is softened through fluctuations in temperatures of over 15 °C. High temperatures initiate chemical alterations which degrades the lens zone of the seed testa (Smetham and Ying 1991) and physical expansion and contraction allows water imbibition (Teixeira *et al.* 2022). However, subterranean clover seed must dry down to 5-7% moisture before imbibing of water can begin (Smetham 2003b). Structural components below the hilum and longitudinal groove of the seed allow moisture to exit but not enter. Eventually an equilibrium, as determined by the maximum air temperature, is reached. Moisture can then enter the seed and initiate germination.

2.2 Morphology

Morphological characteristics are used to differentiate between subterranean clover cultivars (Nichols *et al.* 2013). The leaf size, shape and markings indicate cultivar distinctions. Leaf lamina colour; anthocyanin presence and position on leaves, calyx, and stipules; pubescence on structural components and seed colour enable differentiation among cultivars. Visual differences in morphological characteristics are due to genotype and environment. Leaf size influences light capture. If plants have the same number of leaves, then those with larger leaves are capable of greater light interception, so have more photosynthesis and growth per unit area. However, plants with large leaves may be disadvantaged under New Zealand set stocked sheep grazing systems where large, erect structural components are easily removed. Small, densely leaved cultivars may be more adapted to New Zealand sheep grazing systems that require set stocking over lambing. However, these small leafed cultivars have less competitive capability and may succumb to shading (Smetham 2003b) from grass and broadleaf weeds.

Anthocyanin pigmentation causes a red discoloration of leaves but is transitory, so should not be used for identification purposes. This is a stress response often induced by low temperatures (Teixeira *et al.* 2020c) with differences reported in cultivar responses. Anthocyanin synthesis protects vulnerable plant tissue from abiotic and biotic factors including low temperatures, pathogen infection, and high light irradiance. Teixeira *et al.* (2020c) conducted a series of experiments assessing the anthocyanin level in subterranean clover in response to temperature and its effect on herbage yield. In the experiment, 14 cultivars from all subspecies were established and kept at a temperature of 12.5±2.5 °C until the rosette stage. Plants were then subjected to two weeks of temperatures of <7 °C. Cultivar differences existed ($P<0.001$) with the ssp. *yanninicum*, *subterraneum*, and *brachycalycinum* having 84, 29, and 20% red leaves, respectively. ‘Denmark’ had the lowest anthocyanin response at 11%. There was no correlation between anthocyanin affected plants and DM accumulation.

2.3 Cultivars

Subterranean clover originates from the Mediterranean, Europe’s Western Atlantic Coast and West Asia (Nichols *et al.* 2013). The species has naturalized and been introduced to countries of a similar climate including Australia and New Zealand. The species thrive on pastoral acid soils with an open pasture canopy. Subterranean clover contributes to the legume component of pasture and is successfully utilized in dryland situations across Australia and New Zealand.

2.3.1 Cultivar types

Subterranean clover cultivars utilized in New Zealand originate from Australian germplasm. Commercial sale of subterranean clover seed is based on imported genotype from Australia (Lucas *et al.* 2015; Teixeira *et al.* 2018). There are 45 registered cultivars in Australia of which 32 are ssp. *subterraneum*, eight are ssp. *yanninicum*, and five are ssp. *brachycalycinum* (Nichols *et al.* 2013). Eleven strains originate from a Mediterranean environment, 15 are naturalized ecotypes, 18 have been selectively bred and one is the product of mutagenesis. Of these, 17 have been promoted as suitable for New Zealand conditions since the introduction of subterranean clover (Lucas *et al.* 2015). However, some of these cultivars, such as ‘Mt Barker’ and ‘Tallarook’, sown in the 1930’s have been superseded by superior cultivars. Ten cultivars are currently available for sale in New Zealand. Sales of subterranean clover cultivars are dictated by harvest yield and availability in Australia, as there is no ability to harvest seed at present in New Zealand. Cultivars sown in New Zealand are therefore dictated by cultivar availability rather than for a particular environment (Monks *et al.* 2016). This has created a slow uptake of subterranean clover use in New Zealand, despite its potential.

2.3.2 Australian context

Subterranean clover in southern Australia is the most widespread annual legume sown (Nichols *et al.* 2013). Subterranean clover was accidently introduced to Australia with the importation of animals and contaminated seed. ‘Mt Barker’ was the first recognized and developed cultivar in South Australia. ‘Mt Barker’ was registered in 1907 and sold commercially with additional cultivars discovered and marketed in the 1920s. Subterranean clover is used extensively across Australia and particularly in the wheat belt where it fixes nitrogen into the system and provides off season grazing for livestock (Smetham 2003b). Subterranean clover is predominantly sown as a monoculture in Western Australia as perennial species fail to persist under dry conditions. In eastern areas of Australia where annual rainfall is ~560 mm, subterranean clover is sown with *Phalaris* spp. and occasionally lucerne when rainfall is >350 mm on well drained acidic soils. However, subterranean clover is generally sown as monoculture rather than in a mixed sward which differentiates it from New Zealand practices.

Selective breeding of subterranean clover in Australia has focused on flowering time and seed production (Nichols *et al.* 2013). Flowering time influences vegetative growth and harvestable material of a cultivar before it switches to the reproductive stage, and so limits grazing if managed under best practice (Smetham 2003b). Flowering time influences the suitability of a cultivar for a particular environment. The number of seeds set influences autumn reestablishment and cool season utilization. Typically, later flowering species will produce more herbage mass in a growing season under non-limiting moisture conditions (Nichols *et al.* 2013). Of the 45 cultivars registered in Australia, 10 are early flowering, 20 are mid-season, and 15 are late-flowering.

Selection criteria of Australian breeding has also focused on oestrogenic isoflavones, hardseededness, burr burial, and disease and pest resistance (Nichols *et al.* 2013). Oestrogenic isoflavones can cause temporary and permanent infertility in sheep flocks. Formononetin, genistein A, and biochanin A are the main oestrogenic isoflavones responsible for inhibiting fertility. Formononetin is the primary cause. Heritability of the trait is not well researched, although it appears to be passed onto subsequent generations, suggesting a high heritability. Fungal and viral diseases typically affect species in high rainfall environments with pests such as blue oat mite (*Penthaleus major*), blue-green aphid (*Acyrthosiphon kondoi*), and lucerne flea (*Sminthurus viridis*) being characteristics pests of subterranean clover in Australia. Further traits under investigation include adaptability to acidic soils and increasing salinity; increased hardseededness for cropping rotations; disease and pest resistance, and shallow rootedness which influences seasonal persistence.

2.3.3 New Zealand context

Subterranean clover was introduced to New Zealand in early 1900 (Smetham 2003a). Seed was transported by livestock from Australia and regarded as a weed species until the mid-1920s. Subterranean clover was officially sown into New Zealand pastoral systems in the 1930s. ‘Mt Barker’ and ‘Tallarook’ were initially sown over the entire country beginning on the Canterbury plains in 1930, and then into dry North Island hill country in the 1940s (Smetham 2003b). Subterranean clover was incorporated into grass swards rather than as a monoculture in New Zealand. Plants with a large erect growth habitat are disadvantaged under New Zealand conditions due to continuous grazing practices for periods of the year. Small leaved, prostrate structural growth habitats are more suited to New Zealand grazing management (Smetham 2003a). ‘Mt Barker’ and ‘Tallarook’ cultivars possess these structural characteristics and have successfully adapted to the New Zealand environment. ‘Mt Barker’ is a mid-season flowering cultivar while ‘Tallarook’ is late flowering (Smetham 2003b). Despite no longer being sown, these cultivars remain the dominant resident genotypes in summer dry New Zealand environments.

2.3.4 Whatawhata development

New Zealand is dependent on subterranean clover seed developed for Australian conditions. There are currently no New Zealand cultivars registered (Lucas *et al.* 2015). The importation of subterranean clover seed from Australia is inconsistent (Monks *et al.* 2016). At present, plant variety rights, the small New Zealand market and biosecurity risks hinder seed imports to the country (Lucas *et al.* 2015). Cultivars sold have been developed under Australian conditions and are not well adapted to the unique temperate environment of New Zealand (Monks *et al.* 2016). However, there is potential for a New Zealand cultivar of subterranean clover to be developed to limit dependence on Australian imports. The development of a New Zealand cultivar based on naturalized strains has been investigated. Two localized North Island strains were identified, but research ceased due to the limited feasibility at the time and associated hardseed limitations of the genotypes selected.

These strains have been identified as significantly different from their potential parent material and possible replacements for Australian cultivars as they have adapted to New Zealand conditions and grazing management. A three-year experiment by Dodd *et al.* (1995c) investigated the capability of Australian cultivars and naturalized ecotypes in the North Island. Persistence and production of 45 lines were assessed to evaluate the development potential of a New Zealand cultivar. Strains investigated included 14 lines designated WS from the North Island, six lines designated WB from the North Island, 18 lines designated AK from potential Australian germplasm, and seven from registered Australian cultivars. Strains identified as suitable for New Zealand conditions were similar to 'Mt Barker' but had a later flowering date. The growth habit of suitable lines were prostrate and small densely leaved ecotypes able to withstand continuous grazing. Autumn reestablishment was required to be 200 plants/m^2. Established seedlings were transplanted into pasture swards on 15-20° (easy) and 25-35° (steep) slopes. The trial site was located at Whatawhata Research Station in the northern North Island.

Selected New Zealand strains all flowered within the required 'Mt Barker' and 'Tallarook' time frames apart from WS1726, which flowered later than 'Tallarook'. WS strains consistently had higher autumn seedling densities of 200-400 plants/m^2. However, this was not reported as a significant difference. Spring herbage mass from all strains was lower than 'Mt Barker' and 'Tallarook'. The strains WS1737, WS536, and WBLC had greater ($P<0.05$) total herbage accumulation than 'Tallarook'. Of note in this study was the focus on late flowering ecotypes that produce more total DM than early flowering types under partially affected and non-moisture limiting conditions. Registered Australian cultivars of an early flowering nature were consistently outperformed. However, the rainfall of North Island hill country is not typical for the whole of New Zealand with many areas receiving <1000 mm (Dodd *et al.* 1995c). All the North Island strains and three Australians cultivars met the 200 plants/m^2 criteria. The persistence and late winter/spring growth of the strains WS1436, WS536, WS1740, WS549, WS1737, and AK663 was of note. Spring production was >1000 kg DM/ha.

Dodd *et al.* (1995b) conducted a persistence trial of the same strains at Wairakei. Pumice soils in this region have a low water holding capacity and so can experience periodic dry conditions. Results obtained from this experiment were similar to the Whatawhata trials with less persistence of strains in their final year of measurement at this site. 'Tallarook' was again successful in terms of productivity and persistence. North Island strains that were identified for further research include WS1261, WS1840, WS2040, WS536, and AK662. These strains established a winter plant population of >300 plants/^{2} and set 1000 seeds/m^2.

Dodd *et al.* (1995a) also conducted a production evaluation of the same subterranean clover genotype in the previous persistence trials at the Whatawhata site. However, only nine elite lines were identified and tested in this experiment. Selection was centred on regenerative ability, characteristics of growth and isoflavone accumulation. A significant correlation (P<0.01) was observed between initial seed yield and herbage accumulation the following year. 'Denmark' and WS1801 strain accumulated more DM in the first year then 'Tallarook'. However, persistence of 'Denmark' was poor unlike WS1801 which was noted to have greater persistence than the Australian cultivar under New Zealand conditions. The following year, four of the WS lines out-yielded 'Tallarook'. However, the WS1801 strain was not one of them, with final yield in 1993 of 2,894 kg DM/ha. Across the two years of research, the three most productive strains consisted of AK664, WS536, and WS1436. Herbage accumulated exceeded 2,700 kg DM/ha in both years and seed set was >7,000 seeds/m^2. For dry North Island hill country, the WS536 is potentially suitable. However, for dry east coast regions that receive <1200 mm per annum the WS1801 strain was considered potentially more suitable to generate early season growth (Dodd *et al.* 1995a).

An experiment conducted by Widdup and Pennell (2000) investigated the seed set, autumn regermination, and spring growth of Australian cultivars and New Zealand strains over four years in the South Island. The site was grazed with sheep to simulate New Zealand grazing management. The trial site was at Templeton, Canterbury, with 15 registered Australian cultivars, 100 mid-late flowering ecotypes from the Australasian Subterranean Clover and Alternative Legume Programme (ASCALIP) and five selections from Whatawhata and Palmerston North in the North Island. New Zealand strains expressed a late flowering tendency similar to that of 'Tallarook' and exhibited a prostrate growth habitat with small leaves. Three New Zealand strains set high numbers of seed in their initial year of establishment, with WS1801, AK920, and AK948 producing 23,500, 12,000 and 25,400 seeds/m^2 respectively. Level of hard seed percentage of these strains was 28, 19, and 21% for

WS1801, AK920, and AK948 respectively. However, only AK948 showed improved regeneration over the trial period, with WS1801 and AK920 exhibiting a reduction in regeneration over time. ‘Denmark’, ‘Junee’, ‘Goulburn’, and ‘Karridale’ also produced high seed numbers but of the new cultivars during the experiment, only ‘Denmark’ and ‘Junee’ had an increase in regeneration each year over the four years. Cultivar performance was correlated ($R=0.7$ $P<0.001$) to flowering time in 1994-1995. However, there was no interaction observed in the following year due to environmental limitations. Herbage accumulation of WS1801, AK920, and AK948 was 6,060, 6,810, and 6,920 kg DM/ha from June to November of 1995.

After these trials, WS1801 was selected for potential commercialization based on its productivity in environments with <1200 mm per annum and early season growth (Dodd *et al.* 1995b). Objective 1 is to describe the phenotype of WS1801 which has become the named subterranean clover ‘Whatawhata’. The seed and morphological characteristics will be compared with the cultivars ‘Denmark’ and ‘Woogenellup’ as reported in previous literature. As part of that process, the plant development, herbage accumulation, and herbicide tolerance will be assessed (Objectives 2-4).

2.4 Plant development

2.4.1 Thermal time requirements

2.4.1.1 Germination and emergence

Plant development requires a set amount of thermal time between different phases. Thermal time expresses the relationship between plant development and temperature (Moot *et al.* 2000). Thermal time is calculated by taking the mean temperature of a set day and subtracting the base temperature. Moot *et al.* (2000) conducted an experiment to determine the base temperature and thermal time requirements for germination and emergence of temperate pasture species. The base temperature for subterranean clover was reported to be <1 °C. Moot *et al.* (2000) stated that the thermal time requirement for germination of ‘Mt Barker’, ‘Tallarook’, and ‘Woogenellup’ were 45, 48, and 78 °Cd respectively. 50% field emergence of ‘Mt Barker’ was 112 °Cd. Teixeira *et al.* (2021) reported an average thermal time requirement for 50% field emergence of 100±8.8 °Cd for subterranean clover. This was temperature driven ($P<0.001$), with no cultivar interactions reported ($P=0.32$).

The cardinal temperatures of a plant species depict the range of temperatures at which plant processes occur (Lonati *et al.* 2009). These are categorized as the base temperature, below which plant function ceases, the optimum, at which point plant growth and development is maximized, and the maximum temperature, at which plant processes discontinue. Plant function stops above a certain threshold, despite thermal time accumulation, due to denaturing of enzymes at high temperatures (Teixeira *et al.* 2020b). Plant species capable of operating at a range of temperature fluctuations have improved persistence, as they are able to emerge and generate a canopy faster than their competitors. Lonati *et al.* (2009) reported the base temperature of subterranean clover ('Mt Barker') to be 0.1 °C, the optimum at 13 °C and the maximum 27 °C. In comparison, white clover ('Demand'), was estimated to have a base temperature of 2.5 °C and an optimum of 25 °C. Moot *et al.* (2000) reported that white clover function decreased at 30 °C. The thermal time required for emergence and phenological development of annual clovers is generally lower than that of perennial species, which improves persistence in adverse environments (Lonati *et al.* 2009). Subterranean clover operates at lower optimum temperatures then perennial species. However, this limits the species growth and development at higher temperatures. Teixeira *et al.* (2020b) conducted an experiment to quantify the thermal time requirements and cardinal temperatures of four subterranean clover cultivars. 'Denmark', 'Mont', 'Antas', and 'Narrikup'. Seeds were germinated at 11 constant temperatures ranging from 2.5 to 35 °C. 50% germination was calculated at each of the temperatures. Final germination at 35 °C was below 50% for all cultivars involved, demonstrating subterranean clovers lack of adaptation to high temperatures. This is consistent with it being a winter annual, which avoids germination when temperatures are still too high to reduce the risk of a 'false strike'. A cultivar*temperature interaction was reported ($P<0.02$). Germination was 88% across all cultivars between the temperature range of 5 to 25 °C.

Cultivars with an increase in temperature past the base temperature germinate in a linear fashion until an optimum is reached (Teixeira *et al.* 2020b). Past the optimum, germination of all four cultivars decreased until a maximum temperature was reached at which point germination ceased. However, a difference in optimum temperature for germination exists among cultivars. Cultivar (genotype) origin was suggested as the reason for these optimum temperature differences.

2.4.1.2 Trifoliate and runner initiation

Trifoliate leaf appearance represents the first true leaves of subterranean clover. Initiation of trifoliate leaves increases the photosynthetic area available for light capture and provides energy for further growth and development. Moot *et al.* (2003) determined the thermal time requirements for initial trifoliate appearance, branch development and phyllochron for subterranean clover seedlings. Six environmentally controlled experiments were carried out with either one subterranean clover cultivar (‘Denmark’) and one white clover cultivar (‘Demand’) or five subterranean clover cultivars (‘Campeda’, ‘Goulburn’, ‘Denmark’, ‘Leura’, and ‘Woogenellup’) and white clover (‘Denmark’). Temperature regimes ranged from 6 to 25 °C. Split temperatures were implemented for Experiments 4 and 5 and alternated every 12 hours. An additional field experiment was conducted using four sowing dates. Sowing dates reflected potential autumn rainfall events. Thermal time requirement for initial trifoliate leaf appearance of subterranean clover was estimated to be 230±20.0 °Cd. However, the first trifoliate leaf presence of white clover was 309±10.0 °Cd (Table 2.1). Lonati *et al.* (2009) reported a thermal time requirement of 220 °Cd for initial trifoliate leaf appearance for ‘Mt Barker’. Teixeira *et al.* (2021) reported an average thermal time requirement of 188±16.2 °Cd for initial trifoliate appearance.

Table 2.1 Base temperature (T_b) and the thermal time (Tt) requirements of initial trifoliate leaf appearance of white clover and subterranean clover cultivars under controlled and field experiments at Lincoln University. From Moot *et al.* (2003).

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Appearance of the first leaf improves photosynthetic capacity of the plant with thermal time requirements for subsequent trifoliate leaves decreasing. Main stem phyllochron (time between successive leaf appearance on the main stem) was estimated as 68±6.5 °Cd in comparison with 89±5.4 °Cd for white clover (Moot *et al.* 2003). Lonati *et al.* (2009) reported phyllochron development for annual clovers to be 52-62 °Cd. There was no difference (P>0.05) observed for phyllochron of annual clover species. The low base and optimum temperature of annual clovers in comparison with white clover enables successful germination and establishment in cooler environments as autumn approaches (Lonati *et al.* 2009). At trifoliate leaf stage four to five (safe grazing height) (Teixeira *et al.* 2021) plants start to form axillary buds in the axil of the trifoliate leaves (Moot *et al.* 2003). Total leaf development up to this point increases in a linear fashion. However, with the onset of leaves forming from the axillary buds, leaf production turns exponential and is known as the 'switch'. Mainstem leaf appearance remains linear. Runner appearance was estimated to occur at 434±35.0 °Cd for subterranean clover, while white clover stolons required 486±10.0 °Cd (Table 2.2). The ability to establish and develop a canopy capable for cool season growth provides valuable feed for animals approaching mating and rebuilds pasture covers after a dry period.

Table 2.2 Base temperature (T_b) and the thermal time (Tt) requirements of stolon commencement (five leaf stage) for white clover and runner commencement (six leaf stage) for subterranean clover cultivars under controlled and field experiment conditions. From Moot *et al.* (2003).

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2.4.1.3 *Flowering*

Flowering time is influenced by photoperiod and thermal time accumulation. Photoperiod influences the transition from vegetative growth to reproductive development in subterranean clover (Teixeira *et al.* 2020a). Teixeira *et al.* (2020a) reanalysed Australian and New Zealand data sets of multiple subterranean clover cultivars across different environments. Photoperiod influence on thermal time accumulation to initiate flowering was investigated (Figure **2.1**). In a decreasing photoperiod subterranean clover did not initiate reproductive development despite adequate thermal time. This is shown by the continued accumulation of thermal time from April and July sowing dates. Subterranean clover remained vegetative and continued to grow leaves to form a rosette. Flowering was only induced by an increasing daylength which fits with its description as a long day species (Nichols *et al.* 2013). Once the shortest day was reached and photoperiod started to increase, the plants initiate reproductive development and begin forming runners. Thermal time accumulation then drives subsequent development with cultivar differences in flowering time determined by genetic parameters (Teixeira *et al.* 2020b). 'Early' flowering cultivars post winter solstice required ~700 °Cd while 'Late' flowering ecotypes required ~1000 °Cd (Figure 2.1). Part of Objective 2 is to determine whether the 'Whatawhata' is an 'Early' or 'Late' genotype.

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Figure 2.1 Number of days and thermal time accumulation from time of sowing until 50% flowering of subterranean clover. Data was reported from four experimental sites in Australia and nine subterranean clover cultivars. Photoperiod direction as indicated by the solid (—) line (April-July) represents a decreasing photoperiod with the dashed (– –) line (July-November) reporting an increasing photoperiod. The (●) symbols represent early flowering cultivars ‘Dalkeith’, ‘Nungarin’, ‘Seaton Park’ and ‘Trikkala’. (●) symbols represent late flowering cultivars ‘Karridale’, ‘Larisa’, ‘Meteora’, ‘Mt Barker’, and ‘Woogenellup’. From Teixeira (2019).

Because flowering is constant after the shortest day, the total amount of thermal time required to reach 50% flowering will change depending on the emergence time in autumn (Teixeira 2019). Figure 2.2 shows the relationship between photoperiod and thermal time requirement to reach 50% flowering. As photoperiod decreased, the thermal time required to reach 50% flowering fell from 1680 °Cd to 750 °Cd. This relationship was similar for both 'Early' and 'Late' flowering cultivars. After the shortest day, indicated by the arrow, thermal time requirements decrease in response to increasing photoperiod. 'Late' flowering cultivar thermal time requirements decreased to approximately 1000 °Cd ($P>0.05$). However, early flowering cultivars decreased ($P<0.05$) from 800 °Cd to 650 °Cd in response to increasing photoperiod after the shortest day. Teixeira *et al.* (2021) reported that the total thermal time requirement for phenological maturity of subterranean clover sown in February and March was >2000 °Cd due to the extended period of autumn growth.

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Figure 2.2 Estimated thermal time and number of days to reach 50% flowering of nine cultivars of subterranean clover across three Australian experimental sites. The (●) symbol represent the 'Early' flowering cultivars 'Dalkeith', 'Nungarin', 'Seaton Park', and 'Trikkala' while the (●) symbol represent the 'Late' flowering cultivars 'Karridale', 'Larisa', 'Meteora', 'Mt Barker', and 'Woogenellup'. The shortest day is shown by the arrows. From Teixeira (2019).

Flowering time influences the onset of the species spring flush and length of time subterranean clover swards can be grazed before stock removal. It also determines the length of time subterranean clover remains in the sward before plant die off due to life cycle completion. Smetham (2003b) suggested flowering 70-80 days before dry conditions limit pasture growth was required for successful seed set. Flowering time between the end of

October and early November is adequate for successful seed set in New Zealand. For areas that fall below wilting point in mid-November, and experience prolonged dry conditions to March, early to mid-early flowering cultivars should be utilized. It is recommended that 5 kg/ha of a proven cultivar (pre-1998) and 5 kg of a new productive cultivar be utilized in the 10 kg/ha sowing mix (Lucas *et al.* 2015).

2.5 Establishment

2.5.1 Time of sowing

Summer dry areas in New Zealand are characterized by rainfall <1000 mm per annum (Tozer *et al.* 2016). Soil moisture levels increase with autumn rainfall, with warm temperatures providing a conducive environment for plant emergence. Spring conditions are similar in terms of moisture and have increasing temperatures, which meet the thermal time requirements for phenological development. However, moisture limitations influence the growing time of subterranean clover. Moot *et al.* (2003) reported that subterranean clover sown on the 7th of March produced 7,000 kg DM/ha by mid-September, while subterranean clover sown on the 7th of May produced 1,800 kg DM/ha. Daily growth rates differed ($P<0.05$). This highlights the time of autumn rainfall as a significant factor in the expected productivity of subterranean clover once it is established.

Tozer *et al.* (2016) investigated management practices related to high pasture production from two pasture mixes across four different environmental sites. The pasture mixes consisted of grass, legumes, and herbs and legumes only. The sites included dryland areas in Cheviot, North Canterbury and Poukawa, Hawkes Bay. For both dryland sites, germination of legumes, grasses, and herbs was greater in autumn than spring sowings. However, there was no difference observed between autumn and spring sowing of subterranean clover from either Cheviot or Poukawa. Moisture and temperature interactions influence the ability of subterranean clover to establish and set seed in a dryland environment. Seedling survival is often compromised under spring sowings due to the erratic nature of dryland rainfall. The less developed root system of a juvenile plant compared with an adult established in autumn can often lead to desiccation. Subterranean clover sown in autumn has a long period for runner initiation which also extends the grazing time (Teixeira *et al.* 2021). However, when sown into an increasing photoperiod, the subterranean clover life cycle is shortened. The ability of late flowering cultivars to set seed is also compromised when sown in late spring. Therefore, autumn sowing of subterranean clover is recommended.

2.5.2 Pre-sowing management

Pre-sowing management involves creating an environment conducive for seedling emergence and canopy development. Successful establishment of a plant species depends on temperature, soil fertility, moisture, and plant management (Tozer and Douglas 2016). Legumes require phosphorous (P) for growth and development. Soil fertility assessments are required to ensure adequate nutrient levels for developing seedlings. P is required for nodulation of rhizobium to plant roots and their subsequent function (Vardien *et al.* 2016). Gillingham *et al.* (2008) investigated the response of pasture on easy slopes to nitrogen (N) and P application. The experiment was carried out over seven farms from northern Hawkes Bay to North Otago. An increase in soil Olsen P level from 10 to 20 ug mL increased early spring clover production by 100% over the four most northern sites with an increase ($P<0.05$) in late spring and autumn growth also occurring. Clover showed no response to N application. This is due to clover synthesizing its own supply of N through the symbiotic relationship with rhizobium bacteria. However, legumes will utilize inorganic N in the initial stages of plant establishment, during a lack of rhizobium function and in preference to fixation due to the lower energy cost (Berenji *et al.* 2015).

Hardy *et al.* (2019) investigated the macro element requirement of tedera (*Bituminaria bituminosa)* and subterranean clover. The key elements assessed included P, potassium (K) and sulphur (S). Seed was sown into 20 cm diameter (0.031 m^2) by 20 cm deep pots of nutrient deficient soil. P requirement for 90% maximum yield was 139 mg P/pot. K requirements for 90% maximum yield was 194 mg. A significant response was reported for subterranean clover up to an S concentration of 12 mg/pot, which equated to a 13% increase in herbage accumulation. S requirements are typically met with application of super phosphate which contains 11% S.

For dryland areas, a soil Olsen P level between 15-20 is adequate for subterranean clover growth and development (Lucas *et al.* 2016). Sulphate-S values maximize growth at levels of 8. Molybdenum levels of 5.7 are sufficient to promote clover growth, although accessibility to this nutrient is limited under low pH levels. pH levels above 5.6 are sufficient for most subterranean clover cultivars with 'Antas' subterranean clover requiring a pH of 6.0. However, data was not reported by Lucas *et al.* (2016) to support this.

Prolonged removal of existing vegetation reduces competition for emerging seedlings (Tozer *et al.* 2016). The use of herbicide application removes resident vegetation. Herbicides utilized for broad spectrum removal of weed species include paraquat, diquat, dicamba, 2, 4-D, 2, 2 DB and glyphosate. Alternative methods include hard grazing to below 2 cm with stock assisting with the treading of seed into the ground (Tozer and Douglas 2016). Herbicide application has been reported to increase white clover seedling establishment by 200-300% in comparison with no herbicide application (Tozer *et al.* 2016).

2.5.3 Sowing

The method of sowing influences the survival ability of subterranean clover. On slopes of <25° machinery can access and direct drill subterranean clover (Tozer and Douglas 2016). Direct drilling minimizes soil disturbance and moisture loss through retaining vegetation and reducing soil exposure to air. Precise sowing of seed ensures soil to seed contact and reduces intraspecific competition. Subterranean clover should be sown at a 10-20 mm depth (Lucas *et al.* 2016). However, on slopes of >25° the use of ground-based machinery is considered unsafe (Tozer and Douglas 2016). Alternative options for hill country include aerial application of seed by plane or helicopter, or broadcasting by hand. Establishment of pasture species is less successful with these methods. Hill country environments are typically difficult for establishing high fertility species, with resident species requiring suppression through chemical means. Herbage mass present requires removal through stock to allow adequate soil to seed contact (Tozer *et al.* 2016) and can aid in pushing seed into the ground. However, Teixeira *et al.* (2022) reported no difference ($P=0.59$) in whether subterranean clover burrs were on the surface or buried in their subsequent hard seed break down. The complete removal of existing vegetation can increase exposure to climatic factors and therefore increase seedling mortality (Tozer and Douglas 2016).

Sowing rate is determined by seed size and sowing mix. Subterranean clover seed is typically larger than that of other perennial clover species and so must be sown at higher rates to account for fewer seeds. Subterranean clover should be sown at 10 kg/ha (Lucas *et al.* 2016). In a multi species sowing mix subterranean clover should be sown at 10 kg/ha and white clover at 2 kg/ha. Perennial ryegrass should be sown at 8 kg/ha in non-moisture limiting sites and cocksfoot at 2 kg/ha in dryland sites. Due to the erratic spring rainfall of dryland environments, an early and late flowering subterranean clover cultivar should be incorporated into the seed mix (Lucas *et al.* 2015).

2.6 Grazing management

Grazing of subterranean clover influences the longevity of the sward. Persistence and continued productivity of subterranean clover is dependent on flowering and subsequent seed set, followed by reestablishment in autumn (White and Meijer 1979; Ates *et al.* 2006). This is dependent on the grazing management of subterranean clover during flowering and autumn seedling emergence.

2.6.1 Seed set

Grazing of subterranean clover should only commence when seedlings achieve three to five trifoliate leaves per plant (Grigg *et al.* 2008). Grazing of subterranean clover after the initial emergence period during late autumn and winter period should be lax. Defoliation should not occur to below 1200 kg DM/ha (Lucas *et al.* 2016). Set stocking for lambing can occur on pasture covers of 2000 kg DM/ha (Ates *et al.* 2006). If seed set is a priority, then sheep should be removed a week after flowering commences to ensure adequate seed set for the following autumn (Lucas *et al.* 2016). For moderate seed set, cattle can be grazed from initial flower appearance (Guo *et al.* 2022). Grazing can recommence after seed set (bur burial) has taken place. Grazing during flowering reduces seed set through the removal of inflorescences and burrs (Smetham and Dear 2003). For herbage production to be maximised the following spring 500-1000 seedlings m^2 are required in grass/clover mixes (Smetham 2003a).

Smetham and Dear (2003) investigated the effect of different levels of continuous grazing on flowering and seed set of subterranean clover. Grazing levels included nil (no grazing), lenient (visually one layer of leaves over crown and runners), and hard (few leaves left over crown and runners). Seed yield was 1254 and 71 kg/ha for nil and hard continuous ($P<0.05$) grazing, respectively (Table 2.3). However, there was no difference between lenient and hard grazing.

Seed yield influenced ($P<0.05$) herbage production the following autumn with 5587 and 1405 kg DM/ha produced from the nil and hard grazing, respectively. There was no difference between the herbage mass of lenient and hard grazing. Plant weight and leaf area per plant were related ($P<0.01$) to seed set. The grazing of reproductive structures physically removes seed, with the reduction in leaf area and plant weight, due to the removal of photosynthetic areas available for light capture. This reduces partitioning of resources to seed fill. However, previous studies, reported by Smetham and Dear (2003), stated a 30% increase in seed yield when subterranean clover swards were grazed until initial flower appearance. This removes competition from other species and increases inflorescence production due to enhanced solar radiation through the canopy. The point at which flower appearance begins is dependent on the flowering dates of individual cultivars. However, the minimum amount of seed set suggested was 127, 108, and 82 kg/ha for early, mid, and late flowering cultivars respectively (Smetham 2003b).

Table 2.3 The influence of three grazing regimes on herbage accumulation on October 22nd and subsequent seed yield on 25th of February 1986 for five cultivars of subterranean clover. Subscript letters indicate a significant difference. From Smetham and Dear (2003).

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Ates *et al.* (2013) investigated the influence of stocking rate and the closing date on spring subterranean clover. 10 kg/ha (5 kg ‘Campeda’ and 5 kg ‘Leura’) was sown into an existing pasture of cocksfoot, perennial ryegrass and white clover established three years previously. Stocking rate was 8.3 or 13.9 ewes/ha with twin lambs at foot. Autumn germination following spring grazing was greater ($P<0.05$) at 2850±267 seedlings m^2 at the low stocking rate than at the high rate (2500±217 seedlings m^2). A difference ($P<0.05$) was also reported for closing date. Stock removal produced 3850±398, 2950±242, 2100±126, and 1700±152 seedlings per m^2 the following autumn 2, 4, 6, and 8 weeks after initial flower emergence. However, animal productivity per ha decreased at low stocking rates despite individual animal performance increasing (Ates *et al.* 2006). Ates *et al.* (2006) reported that ewes stocked at 10 and 20 ewes/ha had a lamb liveweight gain of 7.5 and 12.3 kg/ha/day respectively. However, ewes at the high stocking rate lost 4.9 kg/animal and seed set was reduced by 62 burrs/m^2. The increase in total animal production from a high stocking rate reduced seedling numbers the following autumn. The implication was that a stocking rate between the two used would be appropriate for maximizing animal and plant performance.

Olykan *et al.* (2019) conducted an on-farm experiment to assess the potential of seasonal management strategies to promote subterranean clover production in an 800 mm rainfall environment on Wairarapa hill country. Resident subterranean clover species consisted of 'Mt Barker' and 'Tallarook'. Three enclosure treatments were created on upper, middle, and low slopes with grazing spells of four or eight weeks implemented. Subterranean clover increased from 13% to 54% for the 8-week exclusion period and 10% to 28% for the lightly grazed control. Subsequent reestablishment in autumn was higher ($P<0.05$) for the 8-week exclusion than the lightly grazed control with a positive association ($P<0.001$) observed between ground cover spelled and seedling density the following autumn.

2.6.2 Autumn re-establishment

Existing pasture mass requires removal before autumn germination of subterranean clover. Grass competition with subterranean clover can cause smothering of seedlings (Costello and Costello 2003). Grass should be grazed in summer to covers of 700-1000 kg DM/ha to ensure successful germination of subterranean clover when adequate moisture levels are achieved (Lucas *et al.* 2016). Subterranean clover swards should be allowed to reach the four to five trifoliate leaf stage before grazing commences (Teixeira *et al.* 2021). In the case of a prolonged drought, direct drilling can be done to enhance resident subterranean clover populations once dry conditions cease (Costello and Costello 2003). Direct drilling improves the amount of buried resident seed and introduces new germplasm to the soil environment.

2.7 Herbicide

2.7.1 Autumn/winter weed competition

Annual weeds compete, particularly for light. Subterranean clover is susceptible to shading (Smetham 2003b) with a high weed population reducing the photosynthetic capability of the species in a pasture. With the onset of autumn moisture, the bare ground left by a summer dry period is colonized (Tozer *et al.* 2007) by species able to grow and develop a canopy capable of capturing light for photosynthesis. Weed species can dominate a sward with perennial grass species often slow to re-establish. In dryland pastures, grass and broadleaf weed species compete with improved species for resources and can have associated animal health implications (Tozer *et al.* 2010).

Weed species differ across spatial areas, with dryland grass weeds consisting of annual poa (*Poa annua*), barley grass (*Critesion murinum*), soft brome (*Bromus hordeaceus*), and vulpia (*Vulpia bromoides*) which possess a relatively low nutritional value. Seed head emergence of barley grass in late spring deters defoliation by livestock with seed heads potentially causing carcass and pelt damage (Tozer *et al.* 2007; Tozer *et al.* 2010). Tozer *et al.* (2007) conducted a study assessing the competitive capability of AR542 endophyte infected tall fescue (*Festuca arundinacea*) and over sown subterranean and Caucasian (*Trifolium ambiguum*) clovers to suppress vulpia and barley grass. Hard grazing by ewes was also implemented during spring. Barley grass ($P<0.05$), vulpia grass, and eudicotyledonus weeds were reported to be lower for treatments over sown with subterranean clover. Barley grass seed head percentage was also reduced ($P<0.01$). Subterranean clover germination in autumn and canopy cover during spring is reasoned to be the main factor in reducing germination of grass weed species. Tozer *et al.* (2010) conducted an experiment investigating the suppression capability of cocksfoot and perennial ryegrass pastures over sown with subterranean and balansa clover (*Trifolium michelianum*) on the grass weeds vulpia and goose grass (*Bromus hordeaceus*). Fewer vulpia and goose grass plants were observed in cocksfoot than perennial ryegrass swards. However, this was only significant in August ($P<0.05$). Grass weed survival was reduced by over sowing of annual legumes ($P<0.05$) with fecundity also reduced ($P<0.05$).

Broadleaf weeds also utilize bare ground made available by previous summer dry conditions. Broadleaf weeds are typically unpalatable to stock with storksbill seed heads causing similar animal welfare effects to barley grasses (James and Hartley 1977). The removal of broadleaf and weed species with selective herbicide increases subterranean clover yields. Safe herbicide application occurs at the three to four trifoliate leaf stage.

2.7.2 ALS inhibiting herbicides

Herbicide control of annual grass and broadleaf weeds must be selective to reduce impacts on subterranean clover production. Previously the herbicides MCPB (4-(4-chloro-2-methylphenoxy) butanoic acid) and 2, 4-DB (4-(2, 4-dichlorophenoxy) butanoic acid) were recommended for control of broadleaf weeds in pastures with establishing legumes (Taylor *et al.* 2020). However, this can reduce subterranean clover yields by 62% (Evans *et al.* (1989) as reported by Taylor *et al.* (2020). Acetolactate synthase (ALS) inhibiting herbicides are recommended for the control of winter weed populations in subterranean clover swards. Headstart® (flumetsulam) is the only ALS inhibiting herbicide currently recommended for use in New Zealand (Taylor *et al.* 2020). Spinnaker® (imazethapyr) has also been shown to be effective at weed control (Lewis *et al.* 2017; Taylor *et al.* 2020).

Acetolactate synthase (ALS) inhibiting herbicides are a class of herbicides that limit amino acid production in plant species (Cobb and Reade 2010). There are five classes of ALS inhibiting herbicides which consist of Imidazolinones (imazethapyr), Triazolopyrimidines (flumetsulam), Sulfonylureas, Pyrimidinyl (thio)benzoates, and Sulfonyl amino carbonyl-triazolinones. Imazethapyr is utilized in legume crops. Accumulation of residuals in the food chain is limited as only plants and microorganisms can generate all their required amino acids. ALS inhibiting herbicides target the enzyme acetolactate synthase responsible for production of valine/leucine and isoleucine which are necessary for further enzymatic function (LaRossa 2013). The herbicide compound inhibits the enzymes' ability to condensate two pyruvate molecules to form a-acetolactate or pyruvate joining with a-ketobutyrate to form a-acetohydroxybutyrate. Death of plants treated with ALS inhibiting herbicides is inconclusive (Cobb and Reade 2010). However, cell division is markedly reduced in the growing points of treated plants. Chlorosis and wilting of treated plants are observed within a few days with discoloration spreading to the entire plant and death of the plant occurring 10 days after application under optimum plant growth conditions. However, under less-than-ideal conditions, plant death may be prolonged to up to two months. Tolerance of this herbicide is due to rapid metabolism by the desired plant species. This reduces the half-life of the herbicide considerably, thereby diminishing its influence on the tolerant plant species.

2.7.3 Imazethapyr and flumetsulam

Imazethapyr has an active ingredient of 5-ethyl-2-(4-methyl-5-oxo-4-propan-2-yl-1H-imidazol-2-yl)pyridine-3-carboxylic acid (National Center for Biotechnology Information 2022b) and is marketed as Spinnaker®. The herbicide is within the Imidazolinones class of ALS inhibiting herbicides. Flumetsulam has the active ingredient N-(2,6-difluorophenyl)-5-methyl-[1,2,4]triazolo[1,5-a]pyrimidine-2-sulfonamide) (National Center for Biotechnology Information 2022a) and is marketed as Headstart®. Flumetsulam is of the Triazolopyrimidines class of ALS inhibiting herbicides marketed in New Zealand.

Taylor *et al.* (2020) conducted an experiment assessing the effect of ALS inhibiting herbicides on weeds and subterranean clover cultivars utilized in New Zealand. Flumetsulam and imazethapyr inhibiting herbicides were used over seven subterranean clover cultivars. Total subterranean clover DM accumulation after three harvests was 4220 and 5500 kg DM/ha for the control and herbicide treatments respectively. Herbicide application reduced weed populations by 1000 kg DM/ha. Lewis *et al.* (2017) reported a similar interaction for the use of imazethapyr on subterranean clover in a mixed species sward. Subterranean clover yield was increased by 1000 kg DM/ha with the application of imazethapyr with cocksfoot yield reduced by 70% in the first spring after sowing. However, cocksfoot yield after first grazing was no different to the control. The use of imazethapyr increased subterranean clover yield through a reduction in weed competition and cocksfoot suppression which allowed subterranean clover to establish in the sward.

Lewis *et al.* (2017) investigated the response of subterranean clover seedlings to herbicide application. Thirteen subterranean clover cultivars were utilized across four experiments. An herbicide cultivar interaction was reported in Experiment 1 (P<0.001) in which the response of clovers, broadleaf weeds and grass weeds were subject to imazethapyr application. Imazethapyr treated 'Woogenellup' produced the highest yield at 2250 kg DM/ha compared with the unsprayed control at 1340 kg DM/ha. Imazethapyr treated 'Whatawhata' yielded 1830 kg DM/ha with 610 kg DM/ha from its respective control (P=0.003). Imazethapyr treated 'Narrikup' also yielded 1710 kg DM/ha with to 550 kg DM/ha from its respective control. However, imazethapyr treated 'Antas', 'Coolamon', 'Karridale', 'Leura', 'Rosabrook' coated, white, and red clover yielded 60-860 kg DM/ha and were not different to their respective controls. Imazethapyr treated broadleaf and grass weeds were lower at <75 and 140 kg DM/ha ($P<0.001$) compared with 1500 and 1370 kg DM/ha for untreated broadleaf and grass weed controls (Lewis *et al.* 2017). Phytotoxicity response of the cultivars was in accordance with the yield results, with 'Woogenellup', 'Whatawhata', and 'Narrikup' having the highest visual tolerance to herbicide application.

2.8 Conclusions

Subterranean clover is a winter annual legume suited to summer dry environments. Cultivar adaptability allows the species to persist across multiple areas in New Zealand. Flowering time influences the annual herbage accumulation of a cultivar with increasing daylength initiating reproductive development. The base temperature of subterranean clover is <1 °C (Moot *et al.* 2000). Grazing of subterranean clover should cater toward maximum seed set and autumn reestablishment post dry, to ensure persistence of the species in the sward. Cultivars of a prostrate growth habit and multiple small leaves are suited to New Zealand grazing systems (Smetham 2003b). The absence of a New Zealand cultivar warrants further research to determine the viability of such an enterprise. Localized genotype from the North Island possesses potential but requires additional comparison against the productive Australian cultivars currently imported.

3 MATERIALS AND METHODS

3.1 Experimental site

The experiment was conducted at paddock C9A(S) Ashley Dene, Canterbury, New Zealand. Subterranean clover was sown on the 10th of May using a 3 m triple disc drill. The soil type is a Lismore very stony silt loam (Ates *et al.* 2006; Manaaki Whenua Landcare Research and Environment Canterbury 2022). The soil possesses a moderate water holding capacity (49 mm to a 30 cm depth) with no significant rooting barrier to 1 m and is well drained. Figure 3.1 shows the soil mineral components with the soil texture dominated by silt within the initial 0–20 cm of topsoil. The profile contains 18–25% clay, 5–40% sand and 20-35% stones. This progresses to 1–4% clay, 85–95% sand and 50–75% stones at a 40–55 cm depth. Available water to a 20 cm depth is 10%.

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Figure 3.1 Soil information depicting soil texture and water retention capacity to a 100 cm depth. From (Manaaki Whenua Landcare Research and Environment Canterbury 2022).

Error! Reference source not found. shows the soil fertility of the experimental site with sulphur reported to be lower than the medium range recommended for unlimited plant growth. Sulphur super 30 with 500 g of molybdenum (N–0, P–7, K–0, S–30.1) was applied on the 26th of June 2021 at a rate of 208 kg/ha which equated to 62.7 kg/ha of Sulphur.

Table 3.1 Soil fertility data (0–75 mm) from paddock CA9(S), Ashley Dene, Canterbury, New Zealand, 22nd of June 2021.

Analysis	Level found	Medium range
pH units	6.2	5.8-6.2
Olsen phosphorous (mg/L)	21	20-30
Potassium (me/100g)	0.68	0.30-0.40
Sulphate sulphur (mg/kg)	2	10-12
Extractable organic sulphur (mg/kg)	6	15-20
Calcium (me/100g)	9.1	4.0-9.0
Magnesium (me/100g)	0.94	0.40-0.60
Sodium (me/100g)	0.11	

3.2 Paddock history

Resident species consist of those sown by Ates (2009). These included five seed mixtures consisting of 'Aries HD' perennial ryegrass, 'Vision' cocksfoot, and 'Demand' white clover sown on the 7th of October 2002 in a randomized block design. His treatments consisted of 0, 5, 10, and 15 kg/ha of perennial ryegrass with 2 kg/ha of cocksfoot and white clover. Perennial ryegrass at 10 kg/ha and 2 kg/ha of white clover was sown as the fifth treatment. However, white clover failed to persist, so 5 kg/ha each of 'Leura' and 'Campeda' subterranean clover were over sown into the original plot areas on the 18th of March 2005. The subterranean clover seedbank therefore primarily consists of 'Leura' and 'Campeda' with naturalized annual clover suckling (T. *dubium*) and cluster (T. *glomeratum*).

Grass species include those sown by Ates (2009) and annual low fertility grasses such as barley grass (*Critesion murinum*), soft brome (*Bromus hordeaceus*), and vulpia hair grass (*Vulpia bromoides*). These annual grasses are common under dryland conditions with autumn germination allowing colonization of bare ground post summer dry periods (Tozer *et al.* 2010). Seed head emergence of these species in late spring deters defoliation from grazing livestock with barley grass seed heads causing carcass and pelt damage (Tozer *et al.* 2007;

Tozer *et al.* 2010). The pastures also contained several broadleafed weeds. These included mouse ear chickweed (*Cerastium fontanum*), storksbill (*Eradium cicutarium*), shepherd's purse (*Capsella buras-pastaris*), dock (*Rumex obtusifolius*), broadleafed plantain (*Plantago major*) and several species from the Compositae family including, dandelion (*Taraxicum officinale*), hawksbeard (*Crepis capillaris*), and catsear (*Hypochaeris radicata*). Grass and broadleaf weeds were determined through visual assessment of the sward on the 9th of September 2021 to determine weed presence for EWRS scores of herbicide damage in spring.

3.3 Climate data

Climate data was accessed from the Ashley Dene meteorological station on site. The long-term mean was sourced from the Burnham meteorological station. Weather data is reported from the start of the experiment in May 2021 to the end of April 2022 when measurements stopped. Mean air temperature was 12.7 °C over the experimental period and consistent with the long-term mean (Figure 3.2). Temperatures in June 2021 were 1.4 °C higher than the long-term mean, while in February of 2022 the average temperature was 1.2 °C lower than the long-term mean.

A significant rainfall event of 142 mm occurred during the month of May 2021 at the start of the experiment. This exceeded the long term monthly mean by 77 mm. In most months, rainfall after May was lower than the long-term mean (Figure 3.3). The differences were; June -43.7 mm, July -35.3 mm, August -21.1 mm, September -21 mm, October -36.9 mm, November -44.9 mm, December -27.9 mm, January -37 mm, February -31.6 mm, March -40.3 mm and April -47.6 mm. Total rainfall in November was only 2.8 mm and April recorded 1.4 mm.

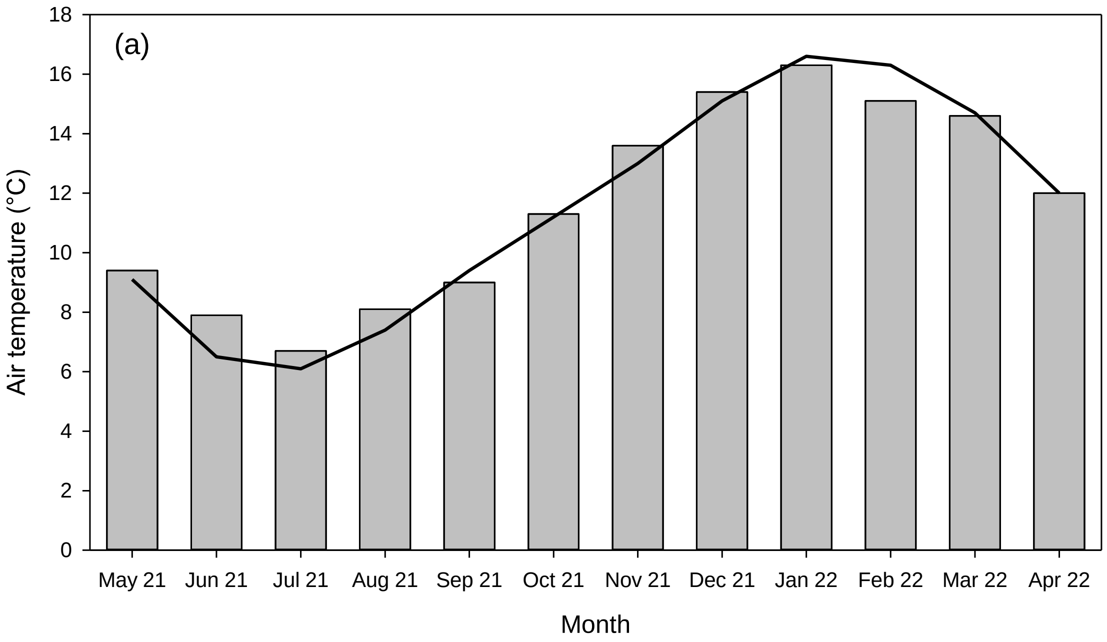

Figure 3.2 Mean monthly air temperature (■) (°C) from May 2021 to April 2022 from Ashley Dene weather station and the long-term (1975-2010) mean (–) from Burnham meteorological station, Canterbury, New Zealand.

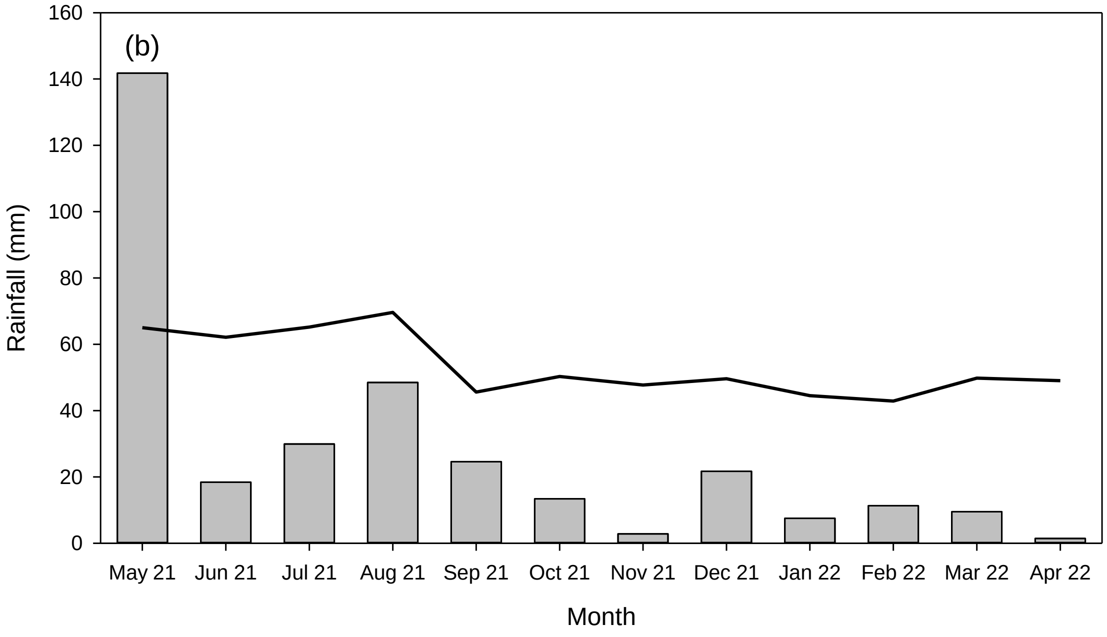

Figure 3.3 Mean monthly rainfall (■) (mm) from May 2021 to April 2022 from Ashley Dene weather station and the long-term (1975-2010) mean (–) from Burnham meteorological station, Canterbury, New Zealand.

3.4 Experimental design

The experiment had four cultivar treatments which were 'Denmark', 'Whatawhata', 'Woogenellup', and the control of the resident cultivars. Treatments were established in paddock C9A(S), Ashley Dene, Canterbury, New Zealand on the 10th of May 2021 and replicated four times to give a total of 16 plots. Replicate 1 was 64 x 3 m wide and Replicates 2, 3, and 4 were 64 x 6 m wide to match paddock size. Total plot area was 0.538 ha. Cultivars were sown as a randomized block design. The area was sprayed on the 5th of May with 4 L/ha of Buster (glufosinate-ammonium) (2-amino-4-[hydroxy(methyl)phosphoryl]butanoate) (National Center for Biotechnology Information 2022c) to remove existing vegetation.

On the 2nd of September 2021 a second treatment was introduced to create a strip plot randomized block design. This involved two herbicide treatments applied to investigate the effect of herbicide application on sown and resident species. Six herbicide strips were applied perpendicular to the sowing direction across the replicates. The ALS inhibiting herbicides imazethapyr (Spinnaker®) and flumetsulam (Headstart®) were randomly applied with one herbicide applied per replicate strip and two per total experiment strip area (**Error! Reference source not found.**). Herbicide was applied at the three to four trifoliate leaf stage when clover plants can tolerate application (Taylor *et al.* 2020). Imazethapyr was applied at 440 mL/ha with flumetsulam applied at 1.0 L/ha (Table 3.2). Non-herbicide strips were used as control treatment. Pegs detailing spray strip area were placed along the relevant replicates at the time of application.

Table 3.2 Herbicide used, active ingredient and their application rate at CA9(S), Ashley Dene, Canterbury, New Zealand on the 2nd of September 2021.

Commercial identification	Active ingredient	Application rate
Spinnaker®	240 g/L imazethapyr	400 ml/ha
Headstart®	50 g/L flumetsulam	1.0 L/ha

3.4.1 Thousand seed weight and sowing rate

Thousand seed weight (TSW) was determined by weighing two lots of 100 seeds and multiplying by five. Germination percentage and rate were measured from two groups of 50 seeds placed on petri dishes and watered before being placed in an incubator at 16 °C. Seeds were germinated when the radicle exceeded twice the length of the seed (Monks *et al.* 2009).

Field emergence was determined through counting individual seedlings along a 1 m drill row 10 times per plot on the 9th of June 2021. Cultivars were sown with a 3 m wide triple disc drill. Replicate 1 had one drill pass while Replicates 2-4 had two drill passes. A high rate of 80 kg/ha of ‘Whatawhata’ was sown, to account for the low germination rate determined in the laboratory test. Seed germination over nine days was 94% for ‘Denmark’, 64% for ‘Whatawhata’, and 98% for ‘Woogenellup’. ‘Denmark’ and ‘Woogenullup’ were sown at 50 kg/ha. These rates are higher than commercial recommendations but were used to represent seed levels that would be present after a year of seed set. Suscon green was drilled on the 11th of May 2021 to reduce the risk of grass grub damage.

Table 3.3 Subterranean clover cultivars, subspecies classification, thousand seed weight (TSW), and sowing rate utilized at CA9(S), Ashley Dene, Canterbury, New Zealand on the 10th of May 2021.

Cultivar	TSW (g)	Sowing rate (kg/ha)	Germination (%)
‘Denmark’	7.25	50	96
‘Whatawhata’	6.45	80	64
‘Woogenullup’	9.35	50	98

3.5 Glasshouse experiment

‘Denmark’, ‘Whatawhata’, and ‘Woogenellup’ were also grown in a controlled glasshouse experiment at Lincoln University from the 10th of March to 2nd of May 2022. Ten seeds of each cultivar were sown to a 2 cm depth in 2.5L pots. The first two emerged plants were pegged and measured for the duration of the experiment. Trifoliate leaf appearance was recorded daily for 28 days post sowing and then twice per week until the 2nd of May 2022. Plants were thinned on the 7th of April 2022 to three or four plants to avoid competition. Glasshouse temperatures did not exceed 25 °C.

3.6 Measurements

Measurements were taken to record phenological development and growth of subterranean clover. Phenological development included emergence after sowing in autumn, number of trifoliate leaves, time of runner initiation and first flower formed on marked runners. Growth and botanical composition were measured using quadrat cuts. The impact of herbicide on subterranean clover, broadleaf weeds, and grass weeds was assessed using the EWRS phytotoxicity scale (Lewis 2017). Canopy ground cover was measured using a handheld Trimble GreenSeeker.

3.6.1 Trifoliate leaves and runner initiation

Five plants per plot were marked on the 22nd of June 2021 to count number of emerged trifoliate leaves. Trifoliate counts occurred from the 21st of July to the 9th of September 2021 for the field experiment and from the 14th of March to the 2nd of May 2022 for the controlled experiment. Runner initiation was determined through the presence of a distinct stem emerging from the rosette. The number of leaves on a runner was recorded from when a runner had extended 20 mm from the plant centre (Moot *et al.* 2003) and had a leaf present. This began toward the end of September in the control plots. Plots were grazed in early October, so a further five plants were marked to monitor development on the 12th of October 2021. At this time two runners were selected on alternating sides of the plant and marked with tape to ensure assessment of the same runner. Total number of leaves on the runner was recorded twice a week from the 12th of October to 4th of November 2021. A further five plants were marked to record trifoliate leaf appearance after seedlings remerged from the 22nd of February 2022. Grazing on the 1st of March 2022 was done as recommended (Costello and Costello 2003) to reduce grass competition. Plants were then remarked on the 8th of March 2022 and leaf appearance again recorded to the 1st of April 2022.

3.6.2 Flower initiation and seed set

Flower initiation was recorded from the 1st of October 2021. At this time the control plots were at the R4 stage while sown cultivars were at R1 of the subterranean clover development scale (Teixeira *et al.* 2021). Flower number and reproductive stage were recorded twice a week. Burr formation (R7) or start of seed set was recorded from the 28th of October 2021. At this stage plants buried their seed head and began to develop seeds. However, insufficient data was collected for burr formation due to dry conditions.

3.6.3 Thermal time

Accumulated thermal time was calculated to investigate phenological development of subterranean clover and growth. Thermal time was determined from Ashley Dene meteorological station data. Thermal time was calculated for individual days and accumulated from the 1st of June 2021, which represented 50% emergence in 2021. Thermal time accumulation restarted from the 4th of February 2022 which initiated the second strike of subterranean clover from which leaf appearance measurements were taken in 2022. Thermal time was calculated from Equation 3.1. Tt represents thermal time for a set day, T_{max} is the maximum temperature, T_{min} is the minimum temperature, and T_b is the base temperature.

Equation 3.1

$$\mathbf{Tt(°Cd)} = \frac{T_{max}-T_{min}}{2} - T_b$$

Thermal time calculations were based on the Jones and Kiniry (1986) methodology. A sinusoidal function of a smooth nature is fitted to daily average air temperatures for three hourly periods. Intervals where T_{min} is lower than T_b were excluded. The cardinal temperatures utilized were T_b=0 °C, $T_{optimum}$=20 °C and a T_{max}=36 °C (Teixeira 2019). The $T_{optimum}$ was derived from the average of the lower and upper optimum values for subterranean clover as reported by Teixeira (2019).

3.6.4 Biomass accumulation

Biomass accumulation was recorded by harvesting all plant material from a 0.2 m² quadrat on the 4th of October (Harvest 1) and 28th of October (Harvest 2) 2021. Samples were taken from representative areas of all plots. Material was sorted into sown subterranean clover, resident subterranean clover, other clover, broadleaf weeds, grass weeds, and dead material. Distinguishing between sown and resident was based on the resident predominantly consisting of 'Campeda' and 'Leura' cultivars. Specific leaf markings, leaf size, petiole hairiness and flower type were the main distinguishing features utilized when sorting harvested material (Nichols *et al.* 2013). After drying in the oven, samples were weighed and proportions of plant species present determined. At Harvest 1 sown and resident subterranean clover were unable to be distinguished and so they were grouped together for that harvest.

3.6.5 Post emergence dry matter yield

Pasture mass was recorded using a plate meter calibrated to 0.5 cm on the 2nd of March and 29th of April 2022. Pasture mass samples obtained from a 0.2 m² quadrat were plotted against height to obtain an adjusted pasture mass (kg DM/ha). A linear regression was applied with the regression forced through zero. The linear equation was y=225x with the coefficient of determination (R^2) equalling 0.99. This was multiplied by the adjusted plate meter reading to calculate pasture mass in kg DM/ha.

3.6.6 Nutritional analysis

DM samples of subterranean clover were retained from Harvest 2 and analysed through NIR scanning on the 26th of April 2022. Samples were ground using a Retsch Ultra Centrifugal Mill ZM 200 with a 1 mm sieve. Ground samples were placed in individual airtight pottles with compressed air used to remove residuals from the equipment to avoid contamination between samples. Ground samples were then analysed through NIR scanning to report their nutritional status.

3.6.7 Canopy cover

Canopy cover as a non-direct measure of light interception, was measured using a Trimble GreenSeeker Handheld Crop Sensor which produced a Normalized Difference Vegetation Index (NDVI). Red and infra-red bursts of light are emitted from the GreenSeeker with the light reflected from the canopy determining the NDVI reading (Taylor 2019). NDVI readings were obtained by placing the device one meter in a perpendicular fashion above the ground and holding down the trigger while walking across the plots. Continuous measurements obtained were averaged at the release of the trigger to give a value between 0 and 1. GreenSeeker measurements were taken every week from the 23rd of September until the 27th of October 2021. Bare ground readings were obtained from measurement of the soil surface devoid of vegetation to calculate background values.

NDVI readings were corrected for using an equation sourced from Oliveira (2015). NDVIr represents the measured index, NDVIs is the bare soil measured index, Cmax the potential maximum radiation intercepted, and NDVImax corresponds to the highest NDVI reading from measurements taken (Taylor 2019). For this experiment Cmax was fixed at 0.95. To convert to canopy cover % the corrected NDVI could be multiplied by 100.

Equation 3.2

$$\boldsymbol{NDVI_{corrected}} = \frac{(NDVI_r - NDVI_s)(C_{max})}{(NDVI_{max} - NDVI_s)}$$

3.6.8 Phytotoxicity assessment

The European Weed Research Society (EWRS) phytotoxicity score for herbicide damage was used to assess the effect of herbicide application on subterranean clover, broadleaf weeds, and grass weeds (Taylor 2019). Visual assessment of plant damage was conducted every seven days from the 9th of September 2021 to the 27th of October 2021, when moisture limitations made it difficult to distinguish herbicide from water stress effects. Entire plots were scored based on the system as outlined in Table 3.4 with the base score of EWRS 1

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3.7 Statistical analysis

All data was analysed through the statistical software Genstat 20th edition. Data, including phenological development measurements, from the randomized complete block design consisting of four cultivars and 4 replicates were analysed as a one-way analysis of variance. Treatment was set as cultivar and reps were used for blocks. Implementation of an herbicide treatment created a split plot design. A two-way split strip plot analysis of variance was used for dry matter yield, canopy cover and EWRS visual scores. Biomass Harvest 2 was unbalanced due to twice as many controls being harvested as herbicide plots. The design was balanced for analysis by limiting the number of reps to two. The treatment structure consisted of cultivar*herbicide. Reps were used as blocks, cultivar for rows and herbicide for columns.

Botanical composition scores were arcsine transformed as they did not fit into the proportions of 0.0-0.3, 0.3-0.6, and 0.6-1.0 groups as outlined by Sokal and Rohlf (1981). SEMs were back transformed for presentation of the results.

Where treatment differences were observed, means were separated by Fischer's protected LSD at the α=0.05 level. If there was an interaction, means were separated with the most conservative LSD. Where no treatment effects occurred, the grand mean is reported with the pooled standard error of the mean. If the F value for the interaction effect are an order of magnitude greater than the interaction then main effects are discussed.

4 RESULTS

4.1 Phenotype description

Morphological characteristics are used to differentiate between subterranean clover cultivars (Nichols *et al.* 2013). The leaf size, shape and markings indicate cultivar distinctions. Leaf lamina colour; anthocyanin presence and position on leaves, calyx, and stipules; pubescence on structural components and seed colour enable differentiation among cultivars. In this section the physical description of the phenotypical characteristics of the 'Whatawhata' strain allows distinction to be made between it and the commercial cultivars 'Denmark' and 'Woogenellup'.

4.1.1 'Whatawhata' identification

Plate 4.1 'Whatawhata' trifoliate leaf on the 1st of November 2021 at Ashley Dene, Canterbury, New Zealand (Photo: Blakely).

Plate 4.2 ‘Whatawhata’ flower and runners on the 1st of November 2021 at Ashley Dene, Canterbury, New Zealand (Photo: Blakely).

Plate 4.3 Autumn trifoliate leaf of ‘Whatawhata’ on the 4th of March 2022 in a controlled glasshouse experiment at Lincoln University, Canterbury, New Zealand (Photo: Blakely).

Plate 4.4 ‘Whatawhata’ seedlings with black flecks and extremely hairy petioles on the 4th of March 2022 in a controlled glasshouse experiment at Lincoln University, Canterbury, New Zealand (Photo: Blakely).

Leaf: Leaf markings resemble ‘Mt Barker’ with a white triangle and brown flecks characteristic of autumn and winter plants (Plate 4.3). Anthocyanin pigmentation is also distinctive during cooler periods of the year. Plate 4.1 shows a summer trifoliate leaf with no brown flecks and a faded white triangular mark.

Stipules: Green stipules with red pigmentation.

Flower: White flower.

Hairiness: Extremely hairy subterranean clover genotype with hairs on the petioles, leaves and runners (Plate 4.4).

Seed colour: Black.

Plate 4.5 'Whatawhata' in a field experiment on the 3rd of March 2022 at Ashley Dene, Canterbury, New Zealand. This shows a small leaved and prostrate growth habit (Photo: Blakely).

4.1.2 'Denmark' descriptive summary

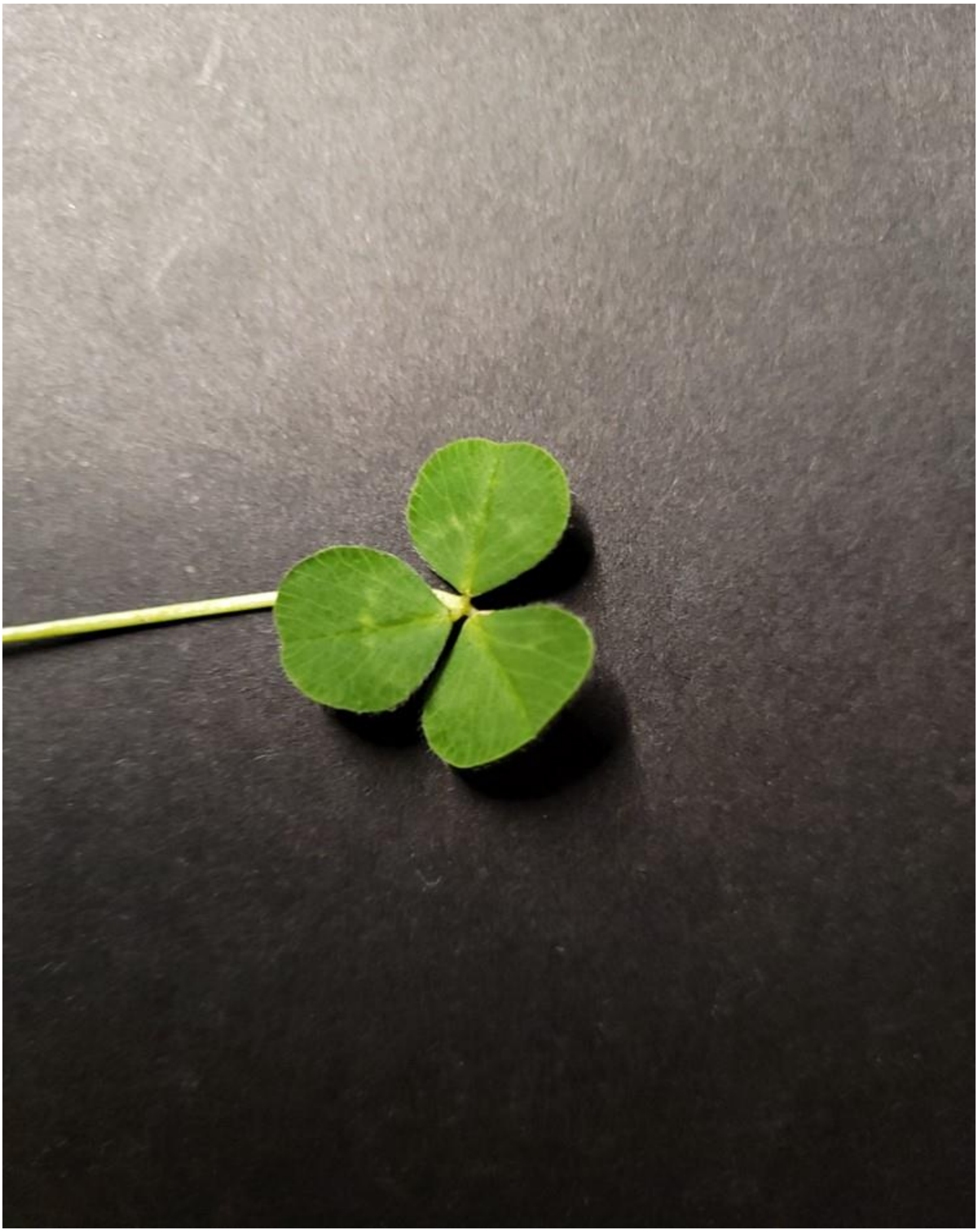


Plate 4.6 Autumn trifoliate leaf of 'Denmark' on the 4th of March 2022 in a controlled glasshouse experiment at Lincoln University, Canterbury, New Zealand (Photo: Blakely).

‘Denmark’ is described as a small leaved cultivar that possesses a prostrate growth habit (Lucas *et al.* 2016). Leaf marks are faint to non-distinguishable with a central diamond stretching a third of the distance to the leaf margin (Wurst *et al.* 2004). Petioles, runners, and peduncles are hairless (Lucas *et al.* 2016). Stipules possess visible red veins. Flowering time is late with flowers possessing white petals and distinct pink veins.

Plate 4.7 ‘Denmark’ cultivar with light leaf marks and relatively hairless petioles on the 3rd of March 2022 in a controlled experiment at Lincoln University, Canterbury, New Zealand (Photo: Blakely).

4.1.3 'Woogenellup' descriptive summary

Plate 4.8 Autumn trifoliate leaf of 'Woogenellup' on the 4th of March 2022 in a controlled glasshouse experiment Lincoln University (Photo: Blakely).

‘Woogenellup’ is described as a large leaved erect cultivar with pale green leaves and long petioles (Lucas *et al.* 2016). The leaf marks consist of flattened triangle dipping toward the centre and then extending halfway to the leaf margins (Wurst *et al.* 2004). Runners are usually hairless but may have hairy runners and petioles in some circumstances. Stipules possess red vein marks. Flowering time is mid-season with flowers being white and peduncles hairy.

Plate 4.9 ‘Woogenellup’ depicting large pale green leaves and extended triangle leaf marks on the 3rd of March 2022 in a controlled experiment at Lincoln University, Canterbury, New Zealand. Petioles are either hairy or hairless (Photo: Blakely).

Plate 4.10 ‘Woogenellup’ plants depicting leaf marks and hairiness on the petioles on the 3rd of March 2022 at Ashley Dene, Canterbury, New Zealand (Photo: Blakely).

4.2 Phenological development

Early development of subterranean clover is determined by temperature when all other conditions are non-limiting. Linear regression was used to predict key phenological stages including initial trifoliate leaf appearance, phyllochron and time of flowering for ‘Denmark’, ‘Whatawhata’, and ‘Woogenellup’.

4.2.1 Trifoliate leaf appearance

4.2.1.1 Leaf emergence 2021

Figure 4.1 shows the accumulation of leaves against thermal time from marked plants. The trifoliate leaf appearance rate was analysed using split line regression to account for the switch from linear to exponential (branching) leaf appearance (Moot *et al.* 2003; Guo *et al.* 2022). Data output included an x-axis intercept which represented the amount of thermal time required for the appearance of the first trifoliate leaf. The x-axis intercept is the slope of accumulative thermal time (°Cd) divided by the y-intercept of the regression output. Initial trifoliate leaf appearance of the Control and 'Woogenellup' were not different in 2021 and required 177 and 230±21.2 °Cd, respectively (Figure 4.1). However, 'Denmark' required more (P=0.007) thermal time for initial leaf appearance (284±21.2 °Cd) than the Control. 'Whatawhata' also (309±21.2 °Cd) required more thermal time accumulation than 'Woogenellup' and the Control to produce its first trifoliate leaf but was not different to 'Denmark'.

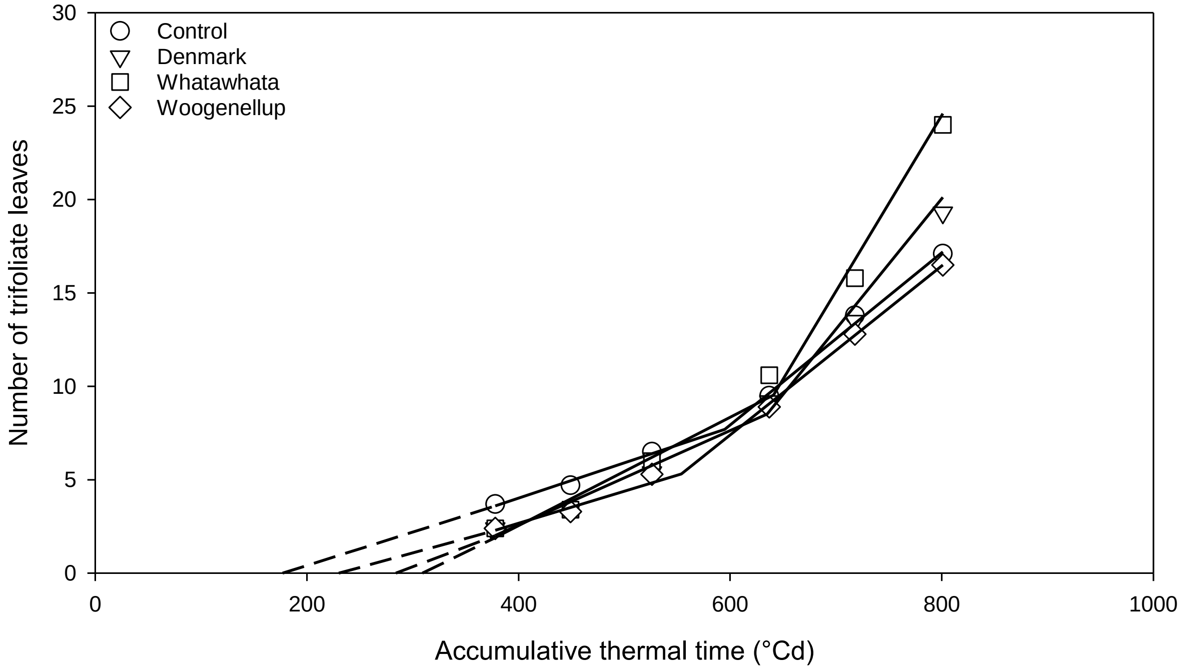


Figure 4.1 Number of leaves against accumulative thermal time (°Cd) of cultivars growing at Ashley Dene, Canterbury New Zealand, 2021. Solid lines (—) report the linear regression of collected data while dashed (– –) lines are extrapolated to report the approximate value for the first trifoliate leaf of the cultivars. The linear equation for the function from the x-axis intercept to x-axis breakpoint (linear phase) is y=intercept±21.2+slope±0.00325 after which leaf appearance becomes exponential and y=intercept±21.2+slope±0.00682. The coefficient of determination (R^2) is 0.99.

Slope 1 describes the linear phase of main stem leaf appearance. There was no difference (P=0.269) between leaf appearance up to first branching. The phyllochron was calculated as the inverse of the slope (Teixeira 2019) for both linear and exponential leaf appearance (**Error! Reference source not found.**). There was no difference (P=0.269) in leaf appearance at the linear phase with the mean phyllochron 51.3±9.72 °Cd/leaf.

Table 4.1 Phyllochron (°Cd/leaf) for linear and exponential leaf appearance with associated slopes and coefficient of determination (R^2) values for cultivars of subterranean clover growing at Ashley Dene, Canterbury, New Zealand, 2021. Values with subscript letters in common are not significantly different at alpha=0.05.

	Linear phase		Exponential phase		
Cultivar	Phyllochron (°Cd)	Slope	Leaf rate (°Cd)	Slope	R^2
Control	57.1	0.019	22.9_{a}	0.046_{b}	0.98
'Denmark'	48.1	0.024	15.3_{b}	0.070_{a}	1.00
'Whatawhata'	36.1	0.028	11.0_{b}	0.094_{a}	0.99
'Woogenellup'	64.0	0.016	22.6_{a}	0.045_{b}	1.00
P value	0.269	0.108	0.005	0.002	
SEM	9.72	0.003	1.94	0.007	

The linear phase of leaf appearance was described by Slope 1 and switches to an exponential phase at the 5-6 trifoliate leaf stage (Moot *et al.* 2003; Teixeira 2019). The breakpoint marks this point where the rosette of subterranean clover becomes apparent from branching and when leaf appearance is exponential. There was no difference (P=0.095) among cultivars at this stage. The mean for breakpoint was 606±24 °Cd (Appendix 3). The phyllochron rate up to the five to six leaf stage was accumulated and added to the thermal time for initial trifoliate requirement to provide a thermal time requirement up to the fourth trifoliate (Teixeira *et al.* 2021), which is considered the earliest time of safe grazing (**Error! Reference source not found.**).

Table 4.2 Thermal time values (°Cd) for the first and fourth trifoliate leaf stage of subterranean clover cultivars growing at Ashley Dene, Canterbury, New Zealand, 2021. Phyllochron (°Cd/leaf) reports the thermal time requirement after the initial trifoliate leaf. Values with subscript letters in common are not significantly different at alpha=0.05.

Cultivar	First trifoliate (°Cd)	Phyllochron (°Cd)	Fourth trifoliate (°Cd)
Control	177_{c}	57.1	348
'Denmark'	284_{ab}	48.1	428
'Whatawhata'	309_{a}	36.1	417
'Woogenellup'	230_{bc}	64.0	422

P value	0.007	0.269
SEM	21.2	9.72

Slope 2 shows exponential growth of the cultivars at the breakpoint also known as the switch (Moot *et al.* 2003). The Control (22.9±1.94 °Cd/leaf) and 'Woogenellup' (22.6±1.94 °Cd/leaf) were not different (P>0.05) in their exponential leaf production. However, 'Denmark' (15.3±1.94 °Cd/leaf) and 'Whatawhata (11.0±1.94 °Cd/leaf) required fewer (P=0.005) thermal units than either 'Woogenellup' or the Control which shows a faster rate of leaf appearance.

4.2.1.2 Leaf emergence 2022

Rainfall during the 2021/22 summer at Ashley Dene caused subterranean clover seed to strike. This enabled the leaf emergence to be measured a second time. Two distinct patterns were observed with most of the seedlings that emerged in the initial strike in January failing to persist. Plants were selected for measurement from the second emergence period in February. Thermal time was accumulated from the start of February. Plots were grazed with sheep on the 1st of March to encourage further emergence by reducing the grass canopy (Figure 4.2). Marked plants at this stage were at the two trifoliate leaf stage. Grazing of the sward limited the ability to calculate initial trifoliate emergence until repegging on the 8th of March 2022. Simple linear regression was performed from this point (Figure 4.2). The difference between the first data point of the linear regression and the initial data before grazing allowed the emergence of the first trifoliate leaf to be calculated. The appearance of the first trifoliate leaf was not different (P=0.574) amongst cultivars including the Control. The mean value was estimated to be 204 °Cd. The slope of leaf emergence was also not different (P=0.439) among cultivars and the Control (0.012±0.003 °Cd). The mean phyllochron was 96.2±19.2 °Cd/leaf (P=0.276) (**Error! Reference source not found.**).

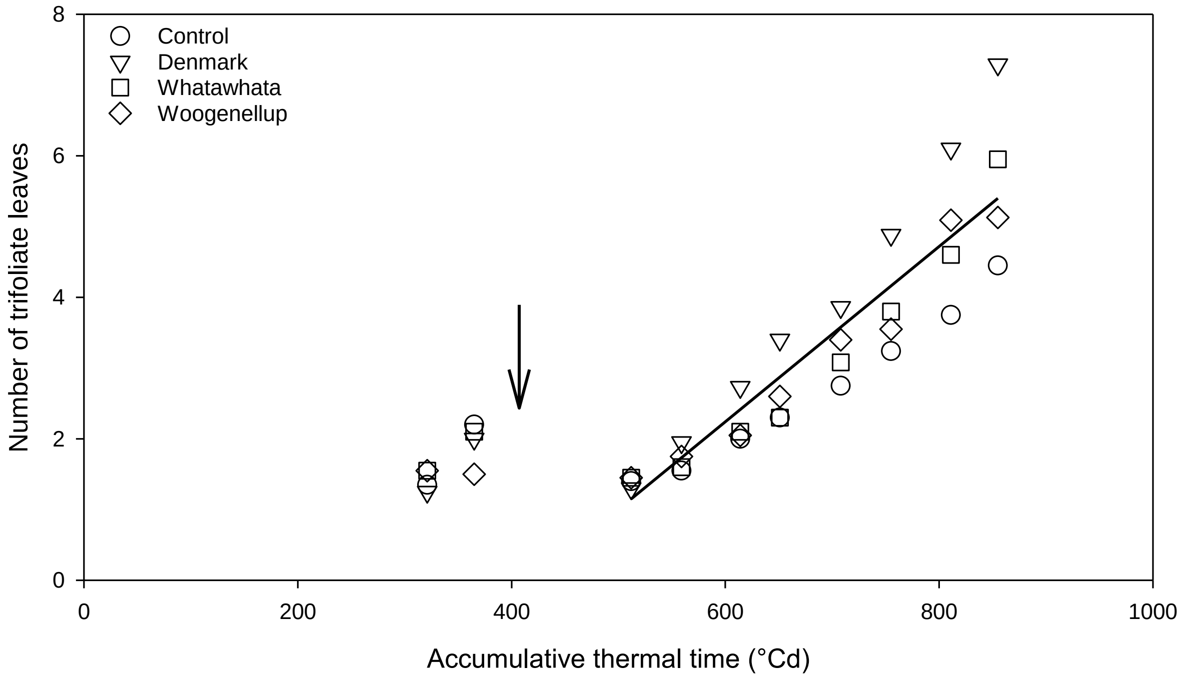


Figure 4.2 Number of trifoliate leaves against accumulative thermal time (°Cd) of cultivars growing at Ashley Dene, Canterbury, New Zealand, 2022. The solid (—) line indicates linear regression for data collected after grazing as shown by the arrow. The singular linear regression reports no difference ($P>0.05$) amongst cultivars. The linear equation for the function is y=intercept±39.1+slope±0.00347. The coefficient of determination (R^2) is 0.87.

Table 4.3 The phyllochron (°Cd/leaf) as determined by the inverse of the slope of cultivars post grazing at Ashley Dene, Canterbury, New Zealand, 2022. There is no significant difference ($P>0.05$) associated with slope values with the mean reported for each factor and the coefficient of determination (R^2).

Cultivar	Phyllochron (°Cd)	Slope	R^2
Control	129	0.009	0.86
‘Denmark’	76.3	0.017	0.89
‘Whatawhata’	82.2	0.013	0.90
‘Woogenellup’	97.2	0.012	0.81
Mean value	96.2	0.012	0.87
P value	0.276	0.574	
SEM	19.2	0.003	

4.2.1.3 Greenhouse results

A split line regression was carried out on trifoliate leaf appearance against thermal time for 'Denmark', 'Whatawhata', and 'Woogenellup' in a controlled experiment at Lincoln University. Trifoliate leaf appearance of 'Woogenellup' required 188±9.29 °Cd which was less (P=0.012) than 'Denmark' at 237±9.29 °Cd and 'Whatawhata' at 260±9.29 °Cd (Figure 4.3). Slope 1 describes linear main stem leaf appearance which was not different (P=0.340) among cultivars. There was also no difference (P=0.230) in phyllochron with the mean 64.4±7.0 °Cd/leaf. Breakpoint at which linear leaf production turns exponential was not different (P=0.521) among cultivars. The mean breakpoint was 521±16.0 °Cd. Slope 2 describes leaf appearance after the breakpoint with there being no difference (P=0.257) among cultivars in leaf appearance rate. There was consequentially no difference (P=0.213) in phyllochron with the mean 12.7±1.26 °Cd/leaf. Thermal time accumulation up to fourth trifoliate leaf stage is reported in **Error! Reference source not found.**.

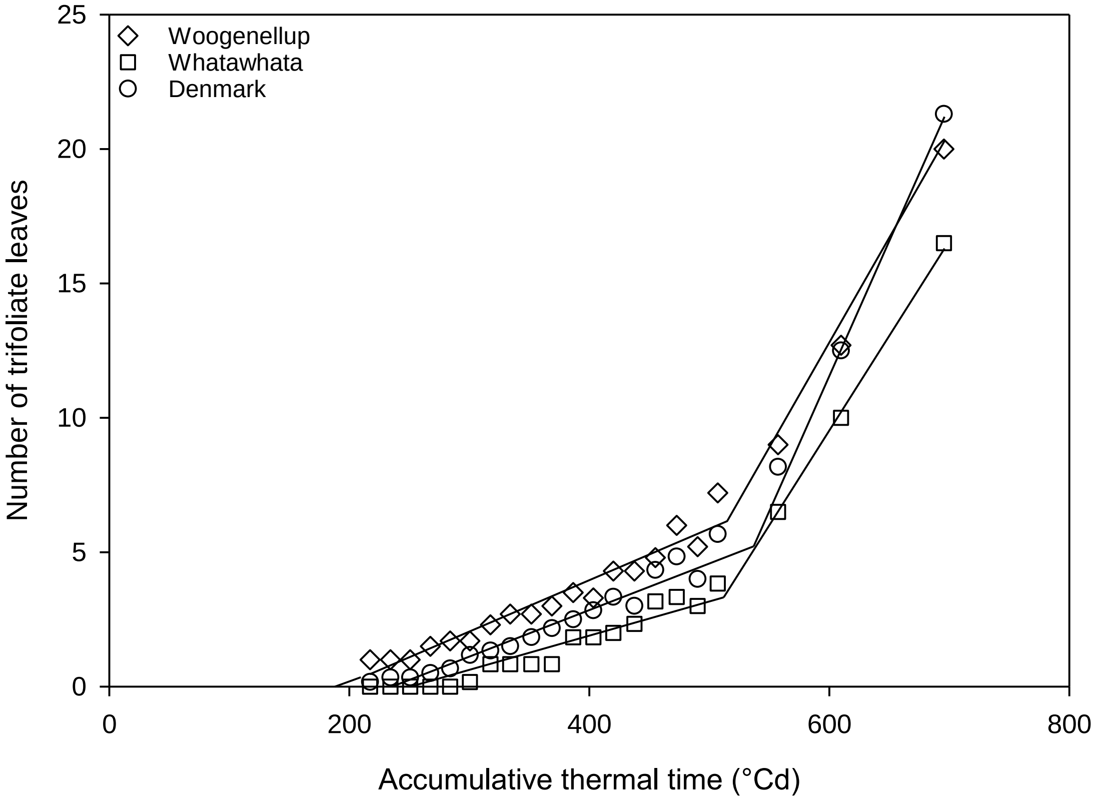


Figure 4.3 Number of trifoliate leaves against accumulative thermal time (°Cd) of cultivars grown at Lincoln University, Canterbury, New Zealand, 2022. Solid (—) lines report the linear regression of collected data. 'Woogenellup' data is extrapolated to give the approximate value for the first trifoliate leaf. The linear equation from the x-axis intercept to x-axis breakpoint (linear leaf phase) is y=intercept±9.29+slope±0.003 after which leaf appearance turns exponential and y=intercept±9.29+slope±0.011. The coefficient of determination (R^2) is 0.99.

Table 4.4 The phyllochron (°Cd/leaf) for linear and exponential leaf appearance, as determined by the inverse of the slope for cultivars grown at Lincoln University, Canterbury, New Zealand, 2022. There is no significant effect (P>0.05) for phyllochron or slopes with the mean reported for each factor and the coefficient of determination (R^2) for the regression function.

	Linear phase		Exponential phase		
Cultivar	Phyllochron (°Cd)	Slope	Leaf rate (°Cd)	Slope	R^2
'Denmark'	58.5	0.017	10.5	0.100	0.99
'Whatawhata'	76.4	0.013	14.2	0.071	0.99
'Woogenellup'	58.5	0.019	13.5	0.078	0.98
Mean	64.4	0.016	12.7	0.083	
P value	0.230	0.34	0.213	0.34	
SEM	7.02	0.002	1.26	0.016	

Table 4.5 Thermal time values (°Cd) for the first and fourth trifoliate leaf stage of subterranean clover cultivars grown at Lincoln University, Canterbury, New Zealand, 2022. Phyllochron (°Cd/leaf) reports the thermal time requirement after the initial trifoliate leaf up to the fourth leaf. Values with subscript letters in common are not significantly different at alpha=0.05.

Cultivar	First trifoliate (°Cd)	Phyllochron (°Cd)	Fourth trifoliate (Cd°)
'Denmark'	237_a	58.5	413
'Whatawhata'	260_a	76.4	489
'Woogenellup'	188_b	58.5	364
P value	0.012	0.230	
SEM	9.29	7.02	

4.2.2 Runner appearance in the field in 2021

Runner initiation occurred on the 21st of June 2021 (shortest day). Trifoliate leaf appearance on two alternating runners was not different (P=0.126) in thermal time requirement to produce the first leaf. The mean value from the shortest day in response to a change in photoperiod was 683±39.5 °Cd. However, the phyllochron was less (P=0.018) for the Control at 44.3±9.45 °Cd/leaf than either 'Woogenellup' (96.1±9.45 °Cd/leaf) or 'Whatawhata' (83.3±9.45 °Cd/leaf) (**Error! Reference source not found.**). 'Denmark' was not different to the Control or 'Whatawhata' at 64.5±9.45 °Cd/leaf. However, it was lower than 'Woogenellup'.

Table 4.6 Phyllochron (°Cd/leaf) as calculated by the inverse of the slope for leaves produced by runners of cultivars growing at Ashley Dene, Canterbury, New Zealand, 2021. The coefficient of determination (R^2) is reported for the linear regression. Values with subscript letters in common are not significantly different at alpha=0.05.

Cultivar	Phyllochron (Cd°)	Slope	R^2
Control	44.3_c	0.0272_a	0.93
'Denmark'	64.5_{bc}	0.0189_{ab}	0.94
'Whatawhata'	83.3_{ab}	0.0149_b	0.88
'Woogenellup'	96.1_a	0.0131_b	0.88
P value	0.018	0.032	
SEM	9.45	0.00291	

Figure 4.4 reports the number of leaves on runners against accumulative thermal time (°Cd). Grey symbols represent a change in the total number of leaves on the runners as leaves start to senesce due to moisture limitations and completion of seed set. These data points were

excluded from the linear regression but are presented here to show the change to senescence as plants near the end of their annual life cycle.

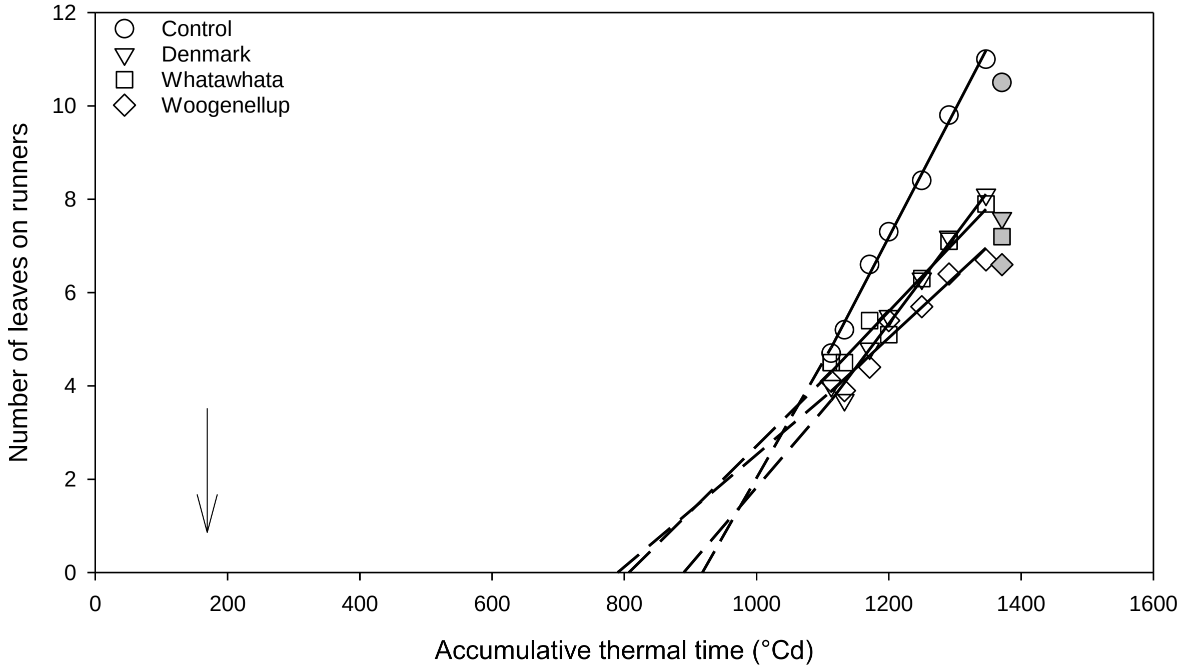


Figure 4.4 Number of leaves on subterranean clover runners against accumulated thermal time (°Cd) for cultivars growing at Ashley Dene, Canterbury, New Zealand. Solid (—) lines report the linear regression of collected data while dashed (– –) lines are extrapolated to report the approximate value for the first leaf on the runners. The linear equation for the function is y=intercept±39.5+slope±0.00291. The (●, ▼, ■, ◆) symbols represent leaf senescence and a decrease in total leaf number. The arrow indicates the shortest day (21st of June 2021) with thermal time accumulation occurring from 50% emergence. The coefficient of determination (R^2) is 0.91.

4.2.3 Flower appearance 2021

The Control and 'Woogenellup' required less (P=0.001) thermal time (°Cd) for initial flower appearance at 1061 and 1051±11.0 °Cd respectively from 50% emergence on the 1st of June, than 'Denmark' or 'Whatawhata' at 1130 and 1119±11.0 °Cd, respectively. There was no difference (P>0.05) in thermal time requirement between 'Denmark' and 'Whatawhata' for their first flower (**Error! Reference source not found.**). The rate of flower appearance was higher (P=0.012) for the Control than all other cultivars.

Thermal time was also accumulated from 21st of June (**Error! Reference source not found.**). The Control and 'Woogenellup' required 894 and 883±10.9 °Cd respectively, which was less (P=0.001) than 'Denmark' and 'Whatawhata' at 962 and 952±10.9 °Cd respectively. The rate of flower appearance was not different (P=0.011) among cultivars.

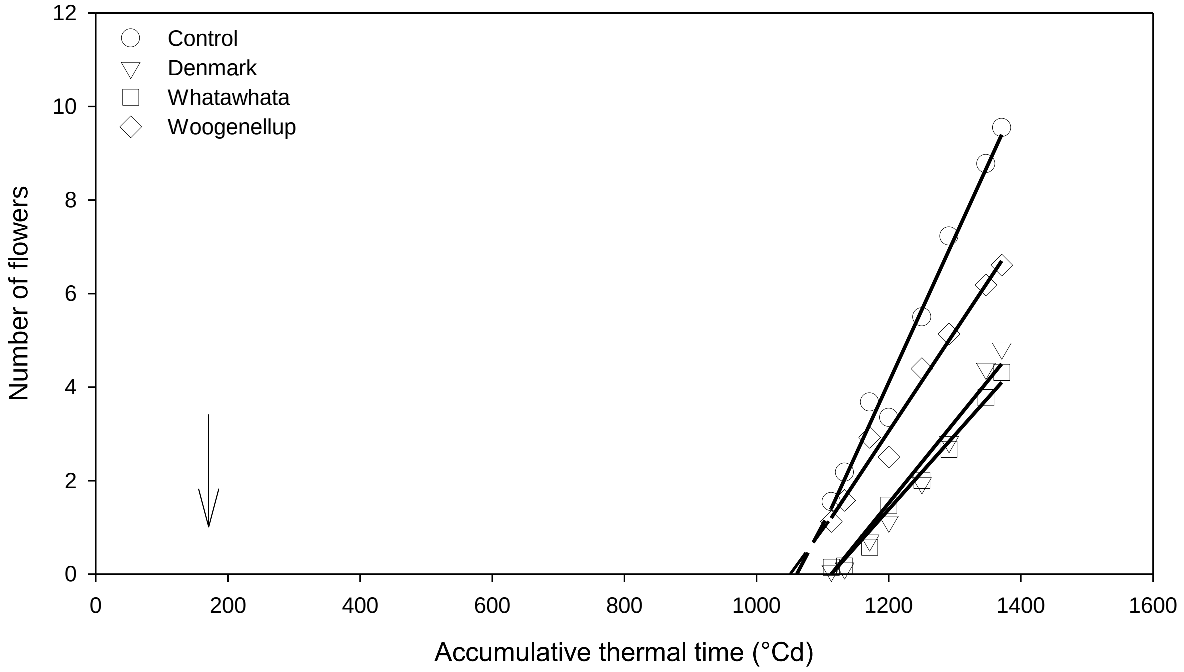


Figure 4.5 Number of flowers formed against accumulated thermal time (°Cd) of cultivars growing at Ashley Dene, Canterbury, New Zealand. Solid (—) lines report the linear regression of collected data while dashed (– –) lines are extrapolated to report the approximate value for the first flower of the cultivars. The arrow indicates the shortest day (21st of June 2021) with thermal time accumulated from 50% emergence (1st of June 2021). The linear equation for the function is y=intercept±10.96+slope±0.002. The coefficient of determination (R^2) is 0.91.

4.2.4 Plant population

The number of subterranean clover seedlings were counted along a 1-meter drill length to calculate seedling number per m^2. Emergence of subterranean clover cultivars sown on the 10th of May 2021 was higher (P<0.001) than the Control treatment. However, there was no difference in number of plants emerged from the cultivars sown (**Error! Reference source not found.**). 'Denmark' had 332 seedlings/m^2, 'Whatawhata' 343 seedlings/m^2, and 'Woogenellup' 385±17.7 seedlings/m^2.

Re-establishment of subterranean clover the following autumn was measured twice using a 0.01 m^2 quadrat. The first measurement occurred on the 25th of February 2022. 'Whatawhata' (181±42.9 m^2) plots had fewer (P=0.026) seedlings present than 'Denmark' (333±42.9 m^2) and 'Woogenellup' (383±42.9 m^2) (**Error! Reference source not found.**). There was no difference in seedling number between 'Whatawhata' and the Control. The Control (213±42.9 m^2) had fewer seedlings than 'Woogenellup' but not 'Denmark'. Seedling emergence was also measured after sheep grazing on the 1st of March 2022. There was no difference (P=0.271) in seedling emergence between cultivars on the 15th of March 2022. The mean number of seedlings was 156±36.1 m^2.

Table 4.7 Number of emerged seedlings (m^2) from cultivars sown on the 9th of June 2021 and seedling re-emergence on the 25th of February and 15th of March 2022 of cultivars growing at Ashley Dene, Canterbury, New Zealand. Values with subscript letters in common are not significantly different at alpha=0.05.

Cultivar	9th Jun 2021	25th Feb 2022	15th Mar 2022
Control	0_b	213_{bc}	136
'Denmark'	385_a	333_{ab}	199
'Whatawhata'	343_a	181_c	103
'Woogenellup'	332_a	383_a	185
P-value	<0.001	0.026	0.271
SEM	17.7	42.9	36.1

4.3 Cultivar growth

4.3.1 Grazing events

Grazing of the plots occurred on the 7th of October 2021 and 1st of March 2022. Total DM removed was based on the metabolizable energy requirements for maintenance of the animals grazed (Nicol and Brookes 2017). Animals were assumed to have a maintenance feed requirement plus a 100 g/day growth rate. Animals removed 205 kg DM/ha from the plots on the 7th of October 2021 and 708 kg DM/ha on the 1st of March 2022 (Appendix 5).

4.3.2 Dry matter production

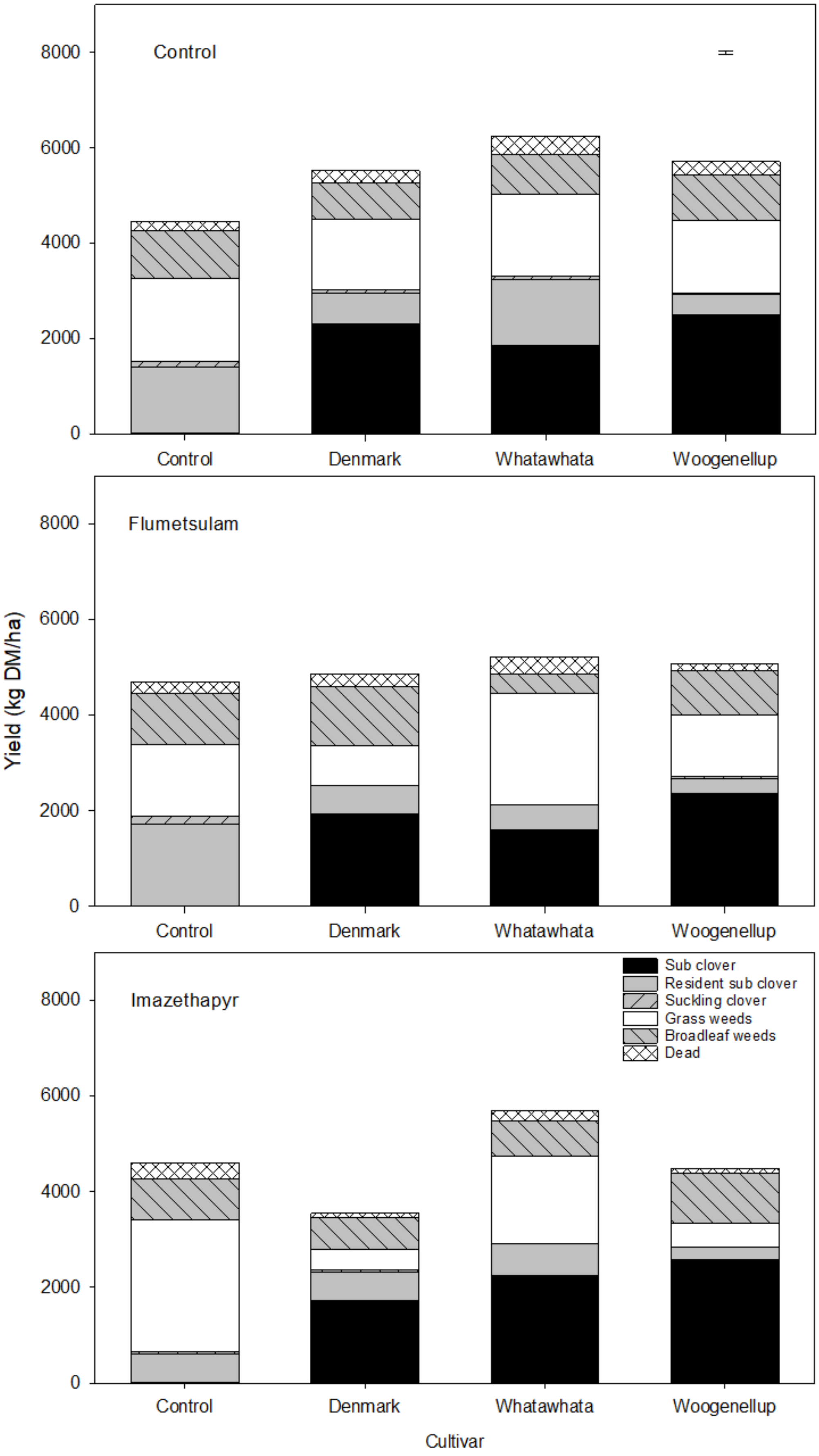
Unless stated there was no interaction effect of cultivar*herbicide for dry matter yield.

4.3.2.1 Total dry matter accumulation: 10 May 2021 to 9 November 2021

Dry matter (DM) yield from two harvests were accumulated from the 10th of May 2021 to the 28th of October 2021. A two-way split strip plot analysis of variance showed no difference in total accumulated yield for cultivar (P=0.335) or herbicide (P=0.448) treatments implemented. The mean DM yield across all treatments was 5012±189 kg DM/ha (Figure 4.6). Total subterranean clover yield in overdrilled plots (~2754±207 kg DM/ha) was higher (P=0.037) than the Control (1359±207 kg DM/ha). The DM yield was not different among sown cultivars but higher (P=0.006) than the Control (Table 4.8). 'Denmark', 'Whatawhata', and 'Woogenellup' produced 1986, 1898, and 2482±166 kg DM/ha respectively. Herbicide treatment did not affect (P=0.695) the yield of sown subterranean clover.

There was less (P=0.006) resident subterranean clover present in 'Woogenellup' plots (324±61.7 kg DM/ha) than any other pasture. 'Denmark' (622±61.7 kg DM/ha) and 'Whatawhata' (863±61.7 kg DM/ha) were not different but had less resident subterranean clover than the Control plots (1250±61.7 kg DM/ha). There was no herbicide effect (P=0.171) on resident subterranean clover. Other annual clover yields were not different among treatments (P>0.05).

Accumulated grass yield was unaffected by cultivar (P=0.284) or herbicide (P=0.697) treatments. The total mean grass yield was 1493±187 kg DM/ha. There was no cultivar (P=0.608) or herbicide (P=0.872) effect on total accumulated broadleaf weeds. The mean broadleaf yield was 878±89 kg DM/ha. A cultivar*herbicide interaction (P=0.001) was reported for dead material. There was less accumulated dead material in 'Woogenellup' (~130±51.5 kg DM/ha) pastures sprayed with an herbicide than 'Whatawhata' plots applied flumetsulam (360±51.5 kg DM/ha) and no herbicide (374±51.5 kg DM/ha).



Control
Flumetsulam
Imazethapyr
8000
6000
4000
2000
0
Yield (kg DM/ha)
Control
Denmark
Whatawhata
Woogenellup
Cultivar
Sub clover
Resident sub clover
Suckling clover
Grass weeds
Broadleaf weeds
Dead

Figure 4.6 Total accumulated dry matter yield (kg/ha) from the 10th of May to the 28th of October 2021 from plots overdrilled with three cultivars and two herbicide treatments at Ashley Dene, Canterbury, New Zealand. The influence of no herbicide (control), flumetsulam, and imazethapyr treatments on the botanical composition of total accumulated dry matter is shown with sward components reported within the graph. There is no significant difference reported between the treatment yields with the pooled SEM reported for total yield.

Table 4.8 Botanical composition of total accumulated dry matter yield of cultivar treatments from plots at Ashley Dene, Canterbury, New Zealand, 2021. P values indicate significant difference in botanical composition yield with the associated SEM for cultivar reported. Values with subscript letters in common are not significantly different at alpha=0.05.

Treatment	Cultivar	Res sub clover	Grass	Broadleaf	Dead
Control	0_b	1250_a	2001	975	252
'Denmark'	1986_a	622_b	918	892	191
'Whatawhata'	1898_a	863_b	1950	668	316
'Woogenellup'	2482_a	324_c	1104	976	183
P-value	0.006	0.006	0.284	0.608	0.181
SEM	166	61.7	392	172	34.6

Table 4.9 Botanical composition of total accumulated dry matter yield of herbicide treatments from plots at Ashley Dene, Canterbury, New Zealand, 2021. P values indicate significant difference in botanical composition yield with the associated SEM for herbicide reported. Values with subscript letters in common are not significantly different at alpha=0.05.

Treatment	Cultivar	Res sub clover	Grass	Broadleaf	Dead
Control	1666	965	1614	896	274
Flumetsulam	1477	788	1482	916	246
Imazethapyr	1632	541	1383	822	187
P-value	0.695	0.171	0.697	0.872	0.395
SEM	152	96.7	176	129	35.7

4.3.2.2 Harvest 1: 1 October 2021

DM yield from Harvest 1 on the 1st of October 2021 was unaffected by cultivar (P=0.151) or herbicide (P=0.254) treatments (Figure 4.7). The mean total yield was 1850±84 kg DM/ha. Total subterranean clover yield in overdrilled plots was not different (P>0.05) to the Control with a mean yield of 848±74.6 kg DM/ha reported. There was no difference between sown cultivar DM yield (P>0.05) with the mean ~1002±100 kg DM/ha. No herbicide effect was reported for sown subterranean clover (P=0.450). Identification of cultivars to differentiate between sown and resident subterranean clover was too difficult to perform at Harvest 1 and so DM yield of cultivar includes resident strains. A lack of overdrilling in the Control treatment allowed all subterranean clover to be classified as resident clover (Figure 4.7). Subsequently, the cultivar*herbicide interaction (P=0.003) reported for resident subterranean clover in the Control plots does not represent an accurate difference as sown and resident subterranean clover could not be differentiated at the time of Harvest 1. Other annual clovers were also not present at Harvest 1.

Grass weed yield was unaffected by cultivar (P=0.209) or herbicide (P=0.363) treatments. The mean grass yield was 603±66.7 kg DM/ha. Broadleaf weed yield was also unaffected by cultivar (P=0.489) or herbicide (P=0.429) treatments. The mean broadleaf yield was 396±46.3 kg DM/ha. Dead material was not present at Harvest 1.

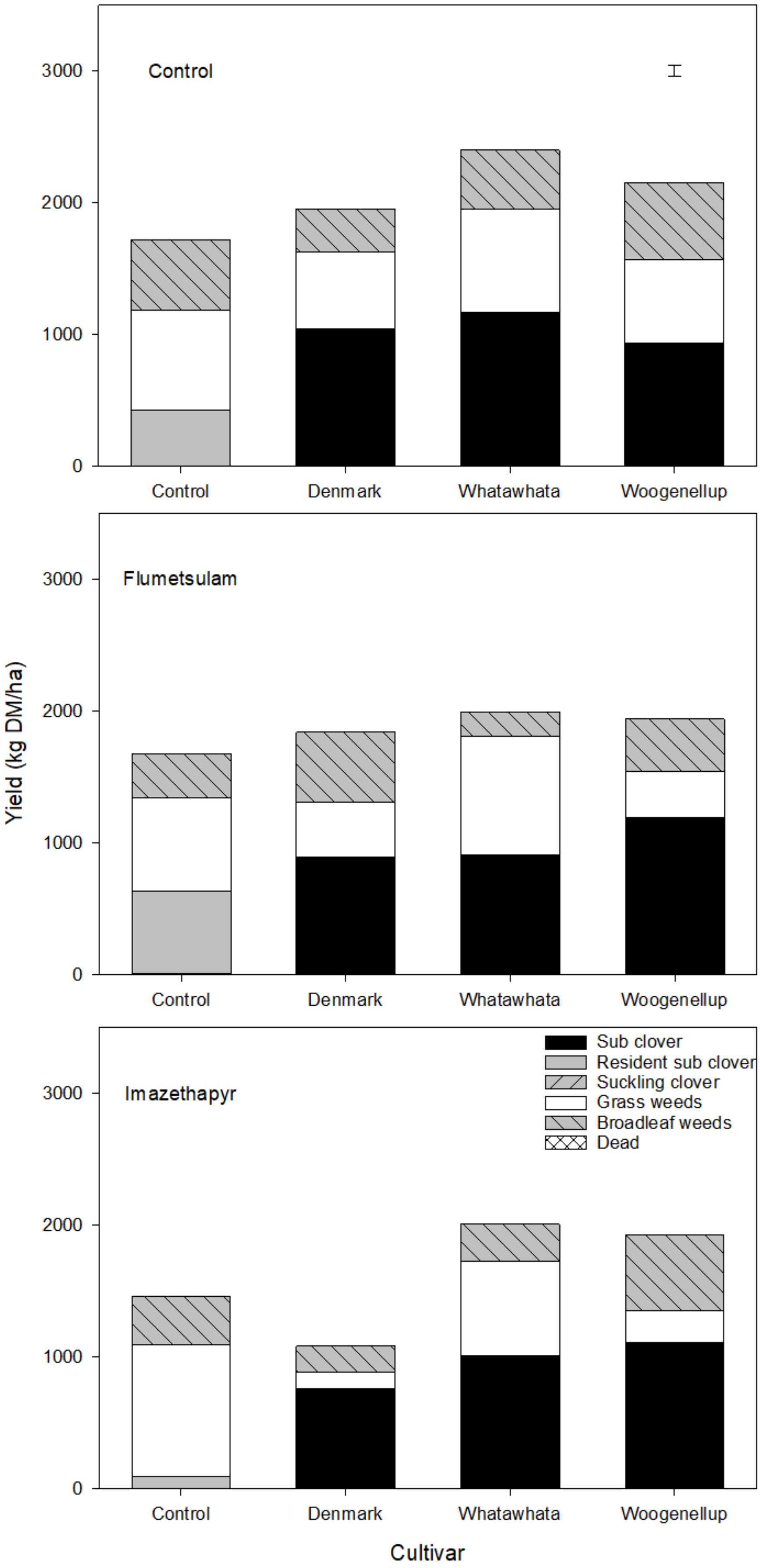

Control
Flumetsulam
Imazethapyr
Yield (kg DM/ha)
0
1000
2000
3000
Control
Denmark
Whatawhata
Woogenellup
Cultivar
Sub clover
Resident sub clover
Suckling clover
Grass weeds
Broadleaf weeds
Dead

Figure 4.7 Dry matter yield (kg DM/ha) of Harvest 1 on the 1st of October 2021 of plots overdrilled with three cultivars and two herbicide treatments at Ashley Dene, Canterbury, New Zealand. The influence of no herbicide (control), flumetsulam, and imazethapyr treatments on the botanical composition of total dry matter is shown with sward components reported within the graph. There is no significant difference reported between the treatment yields with the pooled SEM reported for total yield.

4.3.2.3 Harvest 2: 28 October 2021

There was no difference in yield among the cultivar (P=0.596) or herbicide treatments (P=0.703) on the 28th of October 2021. The mean yield was 3162±131 kg DM/ha. Overdrilled pastures were not different (P=0.075) to the Control in total clover with the mean yield 1558±125 kg DM/ha. However, the cultivar 'Whatawhata' (868±92.9 kg DM/ha) produced less (P=0.006) DM than 'Woogenellup' (1403±92.9 kg DM/ha) but was not different to 'Denmark' (1089±92.9 kg DM/ha) at Harvest 2 (Figure 4.8). There was no herbicide effect (P=0.610) on sown subterranean clover yield. Resident subterranean clover was not affected by cultivar (P=0.054) or herbicide (P=0.306) treatments. The mean resident subterranean clover yield was 669±78.4 kg DM/ha. DM accumulation of suckling clover was 49±15.2 kg DM/ha and was also not affected by cultivar (P=0.256) or herbicide (P=0.50).

Grass yield in Harvest 2 was not affected by cultivar (P=0.356) or herbicide (P=0.937) treatment. The mean yield for grass weeds was 890±134 kg DM/ha. There was also no effect on broadleaf yield for cultivar (P=0.606) or herbicide (P=0.720) treatments. The mean broadleaf yield was 482±57.4 kg DM/ha. A cultivar*herbicide interaction (P=0.006) was reported for dead material. 'Whatawhata' plots had more dead material when applied flumetsulam (360±57.3 kg DM/ha) and no herbicide treatment (374±57.3 kg DM/ha) than 'Woogenellup' applied flumetsulam (149±57.3 kg DM/ha) or imazethapyr (111±57.3 kg DM/ha). Botanical composition of yield for Figure 4.8 are reported in **Error! Reference source not found.**and **Error! Reference source not found.**.

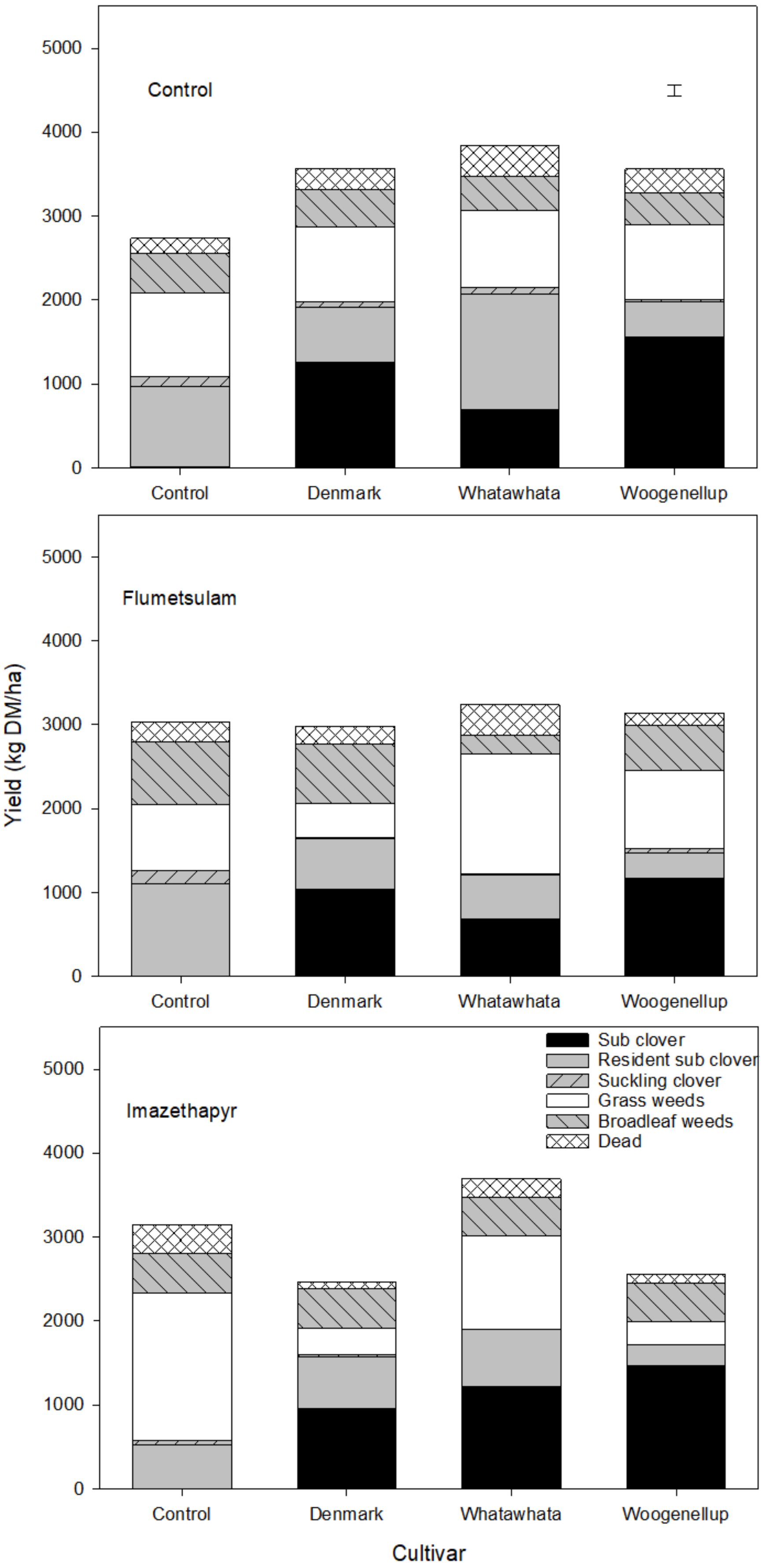

Control
Flumetsulam
Imazethapyr
5000
4000
3000
2000
1000
0
Yield (kg DM/ha)
Control
Denmark
Whatawhata
Woogenellup
Cultivar
Sub clover
Resident sub clover
Suckling clover
Grass weeds
Broadleaf weeds
Dead

Figure 4.8 Dry matter yield (kg/ha) of Harvest 2 on the 28th of October 2021 of plots overdrilled with three cultivars and two herbicide treatments at Ashley Dene, Canterbury, New Zealand. The influence of no herbicide (control), flumetsulam, and imazethapyr treatments on the botanical composition of total dry matter is shown with sward components reported within the graph. There is no significant difference reported between the treatment yields with the pooled SEM reported for total yield.

Table 4.10 Botanical composition of yield from cultivar treatments at Ashley Dene, Canterbury, New Zealand, 2021. P values indicate significant effects (P<0.05) for yield with the associated SEM for cultivar reported. Values with subscript letters in common are not significantly different at alpha=0.05.

Treatment	Cultivar	Res sub clover	Grass	Broadleaf	Dead
Control	0_{c}	866	1176	564	252
'Denmark'	1089_{ab}	622	539	541	179
'Whatawhata'	868_{b}	863	1150	364	316
'Woogenellup'	1403_{a}	324	693	458	183
P-value	0.006	0.054	0.356	0.606	0.21
SEM	92.9	86.7	255	108	55.1

Table 4.11 Botanical composition of yield from herbicide treatments at Ashley Dene, Canterbury, New Zealand, 2021. P values indicate significant effects (P<0.05) for yield with the associated SEM for herbicide reported. Values with subscript letters in common are not significantly different at alpha=0.05.

Treatment	Cultivar	Res sub clover	Grass	Broadleaf	Dead
Control	880	859	919	425	274
Flumetsulam	728	629	889	554	237
Imazethapyr	912	518	861	467	187
P-value	0.61	0.306	0.937	0.72	0.434
SEM	123	115	112	105	53.8

4.3.3 Dry matter yield: 2 March 2022 to 29 April 2022

The post-emergence pasture mass was not different (P=0.783) among cultivar treatments on the 2nd of March 2022. The mean pasture mass was 1794±66.1 kg DM/ha. Pasture yield was also not different (P=0.439) among cultivars on the 29th of April 2022 at 1637±92.5 kg DM/ha.

4.3.4 Nutritional analysis

A nutritional analysis was performed on subterranean clover samples from Harvest 2. The ME content of 'Denmark' and 'Woogenellup' was higher (P=0.023) than the Control (Table 4.12). However, there was no difference in ME between the Control and 'Whatawhata'. The nitrogen% was higher (P=0.037) for 'Whatawhata' than the Control, 'Denmark', and 'Woogenellup' at 0.353%.

Table 4.12 Megajoules of metabolizable energy (MJ ME kg/DM) and N% of cultivars growing at Ashley Dene, Canterbury, New Zealand, 2021. P values indicate significant effects (P<0.05) among the components of nutrition with the associated SEM for cultivar reported. Values with subscript letters in common are not significantly different at alpha=0.05.

Cultivar	MJ ME/kg DM	N%
Control	11.2_b	0.300_b
'Denmark'	11.7_a	0.313_b
'Whatawhata'	11.5_{ab}	0.353_a
'Woogenellup'	11.7_a	0.303_b
P value	0.023	0.037
SEM	0.105	0.012

4.3.5 Botanical composition scores: 29 April 2022

Visual botanical composition scores were carried out on total clover, broadleaf, and grass weeds on the 29th of April 2022. Subterranean clover during this time appeared to be affected by fungal pathogens. To confirm this samples of plants from each cultivar and grass were taken on the 2nd of May 2022 and analysed at Plant Diagnostics Ltd. Results of testing showed the pathogen of infection was primarily from *Rhizoctonia solani* with grass species affected by rust and suspected ectotrophic root-infecting (ERI) fungus (*Gaeumannomyces* and *Magnaporthe* species). *Fusarium* species were also cultured from the roots of grass species analysed. *Puccinia* and *Drechslera* species were present on the leaves of grass and are morphologically similar to *P. coronata* which causes crown rust.

Clover percentage was not different (P=0.083) among cultivars with 17.6% of the sward containing clover (Table 4.13). Broadleaf weed percentage was not different (P=0.113) with the mean percentage 23.6%. Grass weeds were also not different (P=0.076) at 59.2% of the sward.

Table 4.13 Botanical composition scores (%) of clover, broadleaf, and grass weeds on the 29th of April 2022 growing at Ashley Dene, Canterbury, New Zealand. P values indicate statistical difference (P<0.05) among the components of the pasture with the associated SEM for cultivar reported.

Cultivars	Clover	Broadleaf	Grass
Control	10.2	26.8	63.5
'Denmark'	22.8	28.8	49.2
'Whatawhata'	11.2	18.8	70.2
'Woogenellup'	26.2	20.2	53.8
P-value	0.083	0.113	0.076
SEM	4.6	2.99	5.29

4.3.6 Canopy cover

A cultivar*herbicide effect (P=0.033) was reported on the 20th of October 2021. Overdrilled pastures regardless of herbicide treatment had a higher canopy cover (~76.1%) than pastures not overdrilled (~54.7%) and applied an herbicide. From the 16th of September to the 1st of October a control treatment was not in place with only flumetsulam*imazethapyr analysis carried out. From the 7th of October a control treatment was implemented. At all measurement periods a cultivar effect was reported (Figure 4.9).

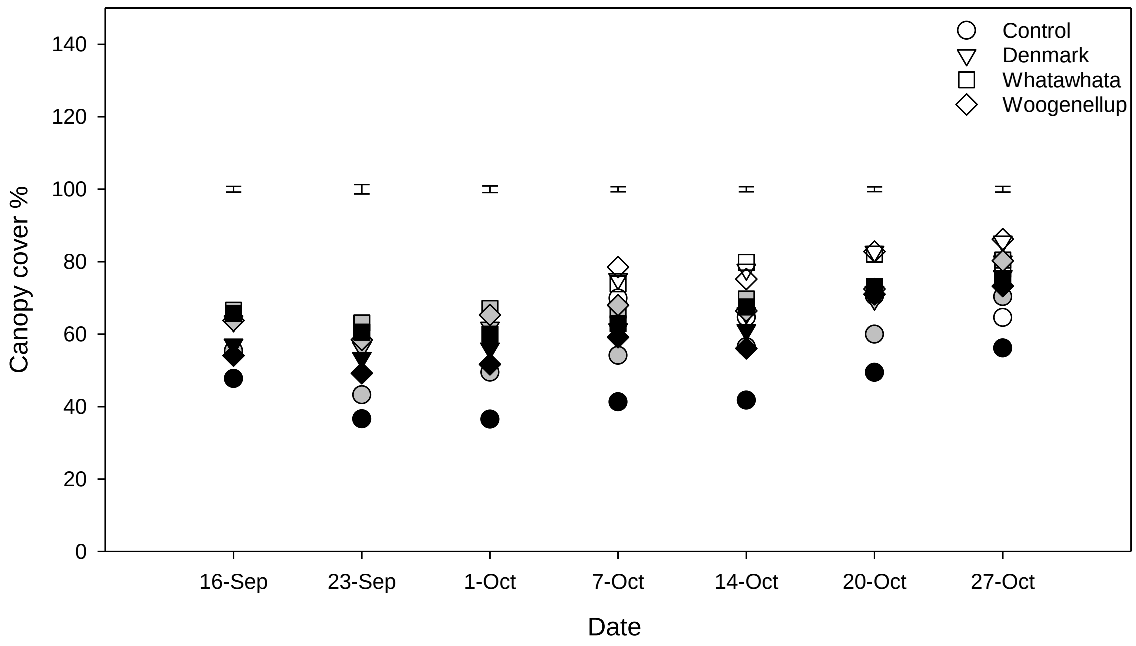


Figure 4.9 Mean canopy cover (%) of 'Denmark', 'Whatawhata', 'Woogenellup' and a Control from the 16th of September to 27th of October 2021 at Ashley Dene, Canterbury, New Zealand. Cultivar identification is presented within the figure with no herbicide (○, ▽, □, ◇) flumetsulam (●, ▼, ■, ◆), and imazethapyr (●, ▼, ■, ◆). Error bars report the SEM for the main effect of cultivar. All effects of cultivar are significant ($P<0.05$).

A cultivar effect ($P<0.001$) was reported on the 16th of September 2021. The canopy cover of 'Whatawhata' (66%) was higher compared with 'Denmark' (60%) and 'Woogenellup' (59%) pastures. All plots containing overdrilled subterranean clover had a higher canopy cover than the Control treatment (52%). There was no herbicide effect ($P=0.074$) on this date. A cultivar difference occurred on the 23rd of September, with 'Whatawhata' having a higher ($P<0.001$) canopy cover of 62% compared with 'Denmark' (55%) and 'Woogenellup' (54%). The Control plots had a canopy cover of 40% compared with the sown cultivars. There was no herbicide effect ($P=0.522$) reported for this date. On the 1st of October pastures that were sown with subterranean clover had a canopy cover of ~60% which was higher ($P<0.001$) than the Control (43%). There was no difference in canopy cover between cultivars. An herbicide effect ($P=0.013$) was reported between flumetsulam and imazethapyr on the 1st of October. However, the means were unable to be separated as only two were present.

Pastures sown with subterranean clover had a canopy cover of ~66% on the 7th of October, which was higher ($P<0.001$) than the Control (51%). Pastures not applied herbicide had a higher ($P<0.001$) canopy cover (74%) than sprayed plots. Flumetsulam (64%) was higher than imazethapyr (56%). ‘Whatawhata’ had a higher ($P<0.001$) canopy cover (70%) than all other treatments on the 14th of October. ‘Denmark’ (66%) and ‘Woogenellup’ (63%) were higher than the Control (51%). Unsprayed pasture had a higher ($P<0.001$) canopy cover (74%) than flumetsulam (65%) and imazethapyr (57%) with there being no difference among the latter. Pastures sown with subterranean clover had a canopy cover of ~78% on the 27th of October, which was higher ($P<0.001$) than the Control plots (63%). Pastures sprayed with flumetsulam were not different to unsprayed plots (~78%) but higher ($P=0.003$) than imazethapyr (70%) treated pastures.

4.4 Herbicide tolerance

4.4.1 Phytotoxicity scores

4.4.1.1 Subterranean clover EWRS scores

EWRS phytotoxicity scores were calculated for clover (Figure 4.10), broadleaf (Figure 4.11), and grass (Figure 4.12) components of herbicide and non-herbicide plots from the 9th of September to the 27th of October 2021. An herbicide effect was reported at each period of measurement after herbicide application for subterranean clover (Figure 4.10). As expected, Control plot EWRS scores were 1.0, which infers no visual sign of phytotoxicity and were lower ($P<0.05$) than sprayed plots at each measurement. There was no cultivar effect ($P>0.05$) reported for clover EWRS scores except for 14 DAT (Appendix 7).

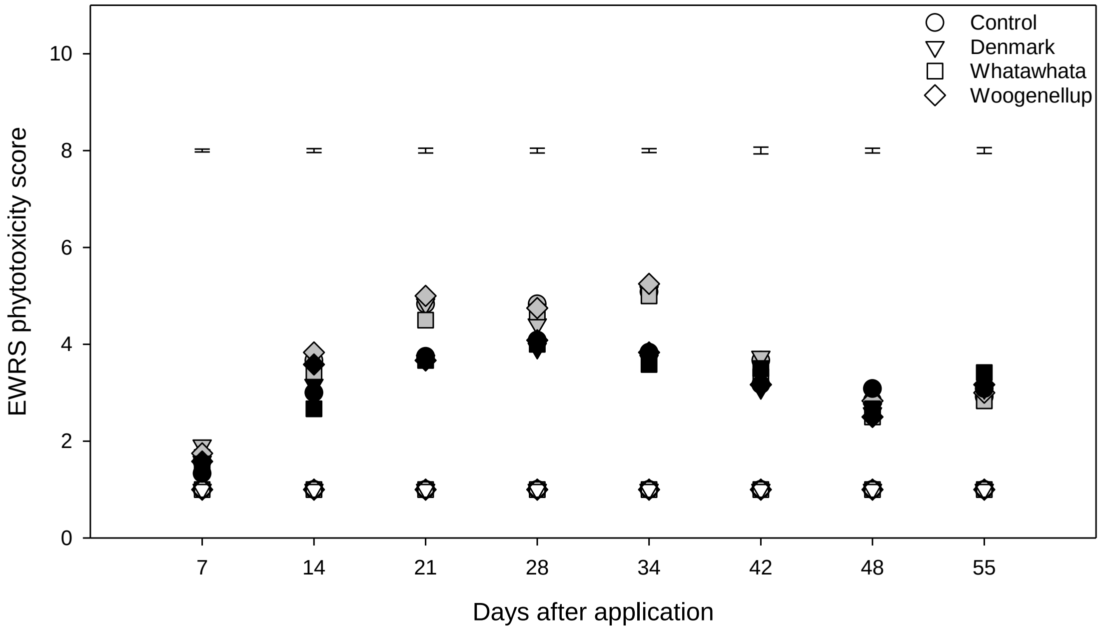


Figure 4.10 Mean EWRS phytotoxicity score of ‘Denmark’, ‘Whatawhata’, ‘Woogenellup’ and Control post herbicide application from the 2nd of September to the 27th of October 2021 at Ashley Dene, Canterbury, New Zealand. Cultivar identification is presented within the figure with no herbicide (○, ▽, □, ◇), flumetsulam (●, ▼, ■, ◆), and imazethapyr (●, ▼, ■, ◆). Error bars report the SEM for the main effect of herbicide. All effects of herbicide are significant ($P<0.05$).

Subterranean clover applied flumetsulam had a higher ($P<0.001$) EWRS score than pastures sprayed with imazethapyr up to 42 DAT. At 7 DAT subterranean clover sprayed with flumetsulam had a EWRS score of 1.7 compared with imazethapyr at 1.5. EWRS clover scores for flumetsulam increased to 4.8 compared with 3.7 for imazethapyr 21 DAT. EWRS scores for flumetsulam decreased to 4.7 with imazethapyr reaching 4.0 28 DAT. EWRS clover scores increased to 5.1 at 34 DAT for plots applied flumetsulam while scores for imazethapyr (3.7) decreased for the same period. Subterranean clover EWRS score was not different ($P>0.05$) for herbicide treatments at 48 (2.7) and 56 (3.1) DAT.

‘Whatawhata’ (2.4) had a lower ($P<0.001$) EWRS score at 14 DAT than the other cultivars. ‘Denmark’ (2.6) and ‘Woogenellup’ (2.8) were not different. However, ‘Woogenellup’ EWRS score was higher than the Control (2.5).

4.4.1.2 Broadleaf weed EWRS scores

An herbicide effect (P<0.001) was reported at each period for broadleaf weed EWRS scores. Broadleaf weeds in Control plots were allocated an EWRS score of 1.0 to indicate no herbicide effect (Figure 4.11). There was no difference in EWRS scores for broadleaf between flumetsulam and imazethapyr plots over the entire measurement period except at 42 DAT. Broadleaf weeds sprayed with imazethapyr (4.5) had a higher (P<0.001) ERWS score than flumetsulam (3.7). Plots not applied herbicide (Control) had a lower (P<0.001) EWRS scores for broadleaf at each measurement. Broadleaf EWRS score for herbicide treatments 7 DAT was ~3.1. Broadleaf weeds applied flumetsulam and imazethapyr reached an EWRS score of 5.4 at 21 DAT. This subsequently declined to 3.9 by 55 DAT.

‘Whatawhata’ (2.6) plots had a lower (P=0.007) EWRS score for broadleaf weeds than ‘Denmark’ (2.9) and Control (2.9) plots but not ‘Woogenellup’ (2.7) at 14 DAT. Broadleaf weed phytotoxicity scores for ‘Whatawhata’ (3.6) plots were again lower (P=0.009) than all other pastures (4.1) at 21 DAT. At 34 DAT the broadleaf EWRS score in ‘Whatawhata’ (2.9) pasture was lower (P=0.012) than the Control (3.4) but not ‘Denmark’ and ‘Woogenellup’. Overdrilled pastures (2.9) had lower EWRS scores for broadleaf compared with the Control (3.5) at 42 DAT. However, broadleaf in ‘Whatawhata’ (2.7) pastures was lower (P<0.001) than ‘Denmark’ (3.1) and ‘Woogenellup’ (3.2) 48 DAT. Overdrilled pastures had lower (P=0.012) EWRS scores for broadleaf (~2.9) than the Control (3.2) 55 DAT.

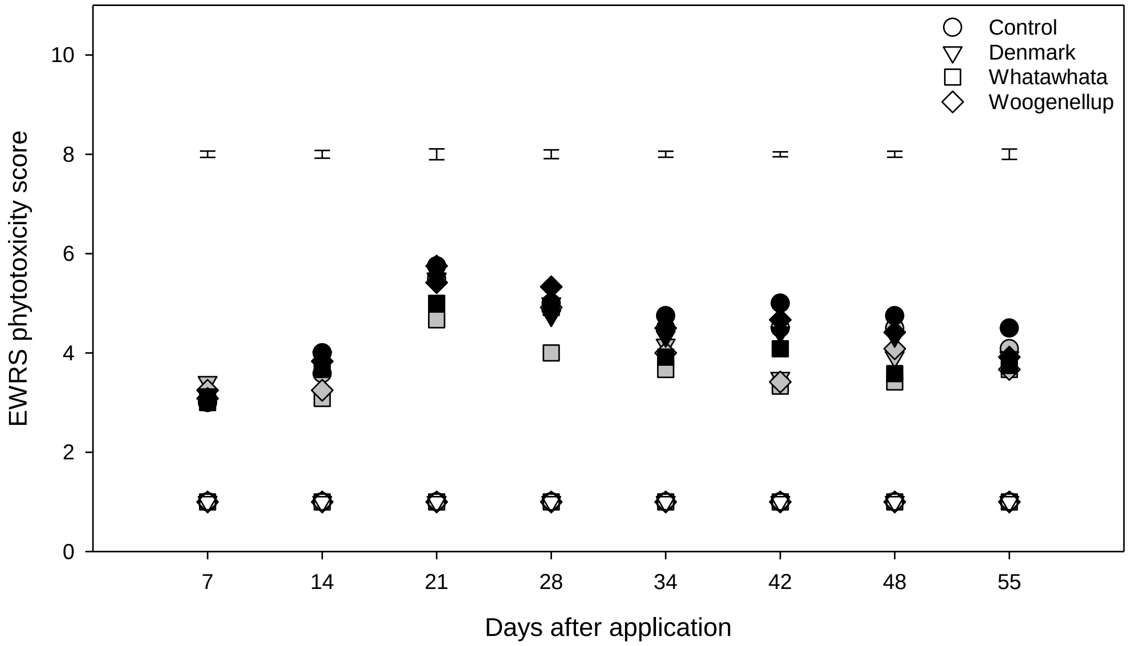


Figure 4.11 Mean EWRS phytotoxicity scores of broadleaf weeds post herbicide application on the 2nd of September to the 27th of October 2021 at Ashley Dene, Canterbury, New Zealand. Cultivar identification is presented within the figure with no herbicide (○, ▽, □, ◇), flumetsulam (●, ▼, ■, ◆), and imazethapyr (●, ▼, ■, ◆). Error bars report the SEM for the main effect of herbicide. All effects of herbicide are significant (P<0.05).

4.4.1.3 Grass weed EWRS scores

A cultivar*herbicide interaction was reported for EWRS scores for grass weeds at 42 (P<0.001) DAT. EWRS scores for grass weeds was higher for Control (~3.1) plots sprayed with an herbicide than all other treatments.

An herbicide effect (P<0.001) was reported for all measurement periods except 7 DAT (P=0.317) (Figure 4.12). EWRS scores for grass weeds applied flumetsulam and imazethapyr was higher (P<0.001) than the Control at all measurement periods after 7 DAT. EWRS scores for grass weeds at 14 DAT was ~1.7 for plots applied herbicide. At 28 DAT the EWRS score for grass was 2.9 for imazethapyr compared with 2.2 for flumetsulam. This decreased to ~2.6 at 48 DAT and remained constant at 55 DAT for both herbicides. Plots containing sown subterranean clover had lower EWRS scores for grass than Control plots at 21 (P=0.003) and 48 (P=0.006) DAT. However, at 34 DAT, 'Denmark' (2.2) was not different to the Control (2.4), with ‘Whatawhata’ and ‘Woogenellup’ scores remaining lower (P=0.002) than the Control. No other difference was reported for the effect of cultivar.

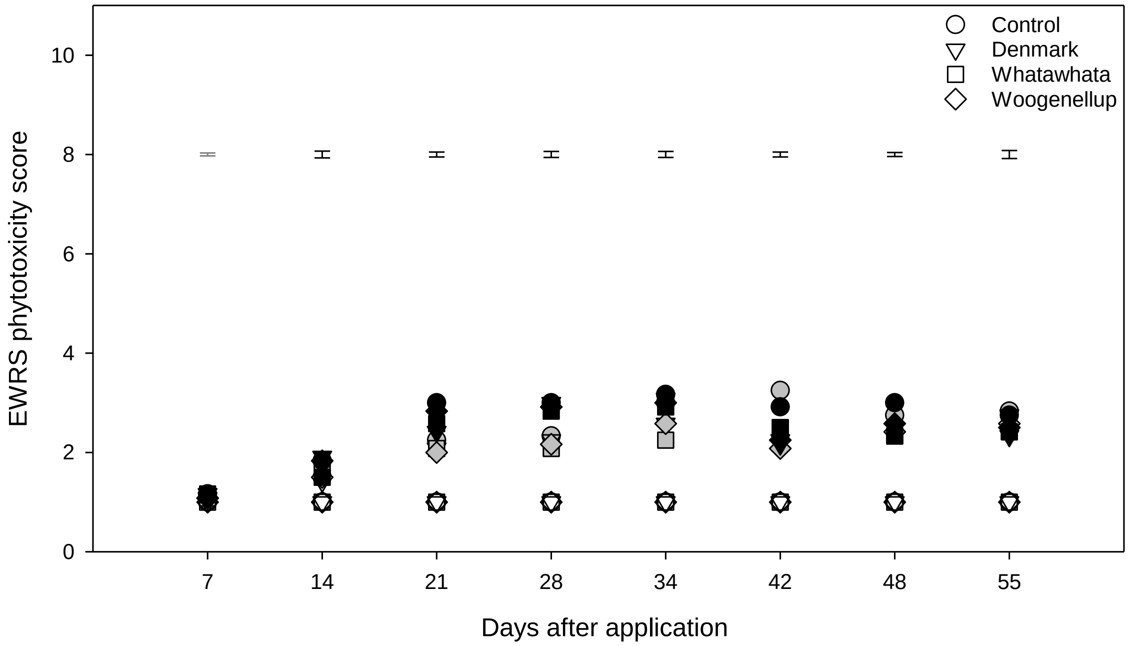


Figure 4.12 Mean EWRS phytotoxicity scores of grass weeds post herbicide application from the 2nd of September to the 27th of October 2021 at Ashley Dene, Canterbury, New Zealand. Cultivar identification is presented within the figure with no herbicide (○, ▽, □, ◇) flumetsulam (●, ▼, ■, ◆), and imazethapyr (●, ▼, ■, ◆). Error bars report the SEM for the main effect of herbicide. All effects of herbicide are significant (P<0.05) except at 7 DAT as highlighted by the grey SEM.

5 DISCUSSION

5.1 Phenotype description

Objective 1 of this study was to describe the phenotype of ‘Whatawhata’ in comparison with the two check cultivars ‘Denmark’ and ‘Woogenellup’. The series of photographs in the results section presents key structural components of the cultivars to allow identification. Plate 4.3 shows the leaf markings of a vegetative plant in autumn/winter of ‘Whatawhata’. This resembles the published leaf marking for ‘Mt Barker’ and is distinct in comparison with the relatively faint leaf mark of ‘Denmark’ (Plate 4.6). ‘Whatawhata’ leaves are small and compact compared with the large light green leaves of ‘Woogenellup’ (Plate 4.9). Anthocyanin pigmentation (red colouration) was also distinctive during cooler periods of the year on ‘Whatawhata’ leaves. ‘Whatawhata’ is also extremely hairy on the structural components including the petioles (Plate 4.4) unlike ‘Denmark’ which is glaborous on the petioles (Plate 4.7) and ‘Woogenellup’ which is inconsistent with petiole hairs (Plate 4.9). Flower characteristics are less distinctive between the cultivars investigated in this experiment. However, the calyx of ‘Whatawhata’ does not have the distinctive red band that is present on ‘Mt Barker’ flowers and so allows differentiation from its probable parent of origin. The stipules of ‘Whatawhata’ are also green with some red pigmentation unlike ‘Mt Barker’ which has red stipules.

5.2 Phenological development

Objective 2 was to quantify the phenological development of ‘Whatawhata’ and compare it with ‘Denmark’ and ‘Woogenellup’. Consistent phenological development of the subterranean clover cultivars in response to accumulated thermal time will enable ‘Whatawhata’ to be classified as an ‘Early’ or ‘Late’ cultivar (Teixeira *et al.* 2020a).

5.2.1 Vegetative phase

Field and controlled experimental data were available for comparison of trifoliate leaf emergence. Based on these results the first trifoliate leaf of ‘Whatawhata’ required 260±9.29 °Cd. There was some variation among the results. For example, leaf appearance data after sowing in 2021 was outside the range of 150-300 °Cd for clover species to produce their first true leaf (Teixeira *et al.* 2021). This was likely influenced by the low and variable emergence rate of ‘Whatawhata’ (64%) in this experiment. However, the thermal time requirement for trifoliate leaf appearance after re-emergence in 2022 was not different among cultivars at ~204 °Cd. This was an estimated value for first trifoliate leaf appearance because grazing prevented conclusive calculation of thermal time requirement at this phase. The thermal time requirement for first trifoliate leaf appearance of ‘Denmark’ were comparable to ‘Whatawhata’ across all data. ‘Denmark’ had a high thermal time requirement of 284±21.2 °Cd in 2021, which is higher than 222 °Cd reported by Moot *et al.* (2003) but falls within the 150-300 °Cd range for annual clovers (Teixeira *et al.* 2021). There was less thermal time variation in the controlled experiment with ‘Denmark’ requiring 237±9.29 °Cd in comparison to published literature at 207 °Cd (Moot *et al.* 2003). Thermal time requirements of ‘Woogenellup’ were consistent with published information across all available data in this experiment (Moot *et al.* 2003; Teixeira *et al.* 2021). Initiation of the first trifoliate of ‘Woogenellup’ required less thermal time than ‘Whatawhata’ in the 2021 field (230±21.2 °Cd) and controlled (188±9.29 °Cd) experiment. ‘Whatawhata’ (260±9.29 °Cd) and ‘Denmark’ (237±9.29 °Cd) both required more thermal time to produce their first trifoliate than the mean value of 188±16.2 °Cd reported by Teixeira *et al.* (2021). However, the thermal time requirements of ‘Whatawhata’ and ‘Denmark’ were nearer the estimate of Lonati *et al.* (2009) of 220 °Cd for initial trifoliate leaf appearance of ‘Mt Barker’. ‘Mt Barker’ is the probable parent of origin from which the ‘Whatawhata’ strain originated (Dodd *et al.* 1995a). The thermal time requirements are therefore likely to be comparable as observed in this experiment along with other phenological characteristics which dictate its management.

Subsequent leaf production after the first trifoliate leaf was not different among cultivars. The mean phyllochron was 64.4±7.0 °Cd/leaf and consistent with leaf appearance of plants subjected to a controlled environment. Similarly, Moot *et al.* (2003) reported a mean value of 68±6.5 °Cd for phyllochron and a range of 63 to 73 °Cd/leaf. However, Teixeira (2019) reported a constant leaf phyllochron of 52 °Cd/leaf from late winter through to autumn which is lower than this experiment. The phyllochron of the cultivars in this experiment fall within the range of published data. Thermal time requirement up to the four-leaf stage required ~404-422 °Cd. Moot *et al.* (2003) reported a mean value of 434±35.0 °Cd and a range of 422 to 456 °Cd for ‘Denmark’ and ‘Leura’ respectively. Teixeira *et al.* (2021) reported a mean value of 400 °Cd at the five to six trifoliate leaf stage with Guo *et al.* (2022) reporting a requirement of 436±49 °Cd for the fourth main stem trifoliate leaf post germination. After the switch to exponential leaf production, which indicated branching, ‘Whatawhata’ (11.0±1.94 °Cd/leaf) and ‘Denmark’ (15.3±1.94 °Cd/leaf) had a shorter rate of leaf appearance than ‘Woogenellup’ (22.6±1.94 °Cd/leaf) in 2021. However, there was no difference in exponential leaf development in the controlled experiment (12.7±1.26 °Cd/leaf). Based on this data it appears likely that the rate of leaf appearance during the vegetative rosette forming stage for ‘Whatawhata’ is the same or slightly shorter than the other cultivars.

5.2.2 Reproductive phase

Runners are initiated after the shortest day in response to a change in photoperiod. Runner extension to flower bud formation is the longest development phase of the subterranean clover lifecycle (Teixeira *et al.* 2021). Leaves on the runners began to appear at 683±39.5 °Cd on all cultivars. The phyllochron on runners of ‘Whatawhata’ (83.3±9.45 °Cd/leaf) was not different to ‘Denmark’ (64.5±9.45 °Cd/leaf). However, phyllochron was shorter for ‘Denmark’ than ‘Woogenellup’ (96.1±9.45 °Cd/leaf). There is little information available on leaf appearance rate of subterranean clover runners and so a lack of data to compare these results with. The start of the runner phase typically requires 487±30.0 °Cd and remains in this phase for >1000 °Cd (Teixeira *et al.* 2021). The runner phase of this experiment was consistent with published data with a change to flowering requiring >1000 °Cd from 50% field emergence. Therefore, ‘Whatawhata’ followed a similar pattern of runner extension to ‘Denmark’ and ‘Woogenellup’ and can be managed accordingly for this phenological stage.

‘Whatawhata’ required 952±10.9 °Cd from the shortest day and 1119±11.0 °Cd from emergence to start flowering. Flowering was characterized as the first flower to appear (Guo *et al.* 2022) rather than 50% flowering (Teixeira *et al.* 2020a) in this experiment. ‘Denmark’ required a similar amount of thermal time to ‘Whatawhata’ to flower while ‘Woogenellup’ required less at 883±10.9 °Cd after the shortest day and 1051±11.0 °Cd from emergence. Teixeira *et al.* (2021) reported that subterranean clover requires 628 to 2600 °Cd from sowing to flower. This range is due to differences in the emergence date which changes the thermal time to the shortest day. After this, in an increasing photoperiod cultivars require 740±79.8 or 1090±94.8 °Cd to flower for 'Early' and 'Late' cultivars, respectively. The cultivars 'Woogenellup' and 'Denmark' are defined as 'Late' flowering cultivars (Teixeira *et al.* 2020a; Teixeira *et al.* 2021). 'Denmark' and 'Whatawhata' required a similar amount of thermal time accumulation to produce there first flower. Thermal time requirements of 'Whatawhata' to flower are therefore consistent with that of 'Late' cultivars Teixeira *et al.* (2020a). These results suggest the phenological characteristics of 'Whatawhata' are comparable to 'Denmark' across all phases of development. However, differences exist between 'Whatawhata' and 'Woogenellup' for the time to first trifoliate leaf and flowering. None the less, the cultivar 'Whatawhata' can be classified as a 'Late' flowering subterranean clover due to its similar thermal time requirement across key phenological stages which fulfils Objective 2. The characterization of 'Whatawhata' as a 'Late' flowering cultivar allows it to be managed accordingly for different regions of New Zealand as outlined by Guo *et al.* (2022).

5.3 Seedling establishment

Cultivars sown on the 10th of May 2021 established a similar plant population of ~353±17.7 seedlings m^2. The sowing rate of 'Whatawhata' was 30 kg/ha higher than 'Denmark' and 'Woogenellup' to account for the 64% germination rate of the seed line. Both sowing rates were higher than the recommended 10 kg/ha for establishing subterranean clover (Lucas *et al.* 2016). However, despite the high sowing rate seedling numbers did not achieve the 500-1000 seedlings/m^2 required to maximise herbage production in spring (Smetham 2003a). Taylor (2019) reported a similar re-emergence pattern of 570-1100±66.8 seedlings/m^2 in a mixed species sward to (Smetham 2003a). The sowing date of this experiment was delayed to the 10th of May 2021 due to a lack of autumn rainfall (Figure 3.3). Cooler temperatures and grass competition also probably reduced the seedling density per m^2 despite the above commercial sowing rate.

Re-emergence the following autumn is dictated by seed set (Smetham and Dear 2003). 'Whatawhata' plots re-established 188±42.9 plants/m^2 on the 25th of February 2022. This was lower than 'Denmark' (333±42.9 m^2) and 'Woogenellup' (383±42.9 m^2) which produced an equivalent number of seedlings to sowing. 'Whatawhata' required more thermal time to initiate flowering than 'Woogenellup' with fewer flowers developed (**Error! Reference source**

not found.) and early dry conditions likely minimised seed set of the cultivar in November. Plant populations were not different at 162±36.1 m^2 after grazing with further dry conditions and fungal disease jeopardizing the persistence of the sward in this experiment. Based on these results it would seem likely that a longer spring period with more rainfall would be advantageous for seed set of ‘Whatawhata’.

5.4 Dry matter yield

Objective 3 was to quantify growth of ‘Whatawhata’ and compare it with ‘Denmark’ and ‘Woogenellup’. Quantification of yield determines potential animal production that can be extracted from the sward compared with other cultivars and plant species. However, this experiment did not investigate the effects of grazing on ‘Whatawhata’ directly and so requires further research to evaluate its performance under New Zealand grazing management.

5.4.1 Total dry matter yield

Total yield of ‘Whatawhata’ pastures was not different to that of ‘Denmark’ or ‘Woogenellup’ from the 10th of May to the 28th of October 2021. The mean DM yield was 5012±189 kg DM/ha. The total legume component of overdrilled pastures (~2754±207 kg DM/ha) was higher than the Control (1359±207 kg DM/ha). Therefore, over sowing of subterranean clover improved the total legume component of the sward (Costello and Costello 2003). The individual cultivar yield of ‘Whatawhata’ was 1898±166 kg DM/ha and was not different to ‘Denmark’ (1986±166 kg DM/ha) and ‘Woogenellup’ (2482±166 kg DM/ha). Subterranean clover was not affected by herbicide treatment with differences in herbage accumulation influenced by cultivar.

Cooler temperatures were suspected of inhibiting herbicide efficacy. However, the temperature at which ALS inhibitor effectiveness is reduced was not reported by the manufacturer (Novachem 2022). Under poor growing conditions the effect of ASL inhibiting herbicides can be delayed by up to two months (Cobb and Reade 2010). (Evers *et al.* 1993) reported that the application of imazethapyr during late winter early spring had a delayed effect on subterranean clover pastures with no difference reported among treatments 20 days after application at the first harvest. This was reported to be due to reduced plant growth. Broadleaf and grass weed content in this experiment were also not affected by herbicide treatment.

Herbicides effects are contradictory to Taylor (2019) who reported an accumulated yield of 8350 and 8160 kg DM/ha for the cultivars 'Napier' and 'Antas' when sown in late April. The remaining cultivars accumulated 6260-6880 kg DM/ha. Herbicide did not affect total pasture yield in accordance with this experiment. However, legume yield was improved with flumetsulam (5260 kg DM/ha) and imazethapyr (5330 kg DM/ha) application compared with unsprayed pastures (4220 kg DM/ha). Lewis (2017) reported a similar result for imazethapyr treated pastures with the legume component of sprayed pastures higher than unsprayed. 'Whatawhata', 'Woogenellup' and 'Narrikup' contributed the highest proportion of clover at 3500-4500 kg DM/ha. Subterranean clover yield in Lewis (2017) was lower than reported by Taylor (2019) due to moisture limitations at sowing which reduced the growth potential. This was reflected in this experiment with rainfall below the long term mean after sowing in May. The experiments of Lewis (2017) and Taylor (2019) were also implemented in autumn which may account for the different response to herbicide application. However, Lewis (2017) reported that mid-winter application of imazethapyr was 40% less effective than when applied in autumn. Subterranean clover was unable to capitalise on vacant ground due to low plant growth with no difference in establishment achieved between herbicide treatments. Reported air temperatures in July were <10 °C (Lewis 2017).

5.4.2 Harvest 1

At Harvest 1 on the 1st of October 2021 subterranean clover cultivars produced ~1000±100 kg DM/ha. Canopy cover (~60%) was not different among cultivars at this date in accordance with individual legume yield. DM yield in Harvest 1 was lower than published values but consistent with the reduced growth rate as affected by sowing date (Moot *et al.* 2003). Moot *et al.* (2003) reported that cultivars sown on the 7th of May produced 1800 kg DM/ha compared with 7000 kg DM/ha from cultivars sown at Lincoln on the 7th of March. The delayed sowing reduced the thermal time accumulation and so growth potential of the cultivars. Lewis (2017) reported that 'Woogenellup' sown on the 1st of March and sprayed with imazethapyr yielded 2250 kg DM/ha while unsprayed pastures produced 1340 kg DM/ha on the 7th of September. 'Whatawhata' applied imazethapyr produced 1830 kg DM/ha compared with the unsprayed pasture at 610 kg DM/ha. Lewis (2017) reported a below average rainfall post sowing with legume yield reduced as described in Harvest 1 of this experiment. Taylor (2019) reported ALS herbicide application to increase the DM yield of ‘Antas’ (~2530 kg DM/ha) and ‘Napier’ (~2100 kg DM/ha). However, the legume component of ‘Coolamon’ was inconsistent while the remaining cultivars were not different to the control in early October.

5.4.3 Harvest 2

Herbage was removed through grazing on the 7th of October 2021 before Harvest 2. At Harvest 2 on the 28th of October 2021, DM yield of ‘Whatawhata’ (868±92.9 kg DM/ha) was not different to ‘Denmark’ (1089±92.9 kg DM/ha). However, the legume component of ‘Woogenellup’ was higher (1403±92.9 kg DM/ha). Under lax grazing the large leaved cultivar ‘Woogenellup’ was able to effectively compete with grass and broadleaf weed species for light and so contribute a higher DM proportion to the total yield of the sward. ‘Whatawhata’, like ‘Denmark’ is a small, leafed cultivar (Plate 4.5) with a prostrate growth habit that is less effective at competing for light when laxly grazed and so yielded less due to its structural characteristics and ability to compete with the infrequently grazed grass. Canopy cover (~78%) was not different among pastures with additional pasture species contributing to the light interception of the canopy. This implied that the yield of each pasture was the same due to no difference in light interception which clarified total herbage accumulation at Harvest 2. DM yield of ‘Whatawhata’ and ‘Denmark’ was less than reported by Lewis (2017) at Harvest 2 for the same cultivars (1240-2730 kg DM/ha) when treated with imazethapyr. However, ‘Woogenellup’ yield coincided with data from Lewis (2017). Taylor (2019) reported cultivars to be affected by herbicide with yield ranging from 1360 to 1860 kg DM/ha in early November. ‘Whatawhata’ and ‘Denmark’ consistently remained below that of prior research at Lincoln which may reflect the colder conditions at Ashley Dene that limited canopy development in early spring, so legume growth was reduced into late spring.

5.4.4 Pasture mass 2022

Subterranean clover herbage accumulation is directly affected by seed set (Smetham and Dear 2003) with establishment controlled by environmental parameters and grazing management. Re-emergence in 2022 was not different in total herbage accumulation

(1794±66.1 kg DM/ha) despite clover seedling/m^2 being lower for 'Whatawhata' (188 m^2) compared with 'Denmark' (333 m^2) and 'Woogenellup' (383 m^2). Pasture mass was the same due to a lack of differentiation between pasture components. Pasture regrowth after grazing (1637±92.5 kg DM/ha) was reduced by moisture availability and suspected pathogen infection which prevented further measurement of the sward. Further research is required to determine genotypes resistance to pathogen infection to prevent loss of seedlings responding to rainfall events.

'Whatawhata' produced a similar amount of DM to 'Denmark' and 'Woogenellup' from the 10th of May to the 28th of October and so fulfils Objective 3. Differences in yield, due to leaf size, enabled 'Woogenellup' to compete with weeds and so produce a higher legume yield at Harvest 2. However, yield was restricted by environmental parameters compared with previous research and did not take into consideration the effects of grazing. Under favourable conditions 'Woogenellup' would have likely out yielded 'Whatawhata' and 'Denmark'.

5.5 Nutritional components

The metabolizable energy content of sown cultivars (11.7±0.105 MJ ME/kg DM), except 'Whatawhata' (11.5±0.105 MJ ME/kg DM), were higher than resident strains (11.2±0.105 MJ ME/kg DM) despite all cultivars being identified as 'Late' flowering. However, resident strains were further advanced in aspects of growth due to less competition in unsown plots which allowed their genetic potential for growth to be expressed. Resident species were therefore able to complete their lifecycle within the environmental constraints of the season and so were entering senescence when samples were collected. Genetic material of the Australian cultivars sown are also likely to be selected for improved energy content compared with resident strains. N% of Australian cultivars (0.307%) was higher than resident species (0.298%). However, 'Whatawhata' (0.352%) had a higher N% than all cultivars analysed. The smaller leaf size of 'Whatawhata' is concluded to contain a higher nitrogen concertation than the larger leaved 'Woogenellup' but this needs confirmation in further studies.

5.6 Herbicide tolerance

5.6.1 Subterranean clover herbicide tolerance

Objective 4 was to describe ‘Whatawhata’ tolerance to herbicide application compared with ‘Denmark’ and ‘Woogenellup’. EWRS scores were employed to determine visual phytotoxicity symptoms for subterranean clover, broadleaf, and grass weeds from the 9th of September to the 27th of October 2021 (Appendix 6). A high EWRS score correlates to a negative herbicide impact with reduced plant yield (Lewis 2017). An EWRS score of 7 is above the commercial threshold with severe yield reductions and plant death expected. Phytotoxicity scores of subterranean clover reported an herbicide effect for sprayed pastures at each point of measurement after application. Imazethapyr was reported to have less of a negative effect on subterranean clover than flumetsulam. However, at 56 DAT there was no difference in visual symptoms (3.1) with subterranean clover in pastures sprayed with flumetsulam recovering to a similar state as plants applied imazethapyr. Phytotoxicity scores did not reach a score of 7 for the duration of the experiment though plants applied flumetsulam reached 5.1 at 34 DAT which caused a loss of production. ‘Whatawhata’ (2.4) had a lower EWRS score at 14 DAT than all other cultivars. However, this did not correlate with an increase in yield with no other cultivar effect reported. The level of pubescence is suspected to increase clover tolerance of herbicide through a reduction in plant uptake (Lewis 2017) which may explain the effect seen in ‘Whatawhata’ at 14 DAT. However, this effect did not continue over the measurement period and so the level of hairiness did not appear to confer any major advantage. This is contrary to Lewis (2017) with plants of a hairy disposition such as ‘Whatawhata’ and intermediate hairy ‘Woogenellup’ having lower EWRS scores which correlated with an increase in legume yield as described in the previous section. Taylor (2019) reported no effect of pubescence increasing tolerance as is consistent with this experiment. There was no difference among flumetsulam and imazethapyr treatments with phytotoxicity score inconsistent with its effect on subterranean clover yield. However, application of the herbicide did increase subterranean clover yield compared with this experiment. Climate parameters are suspected of limiting the growth potential of the cultivars with legume yield likely to have been comparable to that of prior research if rainfall had been higher.

5.6.2 Broadleaf weed control

Broadleaf weed phytotoxicity scores were not different among sprayed pastures except at 42 DAT with imazethapyr (4.5) having a higher ERWS score than flumetsulam (3.7). An herbicide effect was reported at each point of measurement. Phytotoxicity scores for broadleaf weeds reached 5.4 at 21 DAT with some loss in plant yield occurring. However, subterranean clover was unable to capitalise on the weakened state of broadleaf weeds due to herbicide damage with EWRS score of clover reaching a maximum at 34 DAT. Phytotoxicity scores for broadleaf weeds subsequently declined to 3.9 by 55 DAT as plants recovered. Broadleaf in 'Whatawhata' plots consistently had lower phytotoxicity scores regardless of herbicide application. It is unclear why this occurred. The phytotoxicity scores of broadleaf weeds are below the commercial threshold for plant suppression and supports the lack of change among broadleaf proportions in total DM yield. However, the findings of this experiment are not consistent with Lewis (2017) who reported imazethapyr treated weed populations to be reduced by 90% over the growing season. Taylor (2019) reported a weed yield reduction of 1000±140 kg DM/ha from both flumetsulam and imazethapyr. Once again cool conditions and moisture limitations are suspected to be the cause for poor plant uptake of the herbicide and low growth rates of subterranean clover which would have allowed weed suppression to occur.

5.6.3 Grass weed control

Grass weeds had the poorest response to herbicide application of all species measured for phytotoxicity. A cultivar*herbicide interaction was reported at 42 DAT with Control plots having a higher EWRS score (~3.1) than all other pastures as they contained a greater proportion of grass compared with overdrilled plots. Phytotoxicity scores did not differ among herbicides except at 28 DAT with EWRS score of grass sprayed with imazethapyr (2.9) higher than flumetsulam (2.2). Phytotoxicity for grass weeds did not exceed a score of 3.1 with minor discolouration occurring and no loss in production. A cultivar effect was reported at 34 DAT, but the low EWRS score made it inconsequential in terms of effect on DM yield. This is contradictory to that of Lewis (2017) with grass weeds including annual brome and phalaris (*Phalaris arundinacea*) completely removed from the sward with imazethapyr application. Weed suppression was 90% at application with the residual effects of imazethapyr preventing grass regrowth by 80%. However, annual ryegrass was found to recover. Cocksfoot growth was also checked but recovered with the minimal effects on subterranean clover allowing a higher proportion to establish in autumn. Taylor (2019) reported grass weed suppression to be higher for imazethapyr sprayed pastures than flumetsulam. However, there was no difference in total grass weed yield, though they only represented a small proportion (<150±41.8) of the total DM yield. Herbicide application was reported to decrease grass weed content at individual harvests. Grass weed species at Ashley Dene contained a large proportion of barley grass and vulpia hair grass which are known to have inconsistent responses to herbicide application (Tozer *et al.* 2007). Flumetsulam is also less effective at grass weed control with the manufacturer recommending a follow up application if grass weeds fail to regress (Novachem 2022) which may account for the poor response reported.

Application of recommended herbicides for subterranean clover did not improve legume yield or have a significant effect on common pasture weed species. However, cultivars did recover sufficiently to produce a comparable yield to the control treatment with environmental influence limiting the growth potential of the sward and herbicide efficacy. Under favourable moisture and growing conditions, the effects of herbicide may have been more pronounced with results aligning with prior research. However, based on collected information the herbicide tolerance of 'Whatawhata' is consistent with 'Denmark and 'Woogenellup' and so achieves Objective 4 of this experiment. 'Whatawhata', can therefore undergo winter weed management as outlined by (Lewis *et al.* 2017) and (Taylor *et al.* 2020).

5.7 Conclusions

- The phenotype of 'Whatawhata' is small leaved, with a prostrate growth habit and is very hairy on the petioles, trifoliate leaves, and runners. Differentiated from 'Denmark' by the visible leaf mark and structural pubescence while 'Woogenellup' has a large light green leaf with inconsistent hairiness on structural components.
- 'Whatawhata' can be classified as 'Late' flowering cultivar as development is consistent with that of the cultivar 'Denmark' at all stages of phenology. In comparison with 'Woogenellup', the 'Whatawhata' genotype is slightly later to produce its first trifoliate leaf and reach flowering.
- DM yield of 'Whatawhata' is consistent with that of 'Denmark' and 'Woogenellup'.

- ‘Whatawhata’ herbicide tolerance is comparable to ‘Denmark’ and ‘Woogenellup’ with a similar response reported for sprayed and unsprayed legume yield.

6 GENERAL DISCUSSION AND CONCLUSIONS

Subterranean clover is a legume that has adapted to dryland environments through summer dormancy and re-emergence in autumn when sufficient rainfall occurs (Guo *et al.* 2022). Land based professionals have identified it as a suitable species to incorporate into pasture based systems with its adoption widespread across the world (Smetham 2003b). The New Zealand primary industry has also undertaken extensive research to quantify the growth and development of subterranean clover (Moot *et al.* 2003; Teixeira *et al.* 2020a; Teixeira *et al.* 2021) and create best management practices (Olykan *et al.* 2019)for its integration into pastoral systems. However, despite its production potential the registration of a cultivar specifically bred for New Zealand conditions has been neglected since the late 1990's due to low market demand and harvesting difficulties (Lucas *et al.* 2015).

The naturalised genotype classified as 'Whatawhata' is descended from the deregistered 'Mt Barker' cultivar and has adapted to New Zealand grazing management (Dodd *et al.* 1995b). 'Whatawhata' was selected for further research to determine its merits against commonly sown cultivars from Australia in this experiment. 'Whatawhata' behaves like 'Denmark' in terms of its phenological development and growth potential. The assessment of phenological development has allowed this cultivar to be classified as 'Late' flowering cultivar. Its management in New Zealand follows that of a 'Late' cultivar as outlined by (Guo *et al.* 2022). However, in comparison to the large leaved 'Woogenellup' which is potentially capable of more DM yield than 'Whatawhata' and 'Denmark', the small leaved cultivars appear to be less suited to summer dry environments where legume quantity improves animal finishing capability.

New Zealand grazing management unlike Australian practices employs continuous grazing (set stocking) during certain periods of the year. Often this occurs during spring and early summer with pregnant animals preferentially grazing the legume component of the sward to meet energy demands. The large leaved erect growing 'Woogenellup' is particularly susceptible to overgrazing with defoliation decreasing the leaf area available for photosynthesis and so reduces yield. Because of this, the integration of two subterranean clover cultivars has been recommended (Lucas *et al.* 2015) with the small leaved prostrate growth habit of 'Whatawhata' having the potential to replace cultivars such as 'Denmark' if a viable seed industry was to develop. The inclusion of 'Woogenellup' and 'Whatawhata' into summer dry pastures would allow producers to extract the benefits from both cultivars. The large leaved 'Woogenellup' effectively competes with grass species and produces a high DM yield for lactating animals in spring with the smaller 'Whatawhata' replacing the erect large leaved cultivar during continuous grazing.

To achieve this outcome 'Whatawhata' should be autumn sown at least 5 kg/ha (Lucas *et al.* 2016) with a companion genotype and grass species to generate a grass/clover sward. Grazing of the sward can occur at the three to five trifoliate leaf stage (~413 °Cd after emergence) with herbicide application of either imazethapyr or flumetsulam used to supress weed competition. Grazing should be lax over the winter period to allow successful establishment of 500-1000 seedlings/m^2 (Smetham 2003a). However, as found in this experiment grass competition can limit seedling population with grazing allowing subterranean clover to persist in the sward. Lambing can occur on these swards at a reduced stocking rate with the onset of flowering requiring animals to be excluded to allow adequate seed to set and so establish a large seed bank. 'Late' cultivars require a minimum of 82 kg/ha of seed set to ensure persistence (Smetham 2003b). Cattle can be stocked on these swards as they are unable to graze as deep into the sward as sheep (Olykan *et al.* 2019). After seed set normal animal grazing may resume with subterranean clover entering senescence as dictated by the moisture conditions of the season. Re-emergence in autumn requires strategic grazing as determined by moisture and plant species competition.

Further research is required to determine the grazing tolerance of 'Whatawhata' compared with commonly sown Australian cultivars as this was beyond the scope and objectives of this experiment. It is suspected that the cultivar will tolerate close grazing more than 'Woogenellup' but requires further investigation to clarify.

6.1 Conclusions

- ‘Whatawhata is a ‘Late” flowering cultivar and so can be managed accordingly
- Inclusion of ‘Whatawhata’ with large leaved strains could improve animal production but requires investigation under grazing
- ‘Whatawhata’ is a suitable replacement for the cultivar ‘Denmark’

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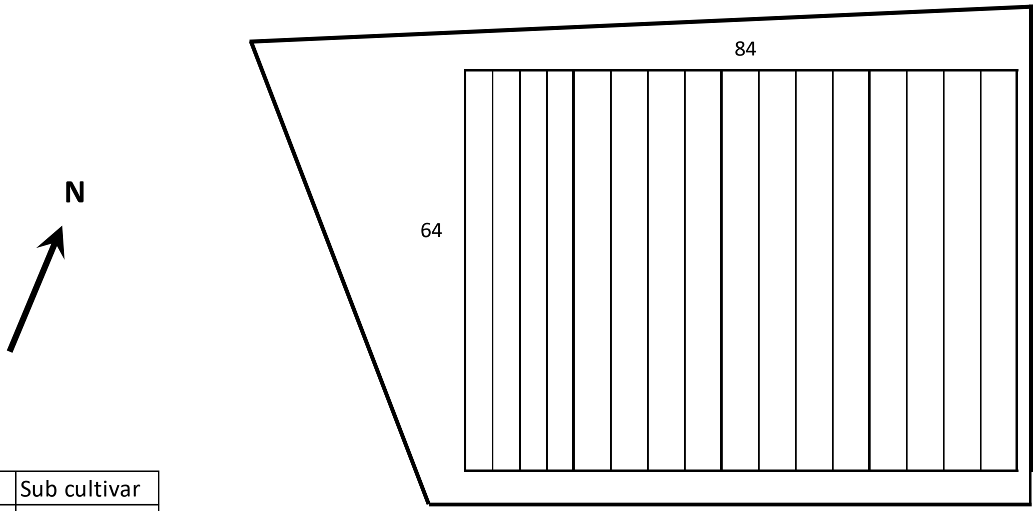
APPENDICES

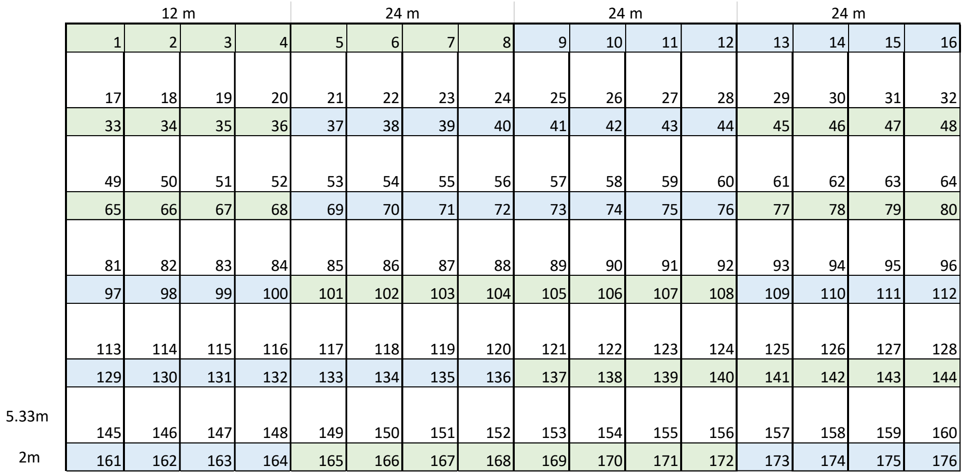
Appendix 1 Experimental design for subterranean clover cultivar comparison in C9(A(S) at Ashley Dene, Canterbury, New Zealand from May 2021 to April 2022.



Treatment	Sub cultivar
1	Control
2	Whatawhata
3	Woogenellup
4	Denmark

Trt no	1	2	3	4	4	3	1	2	2	1	4	3	3	4	2	1
Rep no	1				2				3				4			
Plot no	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

Appendix 2 Experimental design for herbicide application in C9(A(S) at Ashley Dene, Canterbury, New Zealand from September 2021 to April 2022.



	12 m				24 m				24 m				24 m			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64
	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80
	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96
	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112
	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128
	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144
5.33m	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160
2m	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176

Trt no	1	2	3	4	4	3	1	2	2	1	4	3	3	4	2	1
Rep no	1				2				3				4			
Plot no	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

	Rate
2	Spinnaker 400 ml/ha
3	Headstart 1.0 L/ha
1	Control

Appendix 3 Split line linear regression output for subterranean clover growing at Ashley Dene, Canterbury, New Zealand, 2021. The x-intercept (°Cd) reports the thermal time requirement for the first trifoliate leaf. Slope 1 describes linear leaf production while breakpoint x reports the point of change to exponential leaf production. P values report significant effects with the SEM reported. Values with subscript letters in common are not significantly different at alpha=0.05.

Cultivars	x-intercept (°Cd)	Slope 1	Breakpoint x (°Cd)	Slope 2
Control	177_{c}	0.019	595	0.046_{c}
Denmark	284_{ab}	0.024	635	0.070_{a}
Whatawhata	309_{a}	0.028	641	0.094_{a}
Woogenellup	230_{bc}	0.016	554	0.045_{b}
P-value	0.007	0.108	0.095	0.002
SEM	21.2	0.003	24	0.007

Appendix 4 Animal feed requirements as calculated by weight and number of animals to estimate herbage mass removed from plots on the 27th of October 2021 and the 1st of March 2022 from cultivars growing at Ashley Dene, Canterbury, New Zealand.

Animal	Wt. (kg)	No.	DM req.(kg)	Total req.(kg)	Days grazing	DM removed(kg)
Rams	80	25	1.40	35	4	140
Ewes	65	550	0.88	484	1	484

Appendix 5 Herbage mass removed on a per ha basis from experiment site on the 27th of October 2021 and the 1st of March 2022 from cultivars growing at Ashley Dene, Canterbury, New Zealand.

Animal	Plot size (ha)	DM removed (kg/ha)
Rams	0.538	205
Ewes	0.538	708

Appendix 6 Herbicide strip showing visual phytotoxicity on the 9th of September 2022 at Ashley Dene, Canterbury, New Zealand. Note chickweed death was also occurring in unsprayed plots in this figure. Orientation is south parallel with the farm lane on the left directly of Bethels Road (Photo: Wills, A.).

Appendix 7 Effect of cultivar on the mean subterranean clover EWRS scores from the 9th of September to 27th of October 2021. Subscript letters indicate a significant difference ($p<0.05$). Two cultivar*herbicide interaction effects are reported

with no distinction made between the means for the cultivar effect at this period. Ashley Dene, Canterbury, New Zealand.

	DAT							
Cultivar	7	14	21	28	34	42	48	55
Control	1.3	2.6	3.2	3.3	3.3	2.6	2.3	2.3
Denmark	1.5	2.6	3.2	3.1	3.3	2.6	2.1	2.3
Whatawhata	1.4	2.4	3.1	3.2	3.2	2.6	2.1	2.4
Woogenellup	1.4	2.8	3.2	3.3	3.2	2.4	2.1	2.4
P-value	0.162	<0.001	0.361	0.341	0.261	0.147	0.074	0.841
SEM	0.063	0.068	0.07	0.081	0.062	0.062	0.073	0.079
Cultivar*Herbicide		0.017				0.002		

Appendix 8 Effect of herbicide on the mean subterranean clover EWRS scores from the 9th of September to 27th of October 2021. Subscript letters indicate a significant difference ($p<0.05$). Two cultivar*herbicide interaction effects are reported with no distinction made between the means for the herbicide effect at this period. Ashley Dene, Canterbury, New Zealand.

	DAT							
Herbicide	7	14	21	28	34	42	48	55
Control	1.0_c	1.0	1.0_c	1.0_c	1.0_c	1.0	1.0_b	1.0_b
Flumetsulam	1.7_a	3.7	4.8_a	4.7_a	5.1_a	3.5	2.7_a	2.9_a
Imazethapyr	1.5_b	3.1	3.7_b	4.0_b	3.7_b	3.2	2.7_a	3.2_a
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SEM	0.063	0.081	0.11	0.097	0.085	0.144	0.105	0.122
Cultivar*Herbicide		0.017				0.002		

Appendix 9 Effect of cultivar on the mean broadleaf weed EWRS scores from the 9th of September to 27th of October 2021. Subscript letters indicate a significant difference ($p<0.05$). Two cultivar*herbicide interaction effects are reported with no distinction made between the means for the cultivar effect at this period. Ashley Dene, Canterbury, New Zealand.

	DAT							
Cultivar	7	14	21	28	34	42	48	55
Control	2.0	2.9_a	4.1_a	3.6	3.4_a	3.5	3.4	3.2_a
Denmark	2.2	2.9_a	4.0_a	3.6	3.2_{ab}	3.0	3.1	2.9_b
Whatawhata	2.0	2.6_b	3.6_b	3.3	2.9_b	2.8	2.7	2.8_b
Woogenellup	2.1	2.7_{ab}	4.1_a	3.8	3.2_{ab}	3.0	3.2	2.9_b
P-value	0.235	0.007	0.009	0.133	0.012	<0.001	<0.001	0.012
SEM	0.073	0.069	0.123	0.1369	0.116	0.092	0.081	0.088
Cultivar*Herbicide						0.003	0.002	

Appendix 10 Effect of herbicide on the mean broadleaf weed EWRS scores from the 9th of September to 27th of October 2021. Subscript letters indicate a significant

difference ($p<0.05$). Two cultivar*herbicide interaction effects are reported with no distinction made between the means for the herbicide effect at this period. Ashley Dene, Canterbury, New Zealand.

	DAT							
Herbicide	7	14	21	28	34	42	48	55
Control	1.0_b	1.0_b	1.0_b	1.0_b	1.0_b	1.0	1.0	1.0_b
Flumetsulam	3.2_a	3.4_a	5.4_a	4.7_a	4.1_a	3.7	4.0	3.8_a
Imazethapyr	3.1_a	3.9_a	5.4_a	5.0_a	4.4_a	4.5	4.3	4.0_a
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SEM	0.127	0.156	0.217	0.1793	0.123	0.107	0.126	0.209
Cultivar*Herbicide						0.003	0.002	

Appendix 11 Effect of cultivar on the mean grass weed EWRS scores from the 9th of September to 27th of October 2021. Subscript letters indicate a significant difference ($p<0.05$). Three cultivar*herbicide interaction effects are reported with no distinction made between the means for the cultivar effect at this period. Ashley Dene, Canterbury, New Zealand.

	DAT							
Cultivar	7	14	21	28	34	42	48	55
Control	1.1	1.4	2.1	2.1	2.4	2.4	2.3_a	2.2
Denmark	1.1	1.4	1.8	2.1	2.2	1.8	2_b	2.0
Whatawhata	1.1	1.4	1.9	2.0	2.1	2.0	1.9_b	1.9
Woogenellup	1.1	1.4	1.9	2.0	2.2	1.8	2.0_b	2.0
P-value	0.902	0.874	0.003	0.245	0.002	<0.001	0.006	0.060
SEM	0.032	0.058	0.049	0.052	0.06	0.068	0.073	0.066
Cultivar*Herbicide			0.008		0.042	<0.001		

Appendix 12 Effect of herbicide on the mean grass weed EWRS scores from the 9th of September to 27th of October 2021. Subscript letters indicate a significant difference ($p<0.05$). Three cultivar*herbicide interaction effects are reported with no distinction made between the means for the herbicide effect at this period. Ashley Dene, Canterbury, New Zealand.

	DAT							
Herbicide	7	14	21	28	34	42	48	55
Control	1.0	1.0b	1.0	1.0_c	1.0	1.0	1.0_b	1.0_b
Flumetsulam	1.1	1.5_a	2.1	2.2_b	2.6	2.5	2.5_a	2.6_a
Imazethapyr	1.1	1.8_a	2.7	2.9_a	3.0	2.5	2.6_a	2.5_a
P-value	0.317	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SEM	0.061	0.135	0.091	0.129	0.12	0.093	0.088	0.11
Cultivar*Herbicide			0.008		0.042	<0.001		