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Optimization of subterranean clover for dryland pastures in New Zealand.

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# Subterranean clover – Literature Review

Carmen S.P. Teixeira, Dick Lucas, Derrick J. Moot

Lincoln University

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# Executive summary

Sub-clover (*Trifolium subterraneum*) is an economically viable forage option to enhance productivity in New Zealand dryland pastoral systems. This annual clover provides more high quality biomass during late winter and early to mid-spring than the more frequently sown white clover. This is particularly advantageous for high demand livestock such as, lactating ewes.

For example, rain-fed sub clover from March-September yielded up to 7 t DM/ha in Canterbury. Similarly, in Australia sub clover pastures produced 6-7 t DM/ha (early October) with mean crude protein concentrations of 19% in spring.

However, the expansion of the use of sub clover in New Zealand is limited by the lack of agronomic information about the potential of the crop and best management practices to optimize its use. In particular, farmers' perceptions of sub clover are based on research developed more than 30 years ago in the North Island. Newly developed genetic material from Australia and agronomic and grazing management practices to maximise its potential are unfamiliar to most New Zealand farmers. Therefore this review summarizes the most recent international understanding of sub clover responses to environmental and management factors. This knowledge is critical to improve establishment and persistence of sub clover in New Zealand pastures. Specifically, the present analysis has identified new opportunities to widen the use of sub clover in New Zealand. For instance, cultivar cold/frost tolerance, seed hardness and its relationship with different growing conditions and weed management.

Importantly knowledge gaps have been identified. In particular, there is a lack of understanding of sub clover phenological development that limits the ability to estimate flowering and seed set time across environments. This impacts on management decisions related to grazing and closing time and use of different cultivars and combinations of cultivars in different locations. Also, agronomic techniques for effective weed control are limited or unknown in a New Zealand context which increases the perceived risk of production. Understanding the mechanisms that control hardseededness and regeneration of newly

released cultivars is necessary to increase the efficiency of sub clover establishment and persistence. Specifically, some recently introduced cultivars have a higher average hardseed rating than older cultivars. The implication of this on regeneration of plants after the first year requires further investigation, in monocultures and binary mixes.

Finally, creating resilient sub clover pastures may require the inclusion of cultivars with different flowering times, hardseed ratings and frost tolerance to evolve a seed bank capable of performing with greater reliability across the variable locations and climatic conditions on dryland farms.

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# 1 Introduction

The aim of this review is to summarise the status of research work and on-farm practical use of subterranean clover (sub clover) (*Trifolium subterraneum*) since its introduction to New Zealand.

Sub clover has been shown to be of particular value in New Zealand pastures where the performance of perennial legumes such as white clover is limited by dry summer conditions (Dodd *et al.*, 1995b).

Significant gains in pasture yield and quality have been reported when sub clover is well managed (Mills *et al.*, 2008) and in conditions where other legumes do not thrive or persist. Sub clover can improve forage productivity, regenerate the soil through nitrogen fixation (Gillingham *et al.*, 2003) and increase farm profitability (Grigg *et al.*, 2008).

This document presents a brief description of the past and current research and developments of sub clover in Australia and New Zealand. It shows the limitations of the existing knowledge about sub clover with an expected outcome to identify potential areas for research and development to enhance its use in New Zealand dryland pastures.

## 1.1 Geographical distribution

### 1.1.1 Climate and adaptability

Native clovers species are found in three main geo-climatic regions: (a) the Mediterranean basin, (b) western North America, and (c) the highlands of eastern Africa. The *Trifolium* species occur in an extensive range of habitats, including meadows and prairies, open woodlands, semi-deserts, mountains, and alpine peaks. A common feature of these diverse habitats is high solar radiation as few sub clover species tolerate shade (Ellison *et al.*, 2006).

Subterranean clover is an autumn-winter-spring annual pasture legume suitable for dry hill land areas and can be used in combination with perennial grasses (Hannaway, 2004). It originated in the Mediterranean region and parts of western Europe and Asia, but is now a widespread introduced pasture legume in Chile, Uruguay, South Africa, Australia (introduced in 1860), New Zealand (end of the 19$^{th}$ century) and the USA (1930's) (Smetham, 2003a; Nichols *et al.*, 2013).

Within the *Trifolium* genus the *Trichocephalum* clade includes the annual clover species *T. subterraneum* (Ellison *et al.*, 2006). The main characteristic of this species is the negative geotropism which enables the plant to bury its burrs into the ground. This is an adaptation strategy to promote seed germination in dry conditions and avoids the loss of seed to grazing livestock (Smetham, 2011). The word subterranean comes from the Latin: sub (below) terra (earth).

Sub clovers are found in a range of different climatic and soil conditions partly because over many generations the *T. subterraneum* species branched into three main subspecies (Ellison *et al.*, 2006). The subspecies *yanninicum* is reported to adapt to water logged soils while the subspecies *brachycalycinum* has evolved to tolerate more alkaline and dry soils (Pecetti and Piano, 2002). The *subterraneum* subspecies is the most versatile subspecies found in areas ranging from 3.7 to 27.3 °C (mean annual temperature), in elevations from sea level to 2940 m above sea level (a.s.l.), in acidic to slightly alkaline soils (see Table 4, Section 2) and in average annual precipitation ranging from <100 to 1540 mm (Ghamkhar *et al.*, 2015). In Western Australia, this knowledge was synthetized in cultivar recommendations based on the rainfall and soil characteristics. The agricultural area was divided in seven zones where rain fall ranges from 250 mm to 750 mm (Collins *et al.*, 1984). Today it is summarised in commercial websites as general guidelines for farmers to choose the most appropriate cultivar for their conditions (i.e. www.seedmark.com.au) (Seedmark, 2015). The area of annual legumes mainly sub clover, is estimated to be 22 million ha (Wolfe, 2009).

In New Zealand, dry hill and unirrigated lowland pastures have low summer-autumn rainfall and soil moisture levels are below those required for active pasture growth resulting in low (2-5%) legume content (Gillingham *et al.*, 2003). However, previous work reported that annual clovers dominate sunny sites, demonstrating adaptation to dry environment. Sub clover can represent 50% of pasture cover in early summer, for example in December months in southern areas of New Zealand with a late spring season (Power *et al.*, 2003). Annual clovers are probably most competitive in New Zealand's semi-arid environments, which are in the rain shadow of the alpine ranges, inland, above 350 m a.s.l., and have warm summers and cold winters. Annual clovers are also found in dry environments on the northern faces of hill country (Norbury, 2011) in both islands (Figure 1), and on eastern lowland shallow, stony soils from Hawke's Bay to North Otago (Boswell *et al.*, 2003).

Figure 1 and Figure 4 and have been generated from SMap (Landcare Research, 2015) based on the land (soil moisture deficits and steepness) in New Zealand, which indicates potential regions for sub clover use. Figure 2 shows general features of climate (a) temperature and (b) rainfall). Overall, in New Zealand conditions (Figure 2) are similar to the 750 mm rainfall line in Australia which includes the town of Mt. Barker (Collins *et al.*, 1984).

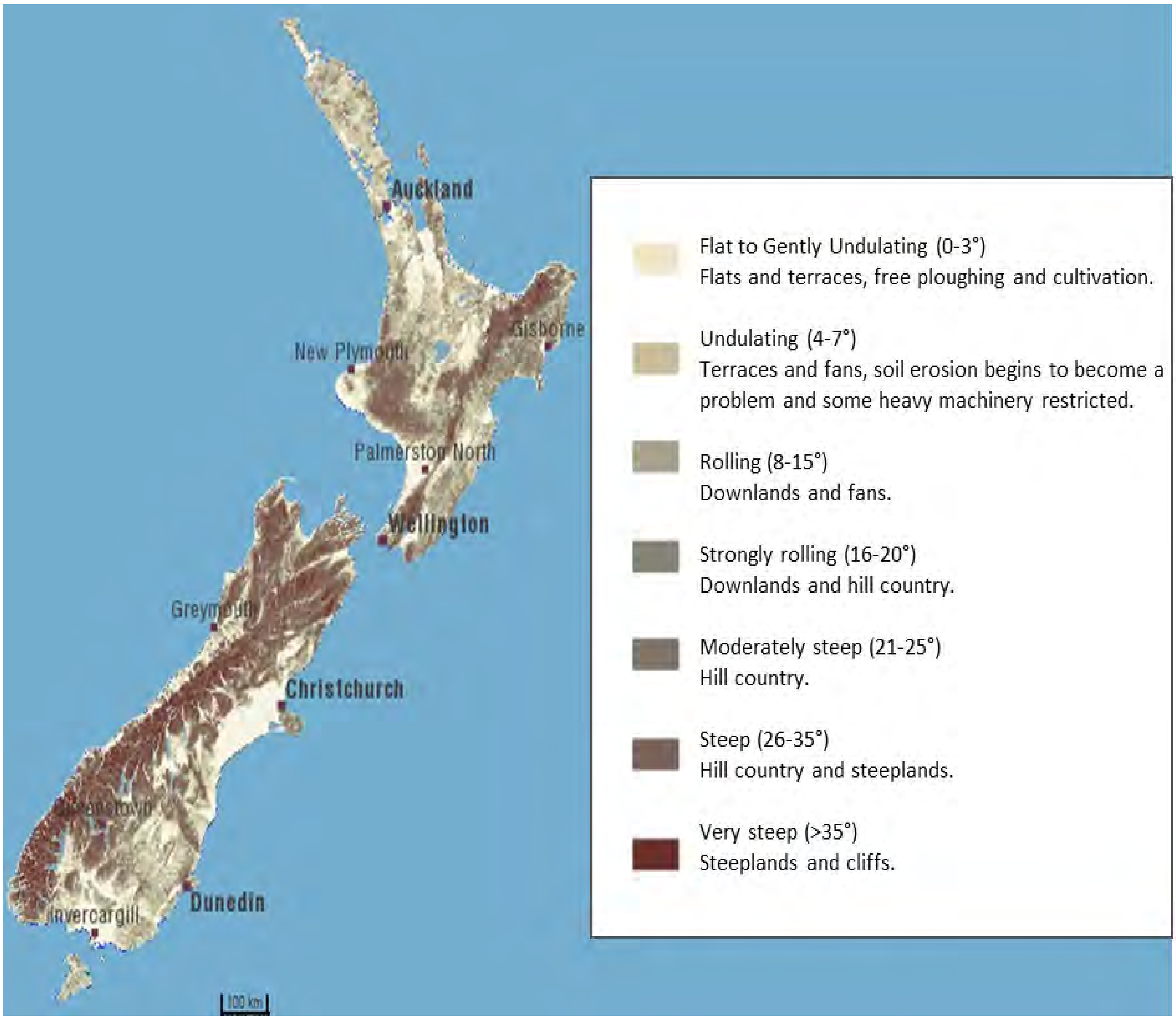



**Figure 1. New Zealand land steepness (0 - >35° slopes). Source: (http://smap.landcare research.co.nz/) (Landcare Research, 2015).**

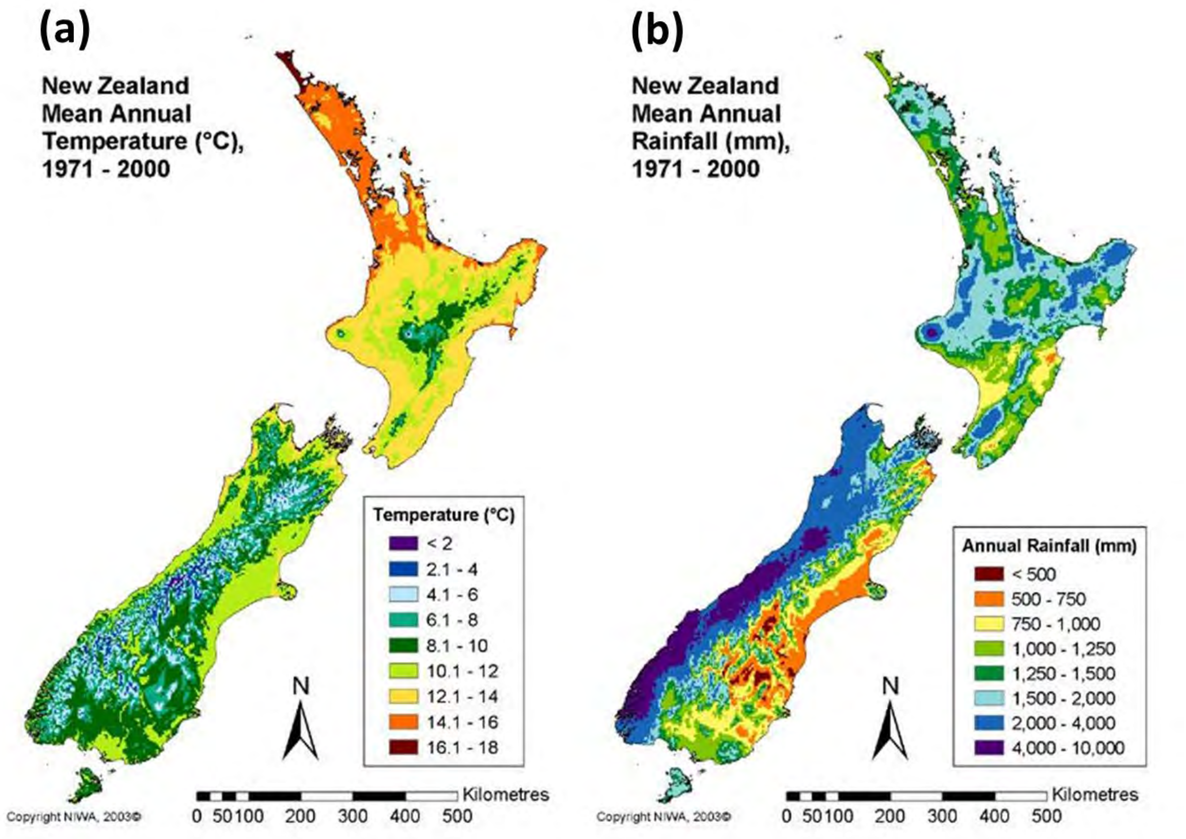



**Figure 2. New Zealand mean annual temperature (a) and mean annual rainfall (b) (1971-2000). National Institute of Water and Atmospheric Research (NIWA, 2014).**

For sub clovers the key adaptive feature is flowering and buried seed before the onset of drought Figure 3 (Francis and Gladstones, 1974; Macfarlane and Sheath, 1984). The earlier flowering cultivars (such as 'Monti') are suited to areas with low rainfall (<500 mm) while the later flowering cultivars (e.g. 'Denmark') are adapted to higher rainfall (>700 mm) (Nichols *et al.*, 2013). This is because the early flowering cultivars set seeds before soil moisture reduces drastically in early summer, for example, in November and December in the South Island of New Zealand (Widdup and Pennell, 2000). In a variable rainfall region (from late October to December) or in years with less than average spring rainfall it is a useful strategy to sow an earlier flowering cultivar which is adapted to the driest parts of lower north faces (Power *et al.*, 2003). Combinations of different annual clovers or mixtures of cultivars with different flowering times are recommended to cope with climate variability, soil (Collins *et al.*, 1984) and topographic variations but data to support there are no data to support these recommendations.

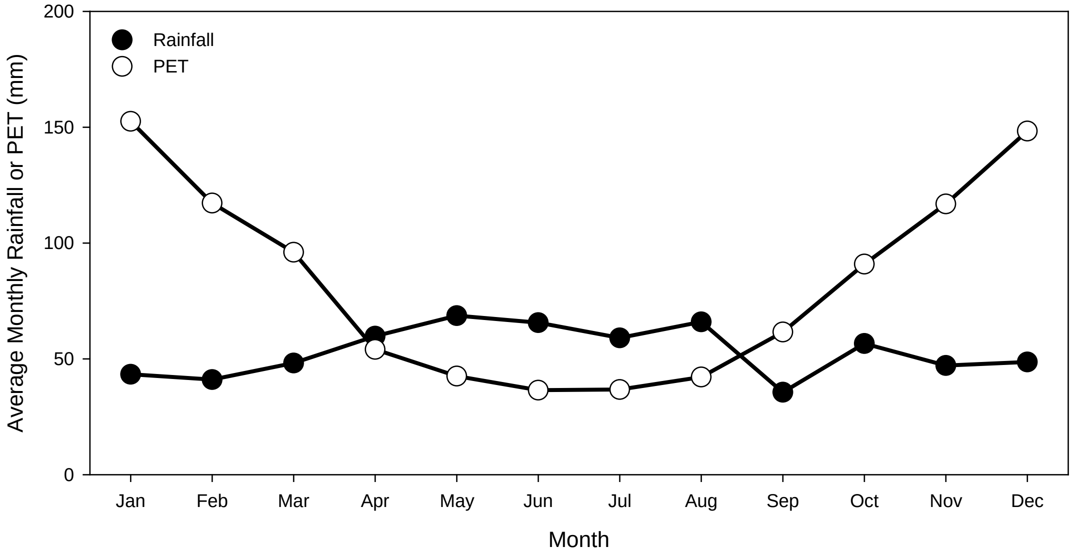



**Figure 3. Monthly Penman evapotranspiration (PET, mm) and rainfall (mm) from the Broadfields meteorological station, Canterbury, New Zealand. The long-term mean (LTM) is for the period 1997–2014 (NIWA, 2014).**

Despite its adaptability sub clover growth is limited at high altitudes (>900 m) due to insufficient thermal time for seed set and production and its frost susceptibility (Power *et al.*, 2003). Therefore, it is important to consider on farm situations (altitude, topography, soil status and moisture) for the successful establishment and growth of sub clover pastures.

The main environmental factors (temperature, rainfall, soil moisture capacity, sunshine and topography) define possible general zones and potential seasonally dry areas that may be suitable for sub clover in New Zealand. The general classification presented here is based on previous reports and pastoral experience combined with climate zones (Figure 1, Figure 2 and Figure 4).


**Figure 4. Soil moisture – profile water availability (moderate-very low) in New Zealand. Source: (http://smap.landcareresearch.co.nz/) (Landcare Research, 2015).**

1. **Dry areas (4-5 months of summer dry)**: Areas in which soil water holding capacity, rainfall distribution and evapotranspiration limit white clover growth (traditional legume) and where lucerne and sub clovers grow. To date there is limited knowledge of white and sub clover interactions in pastures (Williams *et al.*, 1990; Smetham, 2003b).
2. **Moderately dry (2-3 months summer dry)**: Areas in which white clover grows well in some years but fails when precipitation decreases and therefore is less productive. In drier seasons sub clover is more productive than white clover (Mills *et al.*, 2008).
3. **Sunny, steep hills (East Coast of both islands)**: Traditionally, white clover prevails in these areas. Rain runs off steep hills and water loss can be approximately 30%. There is the potential for lucerne and sub clover to contribute to soil regeneration and pasture productivity in these situations.
4. **Non-drought/summer irrigated areas**: White clover is dominant and droughts are rare (i.e. West Coast South Island, Taranaki).

Previous evaluations of sub clover throughout New Zealand have been reported and are summarised in Table 1 and Table 2. Table 3 shows the locations of the New Zealand experiments.

**Table 1. Summary of previous evaluations of sub clover ('Seaton Park'; 'Trikkala'; 'Howard'; 'Woogenellup' 'Clare' ;'Larisa',' Nangeela'; 'Mt Barker' ,'Tallarook') in different regions and soils of the North Island New Zealand.**

| Source | Location | Soil | Main findings |
|---|---|---|---|
| Williams *et al.* (1990) | Kaikohe; Whatawhata, Rawhiti | Wharekohe (Northern podzol); Waingaro (Yellow brown steepland); Kourarau/Wairnarama (Central yellow grey earth) | Late flowering and compact growth plants suited best. |
| MacFarlane *et al.* (1990) | Wairakei (Whatawhata - dry hill country) | Oruanui hill soil | 'Tallarook' produced highest biomass (mean 700 kg DM/ha per year) with the largest soil seed bank (1500 kg seed/ha). |
| Sheath *et al.* (1990) | Porangahau | Soil central yellow grey/ yellow brown intergrade. | No obvious advantage to 'Trikkala' or 'Larisa' (*yanninicum* subspecies) to wet soils. 'Woogenellup' and 'Clare' were the most productive in a lax cutting regime. |
| Chapman and Williams (1990) | Ballantrae | Central yellow brown earth (Makotuku hill soil) | Late or mid late flowering, dense and small-leaved sub clovers were suited to the Ballantrae region (i.e.'Tallarook', 'Larisa' and 'Mt Barker'). |

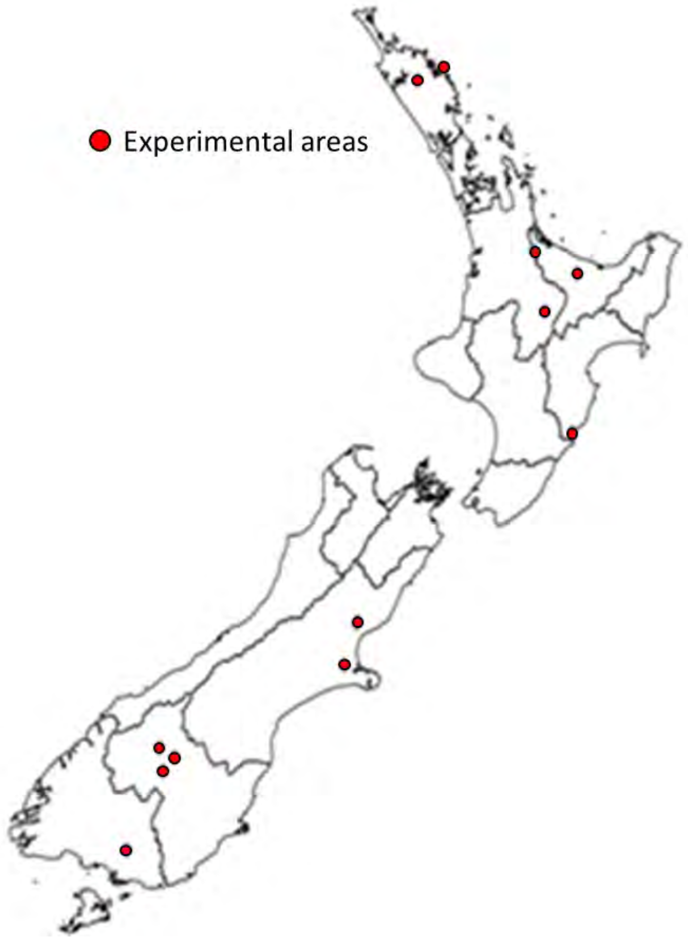
**Table 2. Summary of previous evaluations of sub clover in the South Island -New Zealand.**

| Source | Location | Cultivars | Soil | Main findings |
|---|---|---|---|---|
| Smetham (1968) | Cromwell, Tarras, Wanaka | 'Woogenellup', 'Eden Hope', 'Nangeela', 'Mt Barker', 'Bacchus Marsh', 'Wenigup', 'Portugal 294', 'Bena', 'Tallarook', 'Derrinal', 'Dwalganup', 'Geraldton', 'Yarloop', 'Barrel Medic', 'Clare', 'Kilmore', 'Rutherglen','Tallarook' x 'Wenigu', 'Burnerang' | Molyneux B.G.E. Criffel Y.B.E. Waenga B.G.E. | The most productive strains at all sites were those in the early-mid, mid and late-mid season flowering groups |
| Scott (1971) | Waikari | 'Geraldton', 'Yarloop', 'Woogenellup', 'Clare', 'Mt Barker' and 'Tallarook' | Tipapa Hill Soil | 'Woogenellup' and 'Clare' were best adapted to Waikari environment |
| Williams *et al.* (1990) | Carvossa, Hokonui | 'Seaton Park'; 'Trikkala'; 'Howard'; 'Woogenellup'; 'Clare' ;'Larisa',' Nangeela'; 'Mt Barker' ,'Tallarook' | Tipapa (Yellow grey earth); Kaihiku hill series (Yellow-grey earth) | Avoid frequent grazing at the peak of flowering. Large leaved relatively open cultivars were favoured by a lax grazing regime |
| Hoglund (1990) | Carvossa | 'Tallarook', 'Seaton Park'; 'Trikkala'; 'Howard'; 'Woogenellup'; 'Clare'; 'Larisa'; 'Nangeela'; 'Mt Barker' | Tipapa Hill | 'Woogenellup' had the best combination of reasonable degree of hardseededness degree and large seed size. 'Nangeela' had very low levels of hard seed. White clover did not survive the summer |

| | | | | |
|---|---|---|---|---|
| Smetham and Ying (1991a) | Carvossa | 'Mt Barker'';Trikkala'; 'Howard'; 'Woogenellup','Tallarook' | Tipapa (Yellow grey earth) | Low re generation and lack of seed soften due to insufficient amplitude of daily temperature variation in summer and autumn, mainly for |
| Widdup and Pennell (2000) | Templeton, (Canterbury) | 'Dalkeith'; 'Seaton Park'; 'Trikkala'; 'Gosse'; 'Junee'; 'Woogenellup'; 'Clare'; 'Mt Barker'; 'Goulburn'; 'Karridale'; 'Nangeela'; 'Denmark'; 'Larisa'; 'Leura'; 'Tallarook' and other 100 mid late Australian lines and 5 New Zealand lines | Light Eyre soil | Sardinian material appears to be adapted to the cool, dry Canterbury; the new Australian cultivars surpassed 'Mt.Barker' and ''Woogenellup'' in terms of herbage and seed production. |

**Table 3. Experimental location, coordinates (Latitude and Longitude) and characterization of previous sub clover experiments in New Zealand.**

| *General Environmental Zones** | *Location* | *Latitude* | *Longitude* |
|---|---|---|---|
| 2, 3 | Kaikohe | -35.4019 | 173.8039 |
| | Whatawhata | -37.7927 | 175.9130 |
| | Rawhiti | -35.2330 | 174.2606 |
| 1 | Waikarei | -38.6195 | 176.1038 |
| | Ballantrae | -38.0915 | 176.6906 |
| | Porangahau | -40.3019 | 176.6121 |
| | Carvossa | -42.9583 | 172.7301 |
| | Hokonui | -46.1394 | 168.5293 |
| | Waikari | -42.9694 | 172.7058 |
| | Cromwell | -45.0345 | 169.2017 |
| | Tarras | -44.8366 | 169.4128 |
| | Wanaka | -44.7032 | 169.1321 |
| | Templeton | -43.5531 | 172.4778 |




*New Zealand general environmental zones 1-4 (Section 1.1.1).

In his review Smetham (2003b) provided guidelines for cultivar choice according to specific environments. He suggested for example, the use of early materials such as Riverina (subspecies *yanninicum* as cv. 'Monti') (Nichols *et al.*, 2013) and 'Seaton Park' where soil moisture deficit occurs early in the season. However, he pointed out that there is a lack of data for the driest areas of the South Island, such as Central Otago and the Mackenzie Basin (Norbury, 2011). Data collection from those areas is required.

Recent work has been performed in North Canterbury ('Mount Benger') and Marlborough ('Bonavaree') with new sub clover cultivars such as 'Antas' and 'Monti' (Moot, 2014; Lucas 2015b) and in Marlborough (with older cultivars, 'Mt. Barker' and 'Woogenellup') (Grigg *et al.*, 2008).However, data from the modern sub clover cultivars in other New Zealand environments or soils (such as basalt or alluvial based soils) are unavailable.

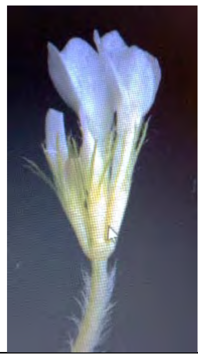
# 2 Genetic Variability

## 2.1 Subspecies

The conventional classification of sub clover divides the *T. subterraneum* species into three main subspecies which are morphologically different. Characteristics such as leaf and stem pubescence, geotropism and burr burial, inflorescence and seed (shape and colour) are traditionally the main attributes to differentiate the plants at subspecies level (Nichols *et al.*, 2013). The subspecies are also adapted to different soil conditions as described (Table 4).

To date there are eight y*anninicum* cultivars: 'Monti', Yarloop', 'Trikkala', 'Riverina',' Gosse', 'Larisa', 'Napier' and 'Meteora'. And five commercial b*rachycalycinum* cultivars ('Rosedale', 'Mintaro' 'Clare', 'Antas', 'Nuba'). More information is required on sub clover cultivar performance over a range of edaphic and climatic conditions.

**Table 4. Summary of sub clover subspecies and recommended soil characteristics. Images are examples of seeds, seedlings and flower of a cultivar of each subspecies. Adapted from: (Brennan and Bolland, 1998; Ellison *et al.*, 2006; Nichols *et al.*, 2013). Photos supplied by D. Hollander, 2015.**

| Subspecies | Soil acidity | Drainage |
|---|---|---|
| *subterraneum* (e.g.. 'Mt. Barker') | moderately acidic soils of pH ($H_2O$) 5.0–8.0 | well-drained sandy-loam to loamy clay soils |
|   |   |  |
| *yanninicum* ('Monti') | moderately acidic soils of pH ($H_2O$) 5.0–8.0 | Poorly drained, high water-holding capacity soils; sandy loam to clay soils. |
|   |   |  |
| *brachycalycinum* ('Antas') | neutral–alkaline soils of pH ($H_2O$) 7.5–9.5 | well-drained stony soils |
|   |   |  |

## 2.2 Cultivars

Most of the cultivars sown in New Zealand have been imported from Australia. Cultivars released since the late 1990's have been selected in Australia for low oestrogen content, persistence and productivity, flowering time and seed maturity, hardseededness and pest and disease resistance (Department Primary Industry NSW, 2004). A large genetic resource either collected from the Mediterranean or naturalised in Australia following European settlement, has made major contributions to breeding and selection programmes (Nichols *et al.*, 2013). 'Antas' and 'Campeda', for example, were originally bred in Italy and are now commercialised in Australia and New Zealand (Nichols *et al.*, 2013; Lucas *et al.*, 2015).

According to the time of flowering, genotypes are organized as early (<105 days), mid-season (106-130) or late (>131 days) groups in Australia (Table 5). This categorization remains to be quantified and validated in New Zealand.

**Table 5. Flowering time (based on observation in Perth, Western Australia) and sub clover cultivars grouped according to Nichols *et al.* (2013).**

| Flower time | Cultivars |
|---|---|
| Early (<105 days) | 'Nungarin', 'Izmir',' Northa', 'Dwalganup', ' Geraldton', 'Dalkeith', 'Daliak', 'Losa', 'Howard', 'Urana', 'Uniwager' |
| Mid (106-130) | 'Bindoon', 'Monti', 'Seaton Park', 'Yarloop', 'York', 'Trikkala', 'Dinninup', 'Rosedale', 'Mintaro', 'Enfield', 'Riverina', 'Esperance', 'Campeda', 'Gosse', 'Junee', 'Narrikup', 'Green Range', 'Clare', 'Woogenellup' |
| Late (>131 days) | 'Bacchus', 'Coolamon', 'Mt. Barker', 'Antas', 'Karridale', 'Larisa,' 'Napier', 'Goulburn', 'Denmark', 'Rosabrook', 'Nangeela', 'Nuba', 'Leura', 'Meteora', 'Tallarook' |

Agronomic attributes of these sub clover cultivars were summarised by Nichols *et al.* (2013).

Some of the cultivars have hairy, broad, heart-shaped trifoliate leaves with distinct marks which are often used to recognise the plants in the field (Department Primary Industry NSW, 2004; Seedmark, 2015). However, these characteristics may change over the growing season and from early to late vegetative stages. Defining these changes over a growing season is required to assist New Zealand farmers with plant identification. The New Zealand Plant Conservation Network (2015) have a general image and description database of *Trifolium* genus, but not specific information on sub clovers. So far, there is no report of visual recognition tools, i.e. Leafsnap (Belhumeur *et al.*, 2011) specifically designed for identification of forage legumes or annual clovers. Other methods to assist plant and cultivar discrimination have been studied (Clark *et al.*, 1995).

### 2.3 Cultivar adaptation in New Zealand

During the 1980's there was a revival of the sub clover research and interest in New Zealand. By that period some New Zealand pastures had naturalised populations of sub clover that were reportedly more than 50 years old. A number of these were typed and collections of ecotypes made (Sheath and Richardson, 1983; Suckling *et al.*, 1983; MacFarlane *et al.*, 1990; Dodd *et al.*, 1995b).

Characteristics of established populations collected from old pastures were determined to assess if they had developed any local differences compared with the Australian commercial lines of 'Tallarook' and 'Mt Barker'. In 1990 a series of papers was published evaluating a number of attributes of sub clover in a range of dry environments and pastures throughout New Zealand (MacFarlane *et al.*, 1990; Sheath and MacFarlane, 1990; Sheath *et al.*, 1990; Williams *et al.*, 1990) (Dodd *et al.*, 1995a) as shown in Section 1.1.1.

In the North Island Suckling *et al.* (1983) noticed variances between the plants grown in New Zealand and their original Australian line. A collection from 51 old pastures in nine regions of New Zealand revealed that 'Mt Barker' types were present in every New Zealand population sampled and constituted 74% of the total collection. Cultivars 'Tallarook' and 'Woogenellup' were 21% and 2% of the collection, respectively. A very late-flowering dwarf type with 'Mt Barker' leaf markings occurred in eight samples, and a larger-leaved, late flowering variant of 'Mt Barker' dominated two old populations from the Auckland district. The predominant forms of 'Mt Barker', 'Tallarook' and 'Woogenellup' collected in New Zealand differed little from the corresponding Australian commercial lines, although New Zealand 'Tallarook' and 'Woogenellup' were less productive, probably because of reduced seed size (with no suggestion if seed size resulted from growing conditions or genotype). 'Mt Barker' showed significant variation among populations which may reflect local adaptation. 'Tallarook' tended to dominate 'Mt Barker' after mixed sowings in a few southern North Island pastures, but there was no scientific evidence to suggest that natural selection had favoured one of the cultivars. Genetic shift in flowering time of some 'Tallarook' plants suggested that crossing with 'Mt Barker' occurred where 'Tallarook' formed a small proportion of the population (Suckling *et al.*, 1983). As a self-pollinated plant the degree of cross pollination is low (<1%) but can be possible and over many years the recombinants may have new traits (Chahal and Gosal, 2002)

As a follow up of sub clover research in the North Island Chapman *et al.* (1986) stated that sub clovers with low oestrogen, late flowering and with prostrate habit should be developed for New Zealand hill country conditions. And Chapman and Macfarlane (1985) added that there was a need to develop improved cultivars to suit best New Zealand edapho-climate conditions. To date there is no evidence that this has happened.

Investigations of Australian-bred cultivars in New Zealand summer-dry environments demonstrated most to be inappropriate for New Zealand climatic conditions and pasture environments. For instance, 'Clare' and 'Woogenellup', are early/mid-season cultivars of erect habit. They had poor persistence in grazed hill land pastures because of grazing damage during the reproductive phase. Later-flowering genotypes of prostrate habit (e.g. 'Mt Barker',' Tallarook') showed much greater reseeding and superior regeneration (Chapman *et al.*, 1986; Sheath and MacFarlane, 1990). Widdup and Pennell (2000) indicated that the late flowering 'Denmark' and 'Leura' were best adapted to moderate to hard grazing because of their small leaves and dense crowns. The authors noted that pasture management is essential to exploit the potential of newly released cultivars.

'Mt Barker' is now of limited seed availability and regarded as surpassed in Australia. Its replacement 'Karridale' is now being substituted by 'Denmark' (released in 1992) (Nichols *et al.*, 2013). 'Denmark' was developed and selected for superior autumn and winter growth rates along with improved disease resistance and adaptation to regions with a 7-8 month growing period (Dodd *et al.*, 1995b; Department Primary Industry NSW, 2004; Nichols *et al.*, 2013). This seems a sensible recommendation for many regions of New Zealand, until more local information is available.

The important factors which the previous research highlighted were late maturity, strong autumn regeneration, long growing season, prostrate habit and high buried seed set (Dear *et al.*, 1993; Dodd *et al.*, 1995b).

## 2.4 Australian cultivars adaptation in Mediterranean Europe

The European research institutions were stimulated to focus on native populations and local sub clover materials because of the unsuitability of the cultivars developed and produced in Australia (Sulas *et al.*, 2005). In his review Nichols *et al.* (2013) indicated that many of the commercial varieties developed in Australia did not perform well in Mediterranean areas mainly because of the management systems, low tolerance to frost and cold climate. These issues may also limit sub clover use in New Zealand. Identification and selection of cold and frost cultivars may be required for widespread adaptation of sub clover in New Zealand because few areas are frost free (Section 7). This suggests there is a case for testing European cultivars in New Zealand.

## 2.5 Sub clover genotypes in New Zealand

An initial identification of New Zealand local sub clover ecotypes started in the North Island with a few lineages outstanding versus the Australian genotypes and cultivars in terms of persistence and herbage yield (Dodd *et al.*, 1995b). These lineages would need to be produced on a large scale and their use in New Zealand would require the seed industry to develop production capacity with equipment and technology (Widdup and Pennell, 2000; Rattray, 2006). Practical seed production aspects involve harvest limitations and price competitiveness with Australia (Rolston, 2003).

**Box 1 Sub clover technology in New Zealand**

The question which remains is what would be a sustainable and economic scenario to promote the use of sub clover in New Zealand: to rely on the importation of seeds of selected Australian cultivars and adapt management strategies or to develop an "in house" sub clover technology suitable to New Zealand pasture systems. The importation of European material for comparative purposes would seem prudent.

# 3 Sub clover life cycle

After sowing, sub clover germinates and emerges in autumn as moisture levels increase. The seedling rosettes produce long, horizontal stems during late winter and spring, which bear 3-6 white florets in each flower head (Thomas, 2003; Department Primary Industry NSW, 2004). The plants grow mainly during early spring (September – October) then set the seed and die during late spring/early summer and remain dormant (hardseed) until next autumn (Scott, 1971; Chapman *et al.*, 1986). Regeneration in the following year is dependent on the level of hardseed and the soil temperatures but the exact conditions remain unclear.

# 4 Physiology

## 4.1 Development

Sub clover has a C3 photosynthetic pathway and responds to environmental signals such as temperature, daylength, light quality which drive the changes from the embryonic to the vegetative phase and later reproductive phase (Bäurle and Dean, 2006).

Sub clover seeds germinate changing from embryonic to post-embryonic development. The seedling progresses from the juvenile state into the adult state (vegetative phase). This progression is gradual mainly with production of foliage components: spade leaf, petiole and trifoliate leaves, branches, axillary leaves and bud, and runners (Ru and Fortune, 1999). The floral transition from the adult vegetative state to the reproductive state is a major and more abrupt change (Bäurle and Dean, 2006) when the plant develops peduncles, elongated branches (runners), flowers, and burrs (Ru and Fortune, 1999).

For pasture legumes Thomas (2003) divided the development into four phases: early seedling, mature plant form, flowering, and post-flowering. All developmental transitions are regulated by environmental signals such as available nutrients, day length, light intensity and quality, and ambient temperature as well as endogenous signals transmitted by plant hormones. Temperature is considered the main factor controlling germination and the time to floral transition in plants (Aitken, 1955b; Nori *et al.*, 2012).

### 4.1.1 Temperature - Thermal time

The duration for organ initiation and development is dependent on temperature and can be related to cumulative heat. Thermal time (Tt), also known as heat units (HU) or growing degree days (GDD), is the sum of cumulative differences between daily mean temperature and a specified base temperature ($T_b$) and it is represented as degree-days (°C d) (Moot *et al.*, 2000).

**Thermal time (Tt) = $\Sigma (T_{mean} - T_b)$**

where: $T_{mean} = (T_{max} + T_{min})/2$ and $T_b$= threshold temperature below which development stops.

Each phenological stage of a plant (germination, emergence and flowering) has a thermal time requirement which may differ among species and cultivars within a species (McMaster and Wilhelm, 1997; Lonati *et al.*, 2009).

#### *4.1.1.1 Germination - emergence*

Seed germination occurs when moisture and temperature requirements are met and it is recognized when the embryo overcomes the seed coat constraints and the radicle surpasses the diameter of the seed (Lonati *et al.*, 2009). Germination starts the quiescent dry seed imbibition and is completed by radicle protrusion through the tissues surrounding the embryo.

There are three phases of seed imbibition as described by Finch-Savage and Leubner-Metzger (2006):

**1)** Dry seeds have very low water potentials, which causes fast water influx during this phase. As this process is driven by water potential, it also occurs in dead seeds. The permeability of the seed coat, being the part of the seed that comes into contact with the ambient water, plays a central role in water uptake.

**2)** In phase 2 there is the rupture of the seed coat.

**3)** And finally in phase 3 the endosperm breaks and radicle protrusion happens.

*In vitro* experiments showed that for sub clover germination ('Dalkeith', 'Leura', 'Mt Barker') the optimum temperature ranged from 12 to 20 °C. However 'Woogenellup' required a higher temperature (Optimum 26 °C; base 1.3± 2 °C; and maximum 35 °C) to germinate (Monks *et al.*, 2009). For 'Mt Barker' 26 °C would inhibit germination as demonstrated by Lonati *et al.* (2009). The germination of 'Mt. Barker' seeds was only 54% under 24°C. These differences among cultivars may have ecological and management implications.

Once cardinal temperatures (base, optimum and maximum) and accumulated Tt requirements have been defined for a species these can be easily transferred to other sites and across seasons assisting the selection of species or cultivars for sowing (Lonati *et al.*, 2009). The thermal time sum required for sub clover germination and field emergence (defined as expansion of both cotyledons) of 'Mt Barker' was determined as 27 and 160 °C days, respectively. For some other cultivars the cardinal temperatures have been defined (approximately 0.1; 13; 27) as well as the thermal time sum for germination (range from 39 to 52 °C) (Monks *et al.*, 2009).

#### *4.1.1.2 Germination and moisture stress*

However, it is important to note that in these laboratory germination tests there is no water constraint. This is not necessarily what happens in the field, especially in dryland pasture conditions (McWilliam *et al.*, 1970). Roberts (1966) described the effects of water deficit on emergence for 'Bacchus Marsh'. McWilliam *et al.* (1970) used tests with aqueous solutions of polyethylene glycol to demonstrate the effects of moisture stress on the germination of grasses and legumes. The germination of 'Woogenellup' seeds was 90% when water was available (solution potential 0 bars, 23 °C). Under moisture stress (-8 bars) germination declined to approximately 15%. Accounting for water deficits in combination with temperature is essential to indicate sub clover suitability for many production systems and regions.

**Box 2. Hydrothermal time**

Recent studies consider the thermal time and moisture components (hydrothermal time models) to assess how seeds perform in a particular temperature and water deficit situations (Alvarado and Bradford, 2002; Larsen et al., 2004). So far, no articles have reported the hydrothermal requirements of sub clover seed germination for different cultivars.

### 4.1.2 Vegetative - Seedling stage

Following germination sub clover plants continue to use seed reserves to elongate the radicle and expand the cotyledons. The terminal shoot bud grows to produce leaves attached to the main stem which does not elongate. The meristem on the main stem develops into the first leaf (spade leaf) and afterwards into the first and sequential heart shape trifoliates described by Thomas (2003).

From the primary stem new trifoliate leaves appear driven mainly by temperature. The mean thermal time requirement for first trifoliate leaf appearance of five sub clover cultivars (Table 6) was 230 °Cd, with a phyllochron (the rate of trifoliate leaf appearance) of 68 ± 6.5 °Cd (Moot *et al.*, 2003).

**Table 6. Base temperature ($T_b$) and thermal time (Tt) requirements for the appearance of the first trifoliate leaf for five subterranean clover cultivars in controlled environment and field experiments (Moot *et al.*, 2003).**

| Cultivar | $T_b$ (°C) | Tt (°C d) | Tt (°C d; $T_b$ = 0 °C) | $R^2$ (%) |
|---|---|---|---|---|
| 'Campeda' | 1.6 | 194 | 216 | 96 |
| 'Denmark' | 1.1 | 207 | 222 | 97 |
| 'Goulburn' | -0.1 | 231 | 230 | 87 |
| 'Leura' | 1 | 235 | 251 | 94 |
| 'Woogenellup' | 0.1 | 234 | 234 | 97 |
| Mean | 0.7 | 220.2 | 230.6 | |

For other annual clovers Nori *et al.* (2012) noticed that changes in the length and direction of photoperiod at seedling emergence affected the phyllochron. When seedlings emerged following the shortest day (winter solstice) into an increasing photoperiod, the phyllochron decreased by 6 (±0.9) °Cd leaf$^{-1}$ hour$^{-1}$ for arrowleaf clover (*T. vesiculosum* Savi). Following the longest day, as photoperiod shortened to approximately 13 hours into the autumn, the phyllochron increased by 21 (±2.6) °Cd $leaf^{-1}$ $hour^{-1}$.

Once a seedling's seminal root is established, its higher region, together with the hypocotylar region of its stem, contracts, pulling the cotyledonary buds and lowermost axillary buds below the soil surface (Thomas, 2003).

### 4.1.3 Vegetative – juvenile /adult stage

The environmental cues daylength, light intensity, and ambient temperature, together with the plant hormone gibberellic acid, significantly influence the transition from juvenile vegetative (a phase in which the plant is unable to flower) to adult vegetative development (Bäurle and Dean, 2006). For sub clover plants there is no clear definition when a juvenile phase finishes and a vegetative adult commences.

The exponential leaf appearance initiates after 434 °Cd at the six total leaf stage. At this point grazing is considered "safe" without causing permanent physical damage to seedlings. Branch initiation was also observed after 437 °Cd (Moot *et al.*, 2003).

The following development of the seedling in all cases leads to the formation of an initial crown of branches growing atop a central root. The first of these grow from the cotyledonary buds and from the lowermost axillary buds on the primary stem. They have arranged leaves each of which subtends an axillary bud enclosed to various degrees by a pair of stipules formed at the leaf base (Williams and Bouma, 1970; Thomas, 2003).

Not all seedling buds develop early into elongating branches. Some remain inactive, providing a pool of buds that retain the potential to develop into branches subsequently. If not grazed, the long petioles of the sub clover leaves raise them well above ground level. In these, the primary shoot elongates very little and stops growing after producing about 10 leaves giving their seedlings an initial rosette habit (Thomas, 2003).

During the early plant stages the size of the leaves tends to increase with an increase in temperature, as demonstrated by Fukai and Silsbury (1976) in controlled environment studies with sub clover. The mean leaf size of seedlings grown at 25 °C was 60% larger (2.63 $cm^2$) than the leaf size of seedlings (~60 days after sowing) grown at 15 °C (1.5 $cm^2$). Similarly, the leaf size increases as adult plants experience warm spring temperatures in Australia. Davidson and Donald (1958) reported an increase in mean leaf size ($cm^2$) from 2.04 (August), to 9.40 in October and finally 12.7 in November. Maximum leaf size was observed at 20 °C (5 $cm^2$); above 27-30 °C observed leaf size was 4.5 $cm^2$ (Fukai and Silsbury, 1976). Few of these studies also report leaf thickness so the specific leaf weight has not been calculated.

### 4.1.4 Reproductive stage

In vegetative plants, the cells in the axil at the base of the youngest developing leaf within a growing bud develop into a new bud capable only of growing out into a leafy branch. When shoots become reproductive, the cells in the axils of some newly formed leaves within their terminal buds develop into flower buds instead (Figure 5). In all cases, the extreme tips of the terminal buds on the flowering shoots remain vegetative. The flowering shoots elongate rapidly and grow strongly to be accountable for seed production (Thomas, 2003).

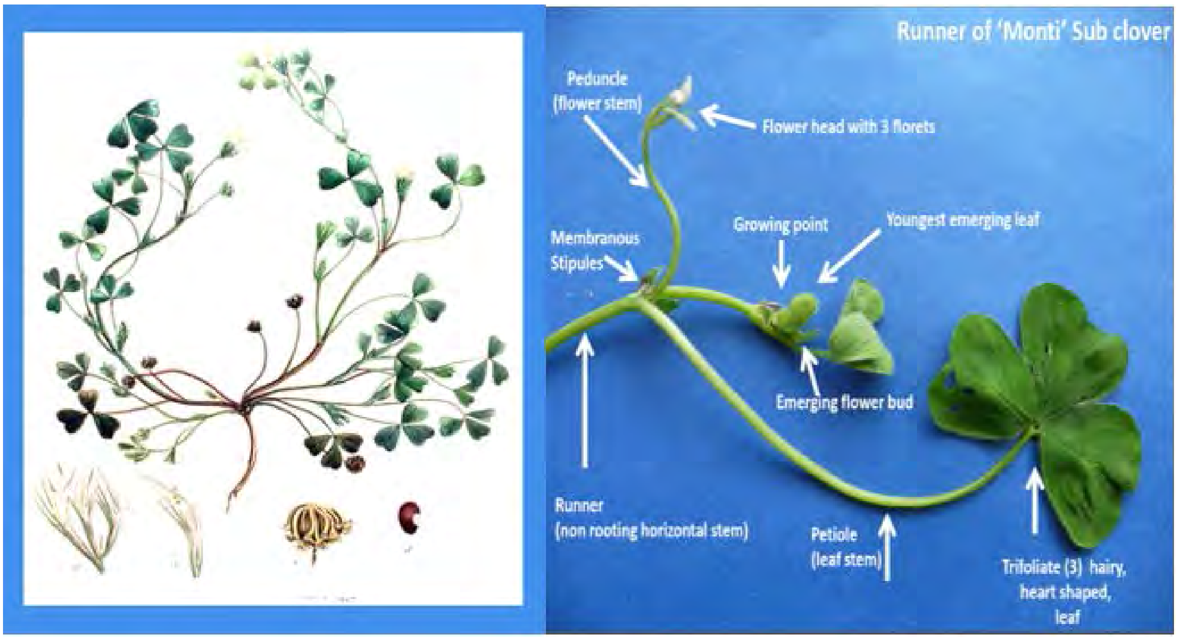



**Figure 5. Diagram of a sub clover plant at reproductive stage: flowers burs and seeds in detail (left) (Kops, 1881) and a photograph of a flower and runner of 'Monti' sub clover (right) (Lucas, 2015).**

The environmental factors which regulate the floral transition overlap considerably with those that control the juvenile-to-adult vegetative transition. Physiological experiments provided the concept of multiple pathways that promote or repress flowering, all of which quantitatively contribute to an activity that switches the shoot apical meristem from producing leaves to forming flowers after reaching a threshold level. The control of flowering in plants is not yet fully understood but for *Arabidopsis* there is evidence that flowering is controlled by gene integrators and activators (Bäurle and Dean, 2006).

Most sub clover cultivars are long day plants with time to flowering controlled mainly by temperature and photoperiod (Aitken, 1955b; Evans *et al.*, 1992). Sheath and Richardson (1983) evaluated the floral morphology and patterns of 14 sub clover cultivars in the North Island (Whatawhata region) and observed that the early flowering cultivars peaked in mid-September and produced relatively few flowers from mid-October onwards. Earlier mid-season cultivars peaked in late September-early 0ctober. Whereas the remaining mid-season cultivars (‘Clare’, ‘Mt Barker’, ‘Howard’, and ‘Larisa’) had maximum flower numbers in mid-late October. Not until the end of November did peak flowering occur in ‘Tallarook’. Sub clover is self-fertile and fertilisation occurs before florets (3-4 florets per peduncle) open (Smetham, 2011). Genetically distinct geographic variety, population or races develop because of environment impact or low degree of cross pollination (Chahal and Gosal, 2002).

The investigation of cultivar performance by Chapman and Williams (1990) showed that high stem density per unit area of pasture was an obvious factor contributing to the superiority of the very late flowering ‘Tallarook’. The high density arose partly from greater branching over a more prolonged period in early-mid spring; ‘Tallarook’ produced an average of 3.0 secondary laterals per primary lateral over the growing season while the other cultivars averaged only 1.2 secondary laterals per primary lateral stem. More branching represented more burrs, and therefore more seeds per plant, because all secondary laterals produced flowers. These factors in turn guaranteed high seedling densities the following autumn, and thus strong regeneration of populations from year to year.

Other characteristics such as high leaf appearance rates, more leaves per stem, more nodes per stem, stem and leaf morphology also contributed to high herbage production and strong annual regeneration for this particular cultivar. A high leaf appearance rate, long petioles, and high stem density all had a positive effect on late winter-early spring herbage accumulation. Based on these results for North Island hill country the authors recommended prostrate late season cultivars, which reseed in late October-November to improve spring herbage production in the Northern high rainfall areas (Chapman and Williams, 1990; Sheath *et al.*, 1990).

However, in very dry environments, such as North Canterbury where moisture deficits commonly occur from November on, late-flowering varieties are at a disadvantage (Hoglund, 1990). Although broad physiological and morphological characters such as flowering date and leaf size influence cultivar performance, it is unclear how these characters exert their influence. For example, it is probably not late flowering alone which results in superior dry matter production, but rather differences in leaf and stem growth and/or flower and burr production associated with later flowering date. The later plants flower, the longer is the growth cycle and the opportunity to exploit favourable growth conditions in spring (Scott, 1969; Widdup and Pennell, 2000). On the other hand, if the strategy is to maximise early spring biomass production for ewe lactation then earlier cultivars are desirable (Lucas, 2015). However, further investigation is necessary to confirm or re-define management-genotype strategies to optimise sub clover production. In particular, explanations of DM yield and persistence differences among cultivars must be considered within flowering date categories. Late flowering cultivars will be ill adapted if drought starts in early November in most years.

**Box 3 Genotype /Morphology and management implications**

Due to the differences among the new released sub clover cultivars it is necessary to investigate morphological features (for instance number of runners, branching, and internode and stem length). Ates *et al.* (2013) demonstrated that grazing regimes caused morphological changes of 'Campeda' and 'Leura'. Those changes had an impact in biomass, flower and seed production. In theory, 'Antas' or 'Woogenellup' with long stems and runners would have the ability to invade new areas more effectively than the small compact cultivars (e.g. 'Tallarook'). These hypotheses need to be investigated. Research to focus on how morphology variations occur with management practices, such as population density and grazing is needed.

#### 4.1.5 Seed Set

Seed setting ability and hard seed production are key attributes that enable survival and persistence of sub clover in pastures. The seed heads bend down and the burrs are pushed into the soil surface after flowering, so the seed survives the summer after the mother plant senesces and dies (Figure 6). Sub clover is named for its ability to produce and bury its seed (unlike most other annual clovers) which is an advantage for intensive systems (Scott, 1969; Francis and Gladstones, 1974).

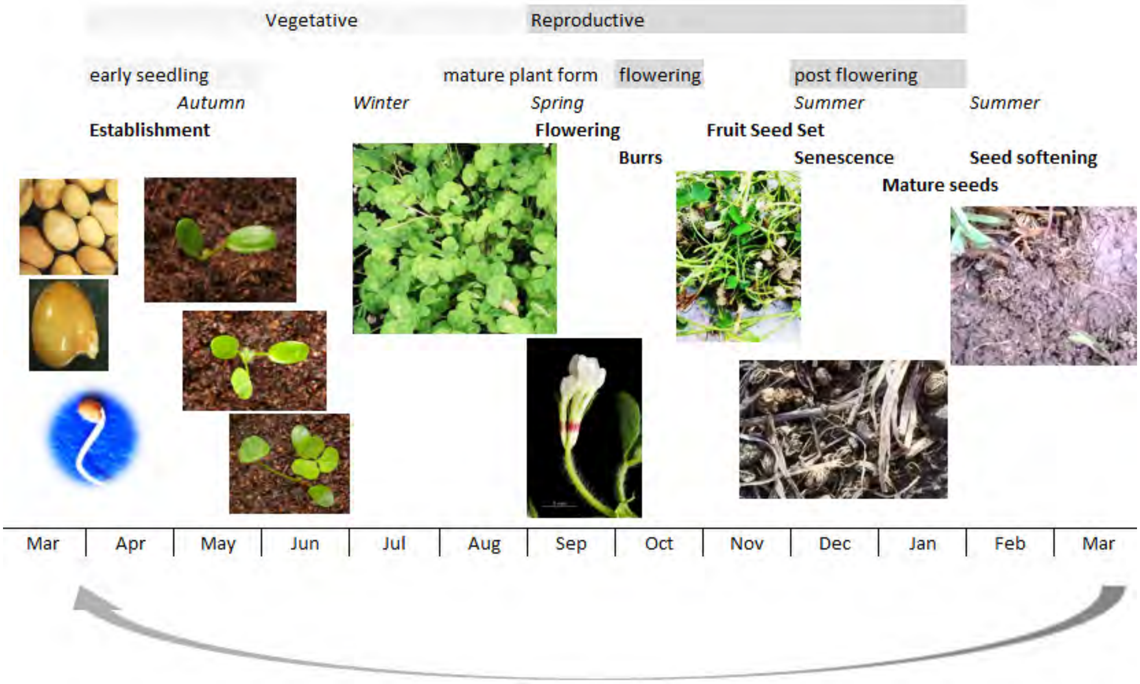



**Figure 6. Outline of the sub clover life cycle in New Zealand. Seeds and seedlings of 'Monti'. Foliage, flowers and burrs unidentified cultivar. Soil surface burrs of 'Narrikup'. Adapted from (Thomas, 2003; Department Primary Industry NSW, 2004). Photos supplied by D. Hollander and C.S.P. Teixeira, 2015.**

Power *et al.* (2003) reported the presence of five annual clover species at two higher (750-910 m.a.s.l.) sunny sites in Central Otago in New Zealand. They indicated that total mid-August-December thermal time (1440 °Cd) was sufficient for production of mature seeds. The authors also indicated that for the same August-December period in Canterbury the 2340 °Cd was ideal for cluster and sub clover.

**Box 4 Thermal time requirements for reproductive phases**

To date no other publications were found indicating thermal time for reproductive phases or seed maturity for sub clover. The previous papers only report days for flowering or seed maturity. Therefore generating this data is important.

## 4.2 Biomass (Yield)

As for other crops yields of pure swards of sub clover vary considerably with cultivar, year, soil and management practices.

Smetham and Jack (1995) measured herbage yields of 18 different lines of sub clover which were grown on a shallow stony soil at Ashley Dene, Lincoln, with a 650 mm rainfall and a cool temperate climate. They found a yield range of ~942 to 5231 kg DM/ha per year. A maximum yield of 9000 kg DM/ha was obtained by Scott (1971) in his cultivar evaluation in New Zealand, North East of Waikari on a Tipapa Hill soil (pH 5.6). He pointed out that the high yields and surprising productivity of 'Woogenellup' was due the fact that the trial was on a sunny face having warm temperatures and little frost damage during winter. In contrast, Chapman *et al.* (1986) reported that yield of 'Woogenellup' was 2123 kg DM/ha/year in Hokonui (Southland -46.115828, 168.533568).

In a mixed pasture, yields are considerably less and differ markedly with cultivar and site (Chapman *et al.*, 1986; Chapman and Williams, 1990).The absolute contribution of sub clover to the yield with a strong perennial grass component ranged from 1300 kg DM/ha on intensively managed pastures to 150-700 kg DM/ha on steep high rainfall North Island hill country (Sheath and Boom, 1985; Smetham and Jack, 1995). Ates (2009) reported mean daily growth rates of subterranean clover in spring of 39-45 kg DM/ha/d. The results suggested that pure sward yields of 4000-5000 kg DM/ha equivalent to total yields of mixed pasture, can be obtained. Yields of 7000 kg DM/ha were observed in experiments at Lincoln (March-September period) (Moot *et al.*, 2003). It is possible that higher yields could be obtained with the new genotypes available, understanding of the plant growth and appropriate management practices. Lucas (2015b) suggested that 'Woogenellup' yields could be as high as 14000 kg/ha and upper limits to productivity of sub clover need to be established. But this hypothesis need to be validated. During a 9 year experiment (Maxclover experiment, Lincoln) among four clovers the sub clover showed the highest yields (>3700 kg DM/ha/year) with cocksfoot and out-yielded white and Caucasian clovers (*Trifolium ambiguum* L.) when summer rainfall was below 650 mm (Mills *et al*., 2008). This demonstrated the potential for sub clover use in New Zealand dryland production systems.

## 4.3 Nutritive value

As with other legumes sub clover has high nutritive value which promotes intake and animal production, mainly early in spring. Ates (2009) observed that the increased clover content (%) in spring increased live weight gain of lambs. Digestibility of sub clover can be as high as lucerne (62 – 80%) (Freer and Jones, 1984; Ru and Fortune, 2000) and with crude protein levels ranging from 27 to 12% in spring (Norman et al., 2005).

## 4.4 Seed production

Sub clover has an annual growth cycle with main winter-spring growth, its ability to set seeds and form a seed bank explains the success of this legume in parts of Australia (Nichols *et al.*, 2009) and New Zealand (Sheath and MacFarlane, 1990).

Cultivars show large differences in seed-setting ability, more particularly under moisture stress conditions (Smetham, 1968). As demonstrated by Ates *et al.* (2013) higher rainfall than normal (in the spring and summer of 2006 (>335 mm) enabled subterranean clover plants to produce a greater number of seeds even at high stocking rates.

In 1974, a study performed in Perth indicated mean seed yields of 491 to 1341 kg/ha (Francis and Gladstones, 1974). The seed yield components for sub clover are calculated as:

***Mean seed weight (mg) x seed numbers per burr x number of burrs per plant x number of plants per unit area.***

In Australia seed yields can be as high as 2000 kg/ha however 500-1000 kg/ha is more typical (McGuire, 1985). In large commercial seed producing areas (e.g. Australia and USA) the harvest requires a specialized vacuum harvester (Hannaway, 2004; Nichols and Dear, 2007). For experimental purposes harvest can be performed by hand removing burrs and seeds on the soil surface and those buried (approximately 1 cm below the soil surface).

In pastures with cocksfoot and ryegrass sub clover seed yields were 713 and 721 kg/ha, respectively. There are few comparable studies of seed set and yields in New Zealand and particularly, of the cultivars released in the last 10 years (Widdup and Pennell, 2000).

### 4.4.1 Past seed production and distribution in New Zealand

Since its initial introduction into New Zealand at the end of the 19th in the Auckland district there was a period of slow acceptance by farmers. Sub clover was then sown on the dry plains of Hawkes Bay, Wairarapa, Marlborough, Canterbury, and in the Manawatu sand country. Initially, the seeds were imported from Australia. Local production began in Marlborough in the late 1920's and seed was first certified in New Zealand in 1938. The first commercial seed originated from the 'Mt Barker' region (South-West Australia), but other types differing in maturity, growth habit, oestrogen content, seed production, and leaf markings have since been collected from various parts of Australia with the most productive registered as cultivars. Up to the end of the 1930's all the seed sown in New Zealand would have been early commercial forms of 'Mt Barker', but 'Tallarook' was also frequently sown, usually in equal mixture (Suckling *et al.*, 1983; Smetham, 2003a). More recently 'Woogenellup' has been oversown into some existing populations (e.g. at 'Tempello' in Malborough) (Grigg *et al.*, 2008). Strain trials indicated that some other cultivars also show promise, but none of these has been widely sown (Suckling *et al.*, 1983; Dodd *et al.*, 1995a) (Widdup and Pennell, 2000) (Rattray, 2006).

Sub clover seeds are imported from Australia and some may have a limited use in New Zealand (Lucas *et al.*, 2015). Seed is not always available for all cultivars (Rolston, 2003; Rattray, 2006). To date, there are approximately five sub clover seed distributors in New Zealand. The commercial cultivars (imported Australian seeds) currently available in New Zealand are: 'Denmark' 'Antas', 'Leura', 'Monti', 'Rosabrook','Woogenellup', 'Narrikup', 'Rosabrook', 'Campeda', 'Leura' and 'Seaton Park'.

# 5 Agronomic Management

### 5.1.1 Sowing period

Temperature and water are key factors for successful pasture establishment. Provided soil moisture is available sub clover will emerge (Roberts, 1966) and establish in early autumn

(Late February/early March in New Zealand) more successfully than late autumn in summer dry regions (Hampton *et al.*, 1999).

Sub clover seeds are approximately 10 times larger than white clover seeds, with a thermal time of requirement range of 41-62 °Cd for germination (Moot *et al.*, 2000). As temperatures decrease towards winter plant germination, emergence and establishment slows down. Field experiments have demonstrated that seeds sown in late March resulted in heavier seedlings that experienced higher temperatures and greater light interception which increased growth by 20% higher than later sown seeds (Ru *et al.*, 1997; Moot *et al.*, 2003).

As demonstrated in Section 4.1.1 thermal time requirements differ according to cultivar (Monks *et al.*, 2009). For 'Mt Barker' sub clover the optimum germination temperature was around 20 °C. At 25 °C germination was reduced to 50% whilst at higher temperatures (30 °C) germination was ~10% (Silsbury *et al.*, 1984). The temperature specificity is one of the plant strategies to prevent summer "false strike" (defined as seedling death caused by out of season rainfall followed by dry conditions) (Dodd *et al.*, 1995a).

Emergence in the field is the result of appropriate temperatures and water availability. According to Roberts (1966) the highest emergence of sub clover seeds was achieved when a water content of 10% by weight (water potential of -0.97 MPa) was maintained for at least 4 days. Very little emergence occurred when the water content was maintained at 2% by weight (water potential 11.7 MPa). This highlights the importance of understanding the hydrothermal requirements of a particular cultivar (Section 4.1.1).

### 5.1.2 Sowing rates

Sowing rates must be adjusted according to seed weights. For sub clover seed weights range from 4.3-12.4 mg (Boswell *et al.*, 2003) depending on the cultivar (Nichols *et al.*, 2013) and the conditions in which seeds were produced (Collins, 1978). For example, the mean seed weight for 'Mt Barker' reported by Nichols *et al.* (2013) was 8.3 mg whereas Lonati *et al.* (2009) worked with a much lighter 'Mt Barker' seed lot of 5.9 mg per seed. A seed sowing rate range of 9 -25 kg/ha has been previously recommended in the literature. In a pure sward, 1000 seedlings per $m^2$ are the minimum recommended to maximise herbage production (Smetham, 2003b). Experiments at Lincoln have used 10 kg seed/ha (Brown *et al.*, 2006) or 2000 viable seeds/$m^2$ based on germination test results and seed weight (Moot *et al.*, 2003).

### 5.1.3 Plant population

A high plant population is the key to ensure maximum herbage production. In New Zealand previous work correlated seedling number in autumn to herbage production in spring: populations above 1700/$m^2$ resulted in the highest herbage production (4000–5000 kg/ha) (Smetham, 2003b). Ates *et al.* (2013) reported strong relationships between established seedling numbers/$m^2$ and total DM and subterranean clover DM production in autumn ($R^2$ =79.5) but a weak relationship of seedling population and DM in the following spring ($R^2$ range 31-81). When mixed with grasses ideally 2000 seedlings/$m^2$ are required (Smetham, 2003a).

### 5.1.4 Sowing method

Where possible, direct drilling is suggested as the most successful sowing method as the sub clover seeds are adapted to germinate from seed burrs buried in the top soil (Section 4.1.4). Direct drilling is efficient in establishing an initial high seedling population and can also be used to increase legume content in grass dominant swards (Ates *et al.*, 2013). Black (1955) stated that the best seed ('Bacchus Marsh') sowing depth was 13 mm in a range of temperatures 7 to 28 $^{o}$C. The drilled seeds should be placed deeper than 13 mm to promote contact to soil and moisture transferal (Hannaway, 2004). Milne and Fraser (1990) reported that most pasture failed to establish because of deep (>23 mm) drilling even though sub clover has a relatively large seed compared with other pastures species (Lucas, 2015). Black (1956) observed that seedling emergence was progressively delayed as depth of sowing increased.

However, where land is steep (26–35° slopes) Newsome *et al.* (2008) and access is difficult on hill country, over sowing by air is the predominant method for establishment of legumes into permanent pastures (Hampton *et al.*, 1999).

Sub clover has been oversown in New Zealand at 2-4 kg/ha on hill country, and included in seed mixtures at a similar rate when drilled into cultivated land (Smetham, 2003b). However in this system merely 5-20% of oversown seed produces an established plant as it is difficult to control seed placement and seedling competition with resident vegetation for water, nutrients and light (Dowling *et al.*, 1971; Hampton *et al.*, 1999; Jennings and Simon, 2009). Moorhead *et al.* (1994) observed that broadcasting Caucasian clover seed was 80% less effective (only 9% of seedlings established) than strip drill (48% of seedlings established). To overcome that sowing rates up to 25 kg/ha increase the chances of seed survival and rapid sub clover cover (Smetham, 2003b) however, this practice adds an extra establishment cost.

Previous work to determine the best establishment methods with white clover are summarised below and are relevant for sub clover.

A) **North Island**: Hume and Lyons (1993) tested seven different drilling possibilities on dry cultivable land. They reported higher emergence of white clover (78%) in a cultivated seed bed compared with those direct drilled (58%). They focussed on grass pastures rather than the legume component and concluded that in a dryland climate early autumn sowing is required for successful establishment and treatments that conserve moisture are essential. Summer fallow followed by spraying or cultivation was the best techniques to establish new pastures. However they pointed out some disadvantages: the costs of cultivation, higher erosion potential, the poor control of rhizomatous weeds and no summer grazing.

   Milne *et al.* (1993) observed that clover densities were highest when sown by cultivation-drilled (~ 280 plants/m$^2$), followed by over sowing (170 plants/m$^2$) and direct drilling (140 plants/m$^2$). The authors do not mention the seed rates used and attribute the poor clover densities from direct drilling due to the seeding depth (26 mm), compared with oversowing of 5 mm.

B) **South Island**: Milne and Fraser (1990) compared three methods on workable dryland sowing (full cultivation, minimum tillage and direct drilling). Their survey revealed that the key factor for rapid dryland pasture establishment was the preparation of a weed free, moist and warm seed bed which resulted in a rapid establishment of dryland pastures.

From this previous work it is clear that the best establishment method is the cultivated drill, however it is more expensive and not feasible in hilly dryland areas. In North Canterbury Costello and Costello (2003) reported that direct drilling was appropriate to establish sub clover in their flat paddocks farm system.

**Box 5 Sowing methods**

So far, no comprehensive sowing method evaluation has been reported specifically for sub clovers. The programmes evaluating best establishment practices in New Zealand combine a mixture of species mostly for the white or red clover pastures and to some extent balansa and Caucasian clover. Progress with “on farm” use of sub clovers requires the development of “best practices” for broadcast seeds on steeper hill areas. In addition, in New Zealand (mainly in the North Island) farmers tend to include sub clover seeds in spring sown seed mixes (R.J. Lucas, Pers. Comm., 03 September 2015). To date there is no published information about the influence of spring sowing on sub clover on flowering patterns, yield or seed production (more details in Section 6.1.1).

### 5.1.5 Chemical seed treatment

For new pasture establishment a chemical seed treatment with fungicides enhances seedling emergence. Diseases will affect the establishment and growth of sub clover. In Australia the most common problems are root rots (*Phytophthora* sp., *Fusarium* sp., *Rhizoctonia* sp., *Pythium* sp., *Aphanomyces* sp.) (Section 5.3). These pathogens are soil borne in New Zealand and infect white and red clover (Skipp and Christensen, 1983; Skipp *et al.*, 1986) and potentially sub clover. But this requires confirmation.

Where a susceptible sub clover cultivar is grown in an area prone to root rot, seed treatment with metalaxyl (commercial Apron XL) (Syngenta, 2013) or Tetramethylthiuram disulfide (commercial name Thiram) is known to improve seedling survival and weight by approximately 50% (Barbetti, 1984; Edmisten *et al.*, 1988).

In previous experiments Brockwell *et al.* (1980) also observed that establishment of clover seedlings was improved using fungicides but the authors pointed out that chemicals did have an adverse effect on *Rhizobium* inoculum and further nodulation. Thiram was slightly toxic whereas Captan (N-trichloromethylmercapto-4-cyclohexene 1,2-dicarboximide) was moderately toxic to the inoculum. Field and glasshouse experiments show that metalaxyl seed dressing had no detrimental effect on *Rhizobium* viability or nodulation in lucerne seedlings (Edmisten *et al.*, 1988).

To prevent diseases such as root rot the recommended commercial application rate of Apron (metalaxyl) is 40 mL/ 100 kg of forage legume seeds (lucerne, clover, trefoil, vetch) (Syngenta, 2013). There is no specification for treatment of sub clover seeds.

### 5.1.6 *Rhizobium* (inoculation and Nitrogen fixation)

One of the reasons to introduce sub clover in a pasture system is its ability to fix atmospheric nitrogen (N) through the symbioses with the soil bacterium (*Rhizobium trifolii)*. In the symbiotic process the plant provides the energy and nutrients for the microorganisms, and in turn benefits from the atmospheric N fixed by the bacteria (Brock *et al.*, 1989). The amount of N fixed by the *Rhizobium*-legume symbiosis can be as high as 140 kg N/ha/year (Ledgard and Steele, 1992) or approximately 30 kg of N per tonne of clover biomass produced (Høgh-Jensen *et al.*, 2004; Lucas *et al.*, 2010). To date it is a recommended practice to introduce the sub clover specific strain group to ensure successful N fixation, especially for new sown areas (Department Primary Industry NSW, 2004).

The optimization of biological nitrogen fixation depends on the host plant, the bacterium and a favourable environment for both. Nutman (1967) reported the interaction between legume varieties and *Rhizobium* strains and confirmed that nodulation differs among plant lines. For instance, in pot experiments 'Campeda', 'Losa' and 'Denmark' showed a high symbiotic performance with the *Rhizobium* strain WSM1325 tested, whereas 'Clare', 'Dalkeith', 'Tallarook' and 'Gosse' had poor or low symbiosis (Drew and Ballard, 2010).

In Australia the decline in sub clover populations has been associated with a decrease in nodulation and soil acidity. The rhizobia does survive in acidic soils but liming provides a more favourable environment for their survival and growth, particularly during and following hot and dry summer months (Slattery and Coventry, 1993). In previous field experiments Evans *et al.* (1988) noticed a significant increase in rhizobium population and the number of nodules in sub clover roots when pH was increased from 4.7 to 6.9 (pH $H_2O$). They also observed that *Rhizobium* population was reduced after the dry summer months.

In New Zealand dry soil-environments, the quantification of $N_2$ fixation by annual clovers has been infrequent (Boswell *et al.*, 2003). The main studies have focussed on nodulation on white and red clovers (Lowther and Kerr, 2011). More recent field studies with sub clover demonstrated that annually 80 kg N/ha/year was fixed, which represented 70% more than N fixed by white clover. The main reason for the higher sub clover N was its greater growth due to its ability to exploit moisture available early in spring (Lucas *et al.*, 2010). The results of this work show the potential for substantial economic benefits of biological nitrogen fixation by annual legumes in dryland pasture systems.

#### 5.1.7 Other establishment challenges

Other factors affecting legume establishment include predation of the seed/seedlings by insects and birds. Slugs for example can reduce seedling populations with a 46% reduction reported by Charlton (1978) in field and controlled experiments performed in New Zealand with 'Woogenellup'. Campbell and Swain (1973) suggested the aerial application of a molluscicide to minimise seedling losses.

Birds can also cause substantial crop damage (Smetham *et al.*, 1994). Bird damage has also been observed at Lincoln University experimental area (C.S.P Teixeira, Pers. Comm., 02 July 2015). In the North Island geese might eat oversown seeds (Rayburn and Greenb, 2014). To date there has been no research to quantify annual clover stand losses caused by birds nor alternatives to solve the issue.

**Box 6 Bird and insect damage**

There is no current ongoing work quantifying sub clover seeds or seedling losses due to bird damage and insect predation.

## 5.2 Weed control

Before the introduction of a new sub clover cultivar it is recommended to remove unwanted species and wherever possible ungerminated weed seeds as well. Ideally, the cultivation of annual crops and the use of herbicides over three to five years would decrease the proportion of weeds and unwanted clovers. This is vital in seed producing areas to reduce the presence of unwanted species or resident cultivars (Department Primary Industry NSW, 2004).

Because sub clover is a poor competitor and sensitive to shading, effective weed control is essential for successful establishment (Dear and England, 1987). Also, high sowing rates of grass species supress sub clover establishment (Lucas, 2015). See also Section 5.1.2.

Ploughing and heavy grazing limit the chances of weeds competing with sub clover however, such methods may only partially reduce weed species competition and dominance in pasture areas (Edwards *et al.*, 2005).

Chemical weed control methods for new sub clover areas include:

- Formulation of 150 g/L paraquat and 25 g/L diquat at 2 L/ha (oversowing method for 'Tallarook' and 'Mt Barker') (Webby *et al.*, 1990).
- A mixture of glyphosate (6 L/ha) and 2,4-D (3 L/ha) reported by Sheath *et al.* (1990). Chapman and Williams (1990) used paraquat (4 L/ha a.i.) and 2,4-D (2 L/ha a.i.) to suppress unwanted plants. Prior to establishment glyphosate 360 (100 mL/10 L) was applied twice before sowing (with 2 weeks interval) (Murray, 2012).

Another technique used in commercial test areas in Australia is the application of Roundup (glyphosate) in combination with Hammer (400 g/L carfentrazone-ethyl; 463 g/L liquid Hydrocarbon) and post emergent products such as Tigrex, Liase, Verdict 520 or Select applied with Hasten (10 weeks after sowing) (Conbly and Hardwidge, 2009). According to product label, Hammer herbicide kills sub clovers (HerbiGuide, 2014) but there is no information on residual effects on subsequent sown seeds or seedlings of sub clover nor information on whether these techniques controlled the resident species.

In established sub clover pastures chemical weed control must consider the target weeds (grasses or broadleaves), the sensitivity and residual effects on sub clovers. In a pure sub clover sward area in Australia, Francis and Gladstones (1974) used propyzamide (Kerb, 1.5 L/ha) to control grasses, and bromoxynil (1 L/ha) to control capeweed (*Arctotheca calendula*) and Patterson's curse (*Echium plantagineum*), sprayed during winter. The following herbicides were used to control barley grass in irrigated subterranean clover areas in Australia (Squires, 1963), with the main conclusions:

A) Sub clovers tested had very low tolerance to sodium 2,2 DPA (grass killer). For 'Tallarook' application of 2,2 DPA at 2.24 kg/ha controlled barley grass and caused slight damage to sub clover. Visual assessments ranged from 5 (no damage) to 2 (yellowing and leaf distortion). Rates of 4.48 kg/ha caused distortion and yellowing of sub clover leaves and 80% reduction in DM production. For 'Bacchus Marsh' 8 months after application of 2,2 DPA there were negative effects on seedling population: seedling stand was reduced by ~5% at application rates of 2.24 kg/ha when compared with untreated plants. However, at 4.48 kg 2,2DPA/ha, the seedling population was 70% lower than unsprayed vegetation.

B) Diquat dibromide tested as early post emergence in winter herbicide (2.2 kg/ha) in 'Yarloop'. The herbicide successfully eliminated barley grass (100%) and tripled spring (November) DM sub clover when compared with unsprayed plots.

C) Paraquat was also tested in 'Yarloop', with rates ranging from 0.28 to 0.56 a.i. kg/ha. These resulted in an average 44% increase in sub clover DM in comparison with unsprayed crops (DM 3520 kg/ha) and achieved a grass control of 70%. Paraquat applied at 1.12 kg/ha gave 97% control of barley grass but reduced sub clover biomass.

Experiments with 'Woogenellup' and 'Nungarian' showed that low rates of Paraquat (110 g/ha) and glyphosate (126 g/ha) could control grasses by approximately 50-80% and increased sub clover biomass production (Dear and England, 1987). On the other hand, Scammell and Ronnfeldt (1998) found that late winter herbicide applications produced the greatest increase in legume composition (59 - 81%) although this coincided with a 10% reduction in legume biomass compared to the 3 weeks earlier applications. Early in the season low herbicide rates of Paraquat and Sprayseed® (1.5 L commercial product/ha) tended to be safer on legumes and achieve a desired level of grass control.

In Australia bromoxynil and MCPA were reported to have negative effects on sub clover plants and this was cultivar dependent. 'Karridale' was very sensitive to MCPA while 'Dalkeith' was more sensitive to 2,4-DB. Cultivar maturity is important because the early maturing cultivars have a short period to recover between herbicide application and flowering and seed filling (Department Primary Industry NSW, 2004). Transgenetic herbicide (bromoxynil 1.5 L/ha) tolerant lines from Dear *et al.* (2003) were tested against the non- transgenic 'Gosse'. The herbage yield of two transgenic lines was unaffected by the herbicide while there was a reduction of 50% of 'Gosse' biomass yield. However, the authors reported that the insertion of *bnx* gene reduced the seed yield and hardseededness of one sub clover line. The controversy around the use of transgenic lines limit widespread adoption of such materials, particularly in New Zealand.

Products registered for broadleaf and grass weed control for mixed species pastures containing clovers in New Zealand are listed in Table 7.

Table 7. Summary of registered products which are recommended to control broadleaves and grass weeds in pastures with white clover and to some extent sub clover in New Zealand.

| Product | Active ingredient | Main weed target | Rate (product) | Additional information | Stage of Application |
|---|---|---|---|---|---|
| Head Start | 50 g/L flumetsulam | Chickweed, amaranthus, dandelion, thistle, willow weed | 0.5-1 L/ha | Do not apply in wet cold conditions. | After 6$^{th}$ trifoliate and before stolon elongation. |
| Preside | 800 g/kg flumetsulam | Perennial Buttercups | 30-65 g/ha | For clover seed crops and sub clover. | After 6$^{th}$ trifoliate and before stolon elongation. |
| Maestro | 750 g/L MCPA | Californian thistle, buttercups | 1.5-3 L/ha | High rates (not defined) may suppress sub clover. | After grazing to reduce clover foliage. |
| Pasture Guard | 680 g/L 2,4-D | Vetch, Thistles, wild mustard, annual buttercups, fireweed | 0.75-2 L/ha | It may cause damage to clovers, particularly subterranean. Use the lower recommended rate. | Graze area prior to spraying to reduce clover leaf and to expose target weeds. |
| MCPB | 385 g/L MCPB | Annual buttercups, thistles, redroot, hedge mustard, black nightshade | 4-6 L/ha | No specific recommendations for sub clover. | One to two trifoliate leaves and >50% ground cover. |
| Select | 375 g/L MCPB and 25 g/LMCPA | Californian thistle | 3-4 L/ha | No information available for sub clover. | Clovers have >3 trifoliate leaves and weeds are at seedling stage. |
| TMax | 30 g/L aminopyrilad | Fathen, willow weed, black nightshade, amaranthus | 1-2 L/ha | Designed originally for forage brassica crops. It has residual effects on clover seeds/seedlings. After application is recommended to wait 6-12 months to sow clover. No specific information about sub clover. | Best result achieved when plants are actively growing and prior flowering. |
| Gallant Ultra | 520 g/L haloxyfop-P | Perennial Ryegrass, Poa, Cocksfoot, Prairie grass | 0.25-1.5 L/ha | Selective control of grass weeds in white clover. No mention about sub clover. Not compatible with atrazine herbicides. | Use high rates if weeds are in advanced growth stage. |
| Fusilade Forte | 212 g/L fluazifop | Grasses: wild oats, goose grass,ryegrass, *cynodom* and *paspalum* | 0.82 L/ha | 21 day withholding period; No information about sub clover. | Apply before grass tillering stage. |

Source: (Novachem Manual, 2006)

**Box 7 Weed control**

Published data about new sub clover cultivars (e.g. 'Rosabrook', 'Monti', 'Antas', 'Mintaro') and their sensitivity to herbicides is limited. The information available mainly through the commercial (seed/chemical) companies is vague and too general (for instance: "high rates may prevent sub clover …"; "sub clover is sensitive to some herbicides until established, good pre sowing weed control is crucial. …"; "Search the advice of your local agronomist for satisfactory results". Detailed knowledge of chemical effects on sub clover new materials needs to be addressed and quantified.

## 5.3 Pests and diseases

### 5.3.1 Fungi

Fungi can cause foliage and root diseases on sub clover. Table 8 summarises the main important fungal diseases in Australia. Due to its importance root rot is described in more detailed.

**Table 8. Main fungal diseases in sub clover and related literature. Image sources: Root Rot , Clover Scorch, Rust, Leaf spot, Cercospora leaf spot, Pepper spot (Department Primary Industry NSW, 2004). Powdery mildew (Tsukiboshi, 2001).**

| Disease | Pathogens | Visual Symptoms |
|---|---|---|
| Root Rot  | *Phytophthora* sp., *Fusarium* sp., *Rhizoctonia* sp., *Pythium* sp., *Aphanomyces* sp. | Red purple stunted plants and brown roots with water soaked appearance (Barbetti and MacNish, 1978; Wong *et al.*, 1986). |
| Clover Scorch  | *Kabatiella caulivora* | Twisted leaves and petioles; plant stand collapse (Bayliss *et al.*, 2002). |
| Rust  | *Uromyces trifolii* | Reddish-brown pustules on leaflets and petioles (Barbetti and Nichols, 1991). |

| | | |
|---|---|---|
| Leaf spot  | *Pseudopeziza trifolii* | Brown-black spots on leaves, petioles and stems (Barbetti, 1985b; Nichols *et al.*, 2014). |
| Cercospora leaf spot  | *Cercospora zebrina* | Leaf and petiole salmon pink or brown lesions and defoliation (Barbetti, 1985a). |
| Pepper spot  | *Leptosphaerulina trifolii* | Small brown pin spots on leaf surface (Department Primary Industry NSW, 2004; Roux, 2012). |
| Powdery mildew Redacted | *Erysiphe polygonii* | Whitish powdery growth on leaflets and premature foliage senescence (Department Primary Industry NSW, 2004; Nichols *et al.*, 2014). |

#### *5.3.1.1 Root rot*

In Australia root rot caused by the complex of *Phytium irregulare*, *P. acanthicum* and *Fusarium oxysporum* represents a major challenge to maintain legume content in pastures. The disease decreases emergence, seedling biomass and root growth (Barbetti and MacNish, 1978; O'Rourke *et al.*, 2012). The symptoms of root rot in sub clover can be characterized by the red purple stunted plants during the two and three leaf stage of growth and tap and lateral roots which have a water soaked appearance and vary from light to dark brown depending on the disease severity (Wong *et al.*, 1986).

Fungal combinations containing *P. irregulare* generally caused the severest root rot at 10 and 15 °C, with less disease severity at 25 °C (Wong *et al.*, 1984). Studies conducted in different years at different sites within the Western Australian wheat belt showed that DNA concentrations of *P. clandestina* and *R. solani* were higher in autumn than in spring (O'Rourke *et al.*, 2009). In New Zealand no evidence of similar work has been performed or published.

The combination of poor soil fertility, nutritional deficiency and root rot incidence was established by O'Rourke *et al.* (2012). They observed, in controlled environment experiments, that nutrient amelioration, particularly application of Hoaglands nutrient solution, K (88.7 mg $kg^{-1}$ of soil) and N (33 mg $kg^{-1}$ of soil) decreased root rot severity by approximately 35% and improved plant growth. Barbetti (1984) also found a negative correlation between plant size and weight and severity of root rot.

##### *5.3.1.1.1 Cultivar susceptibility*

Sub clover cultivars have different tolerance/susceptibility to root rot. For susceptible cultivars (i.e. 'Nungarin' and 'Northam') it was observed that plants did not set seeds and seed maturity was compromised (Dear *et al.*, 1993). In contrast, cultivars 'Trikkala' and 'Riverina' are considered tolerant to root rot (Department Primary Industry NSW, 2004).

Table 9 shows a selection of 14 commercial released cultivars and one New Zealand subterranean clover lineage and their reported susceptibility to root rot pathogens.

**Table 9. Pathogen resistance rating for selected sub clover cultivars (6 = highly resistant, 5=Resistant, 4=moderately resistant, 3=moderately susceptible, 2=susceptible, highly susceptible). Root rot pathogens are: *Phytophthora clandestina* (races 000, 001, 173, 177), *Pythium irregulare*, *Rhizoctonia solani*, *Fusarium avenaceum.*"-"= data not available. Adapted from: (Nichols *et al.*, 2014)**

| Cultivar | Phytophthora 000 | 001 | 173 | 177 | Pythium | Rhizoctonia | Fusarium |
|---|---|---|---|---|---|---|---|
| 'Monti' | - | - | - | 6 | - | - | - |
| 'Seaton Park' | 5 | 5 | 4 | 4 | 1 | 2 | 2 |
| 'Trikkala' | 6 | 5 | 3 | 3 | 2 | 3 | 5 |
| 'Campeda' | - | - | 1 | 1 | - | - | - |
| 'Narrikup' | - | - | 3 | 3 | - | - | - |
| 'Woogenellup' | 1 | 3 | 1 | 1 | 2 | 2 | 3 |
| 'Coolamon' | - | 5 | 2 | 4 | 1 | - | 2 |
| 'Mt Barker' | 3 | - | 1 | 1 | 4 | 1 | 5 |
| 'Antas' | - | - | 3 | 3 | - | - | 3 |
| Karridale | 5 | - | 1 | 1 | 5 | 2 | 5 |
| 'Napier' | - | 5 | 5 | - | 3 | - | 3 |
| 'Denmark' | 6 | 5 | 5 | 6 | 3 | 1 | 3 |
| 'Rosabrook' | - | - | 5 | 6 | - | - | - |
| 'Leura' | 6 | 5 | 1 | 1 | 3 | 4 | 4 |
| 'Whatawhata' selection | - | - | - | - | - | - | - |

Strategies to minimise root rot include management of soil fertility (amendments with fertiliser, K, N and lime) (O'Rourke *et al.*, 2012), seed treatment at establishment (Barbetti, 1984) and the use of resistant or tolerant cultivars (Nichols *et al.*, 2014).

### 5.3.2 Viruses

According to Jones (2012) in Australian pastures the most important virus diseases are Bean common mosaic virus (BCMV) and subterranean clover mottle virus (SCMoV). Potential important diseases are, alfalfa mosaic virus (AMV), beet western yellows (BWYV), cucumber mosaic virus (CMV). Subterranean clover red leaf virus SCRLV and subterranean clover stunt virus (SCSV). Viruses affecting legume pastures have been long detected in New Zealand (Fry, 1959; Wilson and Close, 1973).

#### *5.3.2.1 Subterranean clover red leaf virus (SCRLV)*

The disease is transmitted by the common potato aphid, *Acyrthosiphon solani,* also present in New Zealand (Teulon and Stufkens, 2002). This aphid takes in the virus while feeding on infected white clover during the summer and autumn, before moving onto subterranean clover (Department Primary Industry NSW, 2004)

The incidence of this virus varies from year to year, as the distribution depends on the number of infective aphids entering a stand each season, particularly in seed crops and pure swards. There is no evidence of seed or mechanical transmission of this virus (Jones, 2012).

Sub clover pastures are likely to be at risk from SCRLV when grown in close proximity to perennial pastures of lucerne, red clover or white clover (Ashby *et al.*, 1982; Johnstone and McLean, 1987).

**Symptoms**: The characteristic intense red symptoms develop progressively from the leaflet margins (Kellock, 1971; Johnstone and McLean, 1987). The disease is favoured between 20-25 $^{o}$C and inhibited below 15 $^{o}$C or above 33 $^{o}$C (Helms *et al.*, 1985; Helms *et al.*, 1987 ). SCRLV symptoms can be confused with those of lower leaf reddening from nutrient deficiencies or other stressors (Jones, 2012).

Glasshouse experiments have shown that SCRLV reduced herbage fresh weight of infected 'Mt Barker' plants by >90% (Helms *et al.*, 1985).

In Tasmania, establishment failures occurred sporadically when >90% of emerging seedlings became infected with SCRLV. Extensive infection in spring caused subterranean clover pastures to fail before they could be cut for hay or silage (Kellock, 1971). The disease has been detected in Canterbury mainly occurring in pea, lucerne and clovers (Ashby *et al.*, 1982). SCRLV losses have not been quantified yet in grazed sub clover swards (Jones, 2012).

#### 5.3.2.2 *Cucumber mosaic virus*

The disease occurs as a result of sowing infected sub clover seeds with transmission rates around 9% (Jones, 1991). The spread of this disease is faster in pure clover pastures, and is lessened by the presence of grasses (Department Primary Industry NSW, 2004). In Australia the virus decreased biomass yield by approximately 50%, depending on the cultivar (Jones and McKirdy, 1990). 'Enfield', 'Green Range', 'Nangeela', and 'Yarloop' are all very susceptible cultivars, while 'Dwalganup', 'Larissa', and 'Uniwager' are considered less prone to infection (Jones and McKirdy, 1990; Nichols *et al.*, 2014).

The typical symptom is the leaflets curling downwards (Jones, 2012). The virus is also present in New Zealand legume pastures (Guy, 2014).

#### 5.3.2.3 *Subterranean clover stunt virus (SCSV)*

SCSV is transmitted by four species of aphids: *Aphis craccivora* (the most efficient vector), *Aphis gossypii, Myzus persicae*, *Macrosiphum euphorbiae*, which are present in New Zealand (Teulon *et al.*, 2010). The SCSV virus affects a range of legume crops such as crimson clover (*Trifolium incarnatum*), suckling clover (*Trifolium dubium*), button clover (*Medicago orbicularis*), medics (*Medicago* spp.), broad bean (*Vicia faba*), French bean (*Phaseolus vulgaris*) and peas (*Pisum sativum*) (Grylls and Butler, 1959; Smith, 1966; Teulon and Stufkens, 2002).

The disease occurs in early autumn, when temperatures around 22 $^{o}$C are ideal for sub clover and aphid growth (Gutierrez *et al.*, 1974). There is no evidence this virus is seed-borne (Chu and Vetten, 2003).

**Symptoms**: severe distortion and stunting of the plants and yellowing of new foliage growth. The youngest leaves in the centre of the plant may be small and cup-shaped, while the older leaves turn red (Chu and Vetten, 2003). 'Mt Barker' is very susceptible to SCVS (Chu and Vetten, 2003) while 'Howard' was bred in Australia for SCVS resistance (Foster *et al.*, 2013).

Glasshouse experiments showed a reduction of 20-30% on growth of 'Mt Barker' sub clover diseased plants (Johnstone and McLean, 1987).

Practices to control and minimise sub clover virus diseases suggested in (Jones, 2012) include:

- Avoid new virus introductions by sowing new pastures with uninfected seeds;
- As much as possible avoid sowing new pastures next to old, infected pastures;
- Increase sub clover plant density on pastures  reduce aphid landing rates;
- Sow cultivars that are early maturing, less susceptible to viruses ;
- Reduce grazing pressure to allow swards to grow in spring and healthy plants to overcome diseased ones;
- Spray the pasture with insecticides to kill aphid vectors in late winter/early spring. For instance, Rogor™ ( *a.i.* dimethoate) at 80 mL/10L ((Singh *et al.*, 2004) or bio-insecticide *Bacillus thuringiensis* (Wu and Guo, 2003).

## 5.4 Soil fertility

### 5.4.1 pH

Soil fertility is a determinant in sub clover production (Section 2). The soil pH is an important factor in growth of sub clovers (Ghamkhar *et al.*, 2015). In natural environments the maximum diversity of sub clover was reported in the pH $H_2O$ range 6.9–7.4. Ghamkhar *et al.* (2015) noticed however that there was no sub clover diversity in soils with pH ($H_2O$) <5.7, mainly in loam and clay loam soils. Soil pH also affects the plant-rhizobium symbioses (Section 5.1.6).

### 5.4.2 Phosphorus

Despite being considered a "robust" plant, sub clover is known for the high fertility demand, especially phosphorus (P) and sulphur and micronutrient molybdenum (McGuire, 1985; Frame, 2005). Phosphorus is necessary to promote rhizobium nodulation (Robson *et al.*, 1981). Visual P deficiency symptoms are the reddening petioles and small leaves (Paynter,

1990) and deficient plants are much susceptible to diseases (O'Rourke *et al.*, 2012). Bolland (1985) found a positive correlation between phosphate application, biomass yield and P herbage content and reported maximum herbage yields when 90-100 kg P/ha was applied. Moir *et al.* (2012) demonstrated in a glasshouse experiment that 97% of maximum yield of sub clover was observed at P application rates of 336 mg P/kg soil and 45 equivalent Olsen P (mg/L).

Phosphorus is one of the most important macronutrients limiting growth of annual legumes, for example in highly weathered Australian soils (Paynter, 1990). Management of phosphate fertilizers has become a research priority (Peoples and Craswell, 1992). One of the strategies to optimize phosphorus in farming systems is the use of legume varieties or cultivars which tolerate low P soil status and /or acquire and use P more efficiently (Vance *et al.*, 2003; Moir *et al.*, 2012). For white clover for instance, Caradus (1994) identified genotypes adapted to low-P soils and emphasized that it was possible to improve plant edaphic stress tolerance and persistence in the field.

**Box 8 Sub clover genotypes and *rhizobium***

There is limited information about sub clover genotypes (and its interactions with *rhizobium* strains) adapted to low P soil conditions and low pH and aluminium status which are important in high country soils.

## 5.5 Hardseededness

Hardseededness is an innate mechanism of seed dormancy that controls germination within and between years. The hard seeds have a seed coat which is impermeable and prevents germination even when moisture and temperature conditions are ideal for germination. Ecologically it enables the persistence of legumes under a broad range of management and environmental conditions through the development of soil seed banks (Taylor 2005).

The level of seed coat imposed dormancy (seed hardness) differs among cultivars (genetic makeup) and plants of the same cultivar (determined by environment). To date 'Urana' and 'Izmir' are potentially the "most" hardseeded (Score 10) whereas 'Woogenellup' for example is ranked as "less" hardseeded (Score 1) (Nichols *et al.*, 2013).

Environmental factors such as temperature and humidity during seed set may affect the level of seed hardness. For example, seeds produced under higher temperatures (>27 °C) were less permeable (>90% hardseededness) than those produced under lower temperatures (<21 °C; ~60% hardseededness) (Argel and Humphreys, 1983). However, it was observed that when seed moisture content was below 7% all seeds had a high percentage of hardseededness (Argel and Paton, 1999). Moisture during seed maturation also affects hardseededness, which is reportedly accentuated with earlier flowering lines (Smetham, 2003a). In his work Smith (1988) observed that hard seed levels averaged 80, 70 and 47% in years in which rainfall during seed maturation was 109, 174, 327 mm (the drier the condition, the higher the proportion of hardseed produced). In contrast, previous researchers proposed that the longer plants of subterranean clover were kept growing by watering, the higher the proportion of hard seeds (Quinlivan and Millington, 1962). The more favourable the environment (in terms of moisture availability) and the longer the maturation period, the thicker would be the suberin layer of the seed (Section 5.5.4) and consequently the higher the initial hard seed content (Quinlivan, 1965).

Hardseededness is required by a population of sub clover initially after seed maturation. This prevents premature germination with summer rains that are followed by dry conditions that cause seedling losses ("false strike") (MacFarlane *et al.*, 1990; Dodd *et al.*, 1995b).

Hardseededness declines with time depending on genotype and the diurnal amplitude of temperature fluctuation experienced by burrs and seeds (Smetham and Ying, 1991b). For instance, in Australia it was observed that 98% of 'Woogenellup' seeds were hard at seed maturity. After four months in the field (day temperatures of 40–50 °C and a diurnal variation of >15 °C) the proportion of hardseeds declined to 34%. However, for 'Mt Barker' initially 59% were hard and seed hardness declined to 11% after 4 months (Quinlivan and Millington, 1962). This natural seed softening over time (Taylor *et al.*, 1984; Revell *et al.*, 1998) was demonstrated by Smetham and Ying (1991b) who harvested in a hill site near Waikairi, North

Canterbury. They tested germination and seed softening in controlled environment conditions (15 °C alternating with 35 °C every 12 h). They observed that after 40 days 80% of 'Woogenellup' seeds had softened whereas 60% 'Howard' seeds had softened.

Seed softening in early autumn that results in a false break is undesirable for regenerating pasture. The dilemma is that seedling death from a false strike will reduce pasture yield but seedlings from seeds which soften later (in late autumn) are placed at a competitive disadvantage with resident species (Taylor 2005).

In New Zealand, for a particular sub clover, hardseededness tends to decline more gradually when compared with the warmer Australian climate because of the lower maximum temperatures and a low diurnal variation. Smetham and Ying (1991b) observed that in autumn approximately 76% of 'Woogenellup' seeds remained hard after four months field exposure and consequently the germination was low (approximately 23%). Essentially high temperatures cause physical changes (expansion and contraction of the seed) and chemical alterations (i.e. in seed coat lipids) which permit germination (Smith, 1988; Taylor 2005; Zeng *et al.*, 2005). More details about this process are presented in Section 5.5.6.

For temperate areas (such as Tasmania and New Zealand) cultivars which soften a high proportion of seeds by the following autumn would be ideal (Smetham and Ying, 1991b; Smetham, 2003a).

**Box 9 Seed hardness/softening**

Comprehensive information of the seed hardness/softening process of new released sub clover cultivars under low amplitude of daily temperature variation in NZ summer and autumn conditions is limited.

### 5.5.1 Testing for Hardseededness in sub clover

Technically the hard seed content of any sample is considered to be the proportion of seeds remaining impermeable to deionised water after 14 days in a moist substrate at 15 °C (paper or blotter). The standard testing technique requires four replicates of 100 seeds and is prescribed by the International Seed Testing Association for germination tests on seeds of sub clover (Quinlivan, 1965; International Seed Testing Association, 2003).

To assess the initial hardseededness, seeds need to be removed from inflorescences (burrs). In a small scale research test this can be done by rubbing the burrs gently between corrugated rubber mats (Norman *et al.*, 2002). In large scale commercial seed producing areas in Australia seeds are cleaned and threshed with machinery and this process scarifies the seed (Nichols and Dear, 2007).

### 5.5.2 Assessment of seed softening (seed hardness decline)

Seed softening can be assessed in a controlled environment or in natural field conditions. A standard procedure to compare seed softening of seed lots consists of placing burr samples in an oven tray with daily temperature fluctuation which simulates dry summer field conditions (Taylor 2005). The rate of sub clover seed softening under a temperature regime of 15/60 °C was well correlated with observed seed softening in Australian (Perth) fields (Quinlivan and Millington, 1962). Variations of this method (i.e. 15/35 °C) have been used to simulate seed softening in cooler temperate regions (Smetham and Ying, 1991b). The rate of hardseededness and seed softening may be an issue for the cooler New Zealand conditions

Seed softening in natural field conditions is measured by placing burrs into strips of fine mesh cotton bags. The bags are laid on the soil surface or buried to experience mainly the natural summer temperatures (Norman *et al.*, 2002). In both cases (controlled cabinets or bags in the field) the proportion of soft (germinated) and hard seeds is monitored over time.

### 5.5.3 The seed coat and seedhardness

In legume seeds, the outermost layer of the seed coat is called the palisade layer and consists of malpighian cells covered by the cuticle (Smýkal *et al.*, 2014). The cuticle covers the whole seed, except for the hilum, and is considered the critical barrier to imbibition (Shao *et al.*, 2007). The degree of seed coat impermeability increases with the thickness of the seed coast and its cutin and suberin content (Argel and Paton, 1999).

### 5.5.4 Suberin

For a long time suberin has been proposed to be the main biochemical component of hardseedness in sub clover seeds (Quinlivan, 1965). Suberin is an extracellular tissue sealing polymer that is deposited together with suberin-associated waxes at distinct locations during plant growth that is species-specific (Kolattukudy, 1981; Nawrath, 2002). The water barrier properties of suberin are attributed to the hydrophobic nature of the lypids deposited in the cell wall (Franke *et al.*, 2012).

The synthesis of suberin increases when plants undergo stress conditions. For example this may create a barrier against water and solute loss and protect against opportunistic pathogen invasion (Bayliss *et al.*, 2002; Nawrath, 2002; Franke *et al.*, 2012).

To date suberin is chemically described as a biopolyester mainly comprised of ω-hydroxy acids (around 11-43%) and α,ω-dicarboxylic acids (which range from 24 to 45%) and lower amounts of fatty acids (1-10%) and alcohols. Glycerol and minor amounts of aromatic phenyl propanoids are also part of the suberin polyester within a range of 14-26% and 0-10%, respectively (Franke *et al.*, 2012).

### 5.5.5 Pigments: anthocyanin

There are also suggestions that the purple anthocyanin in the seed coat of many cultivars may act as an inhibitor of seed germination, and it could be assumed that this would be removed by any leaching process (Smetham, 2011). Pigments and seed colour were correlated with seed hardness of large seeded legumes (Smýkal *et al.*, 2014) but it was not observed in sub clover seeds (Slattery *et al.*, 1982).

### 5.5.6 The seed softening phase

In natural conditions seed softening (or hardseededness break down) happens in two phases. The first phase of softening requires a minimum thermal input. The second requires temperature fluctuations (Taylor 2005). Different requirements for the first and second stage of seed softening determine when seeds soften within a year and what proportion of hard seeds remain hard in a seedbank (Taylor *et al.*, 1984; Norman *et al.*, 2002).

Taylor (2005) further report stages of permeability during seed softening:

- Impermeable preconditioning (when seed is still impermeable)
- Slowly permeable at some point on the testa (that might be other than the lens, such as the micropyle or hylum).
- And finally, rapidly permeable at the lens.

### 5.5.7 Suberin and seed softening

So far there is limited data correlating suberin content and legume hardseededness/softening. And almost certainly none for sub clover seeds. Possibly the reasons are the complexity of suberin chemical constitution, restricted knowledge, methodological limits (Kolattukudy, 1981; Nawrath, 2002) and the relatively low status of sub clover compared with other legumes such as soybean or peas. Slattery *et al.* (1982) found no correlation between permeability and phenol content of 'Bacchus Marsh' seeds (important to note that phenols are only one of the many components of suberin). On the other hand, Zeng *et al.* (2005) demonstrated a positive relationship with seed coat lipid content and sub clover hardseededness of 'Dalkeith'.

**Box 10 Seed coat dormancy**

In recent reviews about seed coat and suberin Franke *et al.* (2012) and Smýkal *et al.* (2014) address the genetic aspect controlling seed coat imposed dormancy of *Aradapdosis* and important legumes (soybean, peas, beans, *Medicago* sp.) with emphasis in pigments and suberin biosynthesis. It is unclear if similar mechanisms are applied for sub clover (different sub clover subspecies or even cultivars) and how important hardseededness is in New Zealand conditions. Work on the genetics of seed coat dormancy relations with the different field growing conditions and management practices (GxExM) is required.

# 6 Time to flowering

### 6.1.1 Days to flower

Flowering time is relevant information for cultivar choice (fit for the environment) and in terms of management (grazing strategies and reseeding) (Section 1).

Table 5 showed how the main commercial sub clover cultivars are organized according to days to flower (from the sowing date) based on Australian observations (Nichols *et al.*, 2013). For a cultivar the days to flower at a given location is relatively constant between seasons, when sown at a similar date provided plants are not under extreme stress conditions (Evans *et al.*, 1992; Nichols *et al.*, 2013). However, significant genotype site, genotype latitude, and genotype sowing date interactions occur (Aitken, 1955b) (Evans *et al.*, 1992). At warmer and lower latitudes, early-flowering genotypes flower earlier, while later flowering plants flower later (Nichols *et al.*, 2013) as observed in many previous studies in Australia and New Zealand (Table 10).

**Table 10. A summary of main Australian and New Zealand published work reporting sub clover days to flower - minimum and maximum observed days to flower for a particular location.**

| **Location: Australia** | Author | Sowing month | Min Days to Flower | Max Days to flower |
|---|---|---|---|---|
| Condobolin (NSW) | (Young *et al.*, 1994) | March | 111 | 154 |
| | | April | 99 | 140 |
| | | May | 106 | 118 |
| | | June | 84 | 92 |
| | | July | 77 | 84 |
| Adelaide | (Cocks and Phillips, 1979) | March | 87 | 213 |
| Katanning | (Evans *et al.*, 1992) | April | 87 | 174 |
| | | May | 100 | 158 |
| | | June | 74 | 134 |
| | | August | 68 | 101 |
| | | September | 60 | 113 |
| | | October | 51 | 92 |
| Launceston | (Evans *et al.*, 1992) | May | 129 | 164 |
| | | June | 107 | 146 |
| | | July | 92 | 125 |
| | | August | 75 | 107 |
| | | September | 61 | 105 |
| | | October | 54 | 118 |
| | | November | 44 | 100 |
| Melbourne | (Aitken, 1955a) | April | 162 | 209 |
| | (Collins and Aitken, 1970) | July | 113 | 113 |
| | | August | 100 | 100 |
| Shenton Park | (Collins, 1981) | May | 109 | 111 |
| WaggaWagga | (Dear *et al.*, 1993) | March | 131 | 208 |
| | | May | 112 | 172 |
| | (Smetham and Dear, 2003a) | June | 105 | 165 |
| **New Zealand** | | | | |
| Ballantrae | (Chapman and Williams, 1990) | April | 132 | 175 |
| Carvossa | (Hoglund, 1990) | May | 130 | 160 |
| Cromwell | (Smetham, 1968) | February | 225 | 271 |
| Christchurch | (Smetham *et al.*, 1994) | May | 129 | 176 |
| Templeton | (Widdup and Pennell, 2000) | May | 133 | 176 |
| Whatawhata | (Dodd *et al.*, 1995b) | May | 114 | 214 |
| | (Sheath and Richardson, 1983) | May | 131 | 207 |

Days to flowering is an indicator of cultivar maturity but is not transferable to different locations or sowing dates as shown in Figure 7 and Table 10. A more accurate and transferable parameter is the thermal sum required to achieve a certain phenological stage (Evans, 1959; Monks *et al.*, 2010; Nori *et al.*, 2014) for example the reproductive stage (Section 4). It is clear that overall for a similar sowing period (e.g. May) the plants flower on average 25-40 days later in New Zealand than they do in Australia as observed by Smetham (1968) and Sheath and Richardson (1983).

### 6.1.2 Thermal time and Photoperiod

The effects of temperature and photoperiod in sub clover flowering time are well documented in Australia and to a less extent in New Zealand. A re-analysis of previous experiments performed by Smetham (1968) in Central Otago, New Zealand (latitude -45.035) revealed a coherent relationship between thermal time and maturity of the cultivars. Those classified as early ('Yarloop' and 'Geraldton') required 1684 $^{o}$Cd while the late 'Tallarook' flowered after 2181 $^{o}$Cd. Those considered intermediate ('Clare', 'Woogenellup', 'Bacchus Marsh', 'Mt Barker') required approximately 1810-1880 $^{o}$Cd to flower. However, it is clear that in Australia the various sub clover genotypes have been studied across different sowing times (March-November) whereas in New Zealand sub clovers were sown and studied mainly in autumn (February-May).

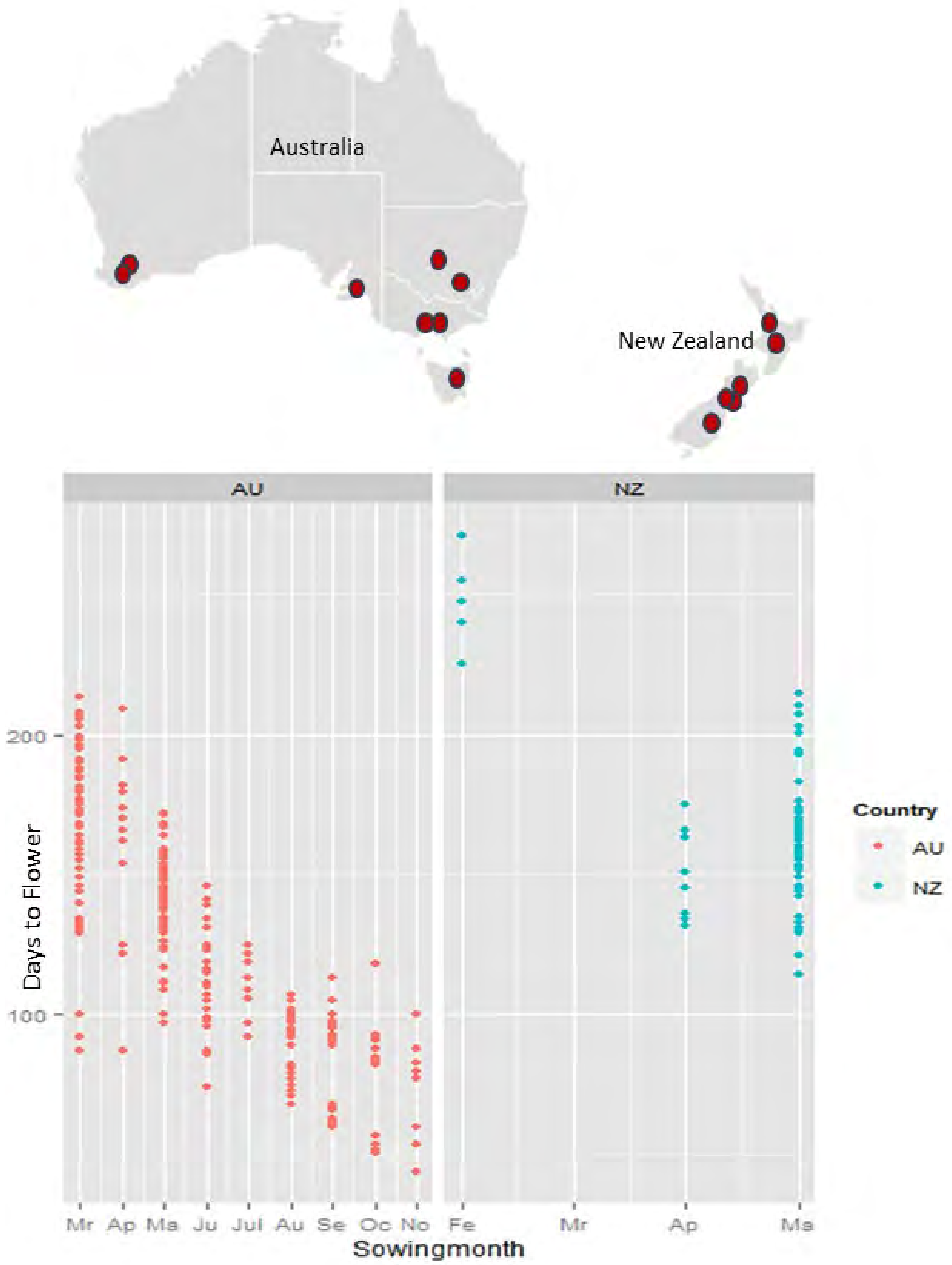



**Figure 7. Days to flower according to sowing month observed in Australia (AU) and New Zealand (NZ). Triangles on map indicate sub clover experimental locations which consider flowering times. Graph shows the relationship between sowing month and days to flower observed in Australia and New Zealand from published data (Table 10).**

Evans *et al.* (1992) correlated thermal time and photoperiod (average photoperiod from sowing to flower) of several cultivars in two locations. They developed a simplified mathematical model (linear equations) and stated that:

"*Differential genotypic sensitivity to mean temperature and photoperiod can explain most of the variation in flowering behaviour of subterranean clover observed in the field. Satisfactory models of flowering behaviour of individual genotypes can be constructed by use of a simple linear model to link flowering times from a range of sowing dates in one or two locations, or from several geographically dispersed locations, with relevant meteorological information*".

Photothermal regime regulates flowering behaviour of different cultivars. More recently Iannucci *et al.* (2008) and Nori *et al.* (2014) quantified relationships describing time to flowering as a function of temperature and photoperiods for different annual legume species. However, Evans *et al.* (1992) and Iannucci *et al.* (2008) used the average photoperiod to develop their photothermal indices whereas Nori *et al.* (2014) pointed out that photoperiod at the time of the first trifoliate leaf appearance was more accurate (inductive phase) for selected annual clovers. The reason may be related to when plant cells detect a functional circadian system and how input photoreceptors enable them to perceive the length of light and dark periods (Millar, 2004; Bäurle and Dean, 2006).

**Box 11 Photothermal requirements**

Even though there is a substantial amount of information about flowering in sub clover cultivars no recent studies have investigated the relationships between thermal requirements and inductive photoperiod in sub clovers. Particularly in New Zealand, previous studies only focus in autumn sowing (February-May). In addition, no further attempts to re-analyse and translate "days to flower" into photothermal time response of studied cultivars. No information about thermal time or days to reach seed maturity for newly released cultivars or quntification of time from floewring to physiological maturity.

# 7 Cold sensitivity and tolerance

Autumn-winter legumes tend to grow during the cool and wet season because growth is limited by the dry summer months (Francis and Gladstones, 1974). Sub clover is an annual legume which produces a considerable amount of biomass very early in spring (Mills *et al.*, 2008). The plants need to grow at low temperatures to extend the growing season (Chapman and Macfarlane, 1985).

When plants are exposed to low winter temperatures a series of biochemical events are initiated that results in acclimation of these plants to the lower growth temperature becoming more freezing tolerant. This process is generally referred to as cold acclimation and involves the accumulation of root soluble sugars, changes in the activity of enzymes involved in foliage sucrose synthesis (Hekneby *et al.*, 2006) and biosynthesis of red/purple pigments such as anthocyanin (Xin and Browse, 2000). In controlled environment experiments Caradus (1995) observed that the most frost (-4 to -16 $^{o}$C) tolerant clovers were *T. arvense, T. dubium,* and *T. hybridum.* In contrast, sub clover ('Tallarook') was one of the most frost sensitive. After one week at sub zero exposure sub clover had 83% more foliage damage (expressed as green leaf dry weight) than *T. dubium* (suckling clover). As previously described, in natural environments seedling frost tolerance determines the distribution of clover on areas more or less prone to frost events (i.e. south slopes) (Scott, 1971; Power *et al.*, 2003). Smetham (1968) reported frost damage in field experiments in Otago and the most affected strains were 'Geraldton', 'Dwalganup', 'Clare', 'Nangeela', 'Wenigup', 'Tallarook' x 'Wenigup' and 'Rutherglen'. In the North Island (Wairakei Research Station (latitude 38°38'S, elevation 400 m a.s.l.) MacFarlane *et al.* (1990) also observed that 'Clare' and 'Larissa' had 80-100% of foliage damage which negatively affected plant yield. 'Seaton Park', 'Woogenellup' and 'Tallarook' were the most frost tolerant.

Other studies showed that in winter wet- summer dry environments (e.g. Kaikohe podzol soil and Rawhiti, central yellow brown earth) the subspecies *yanninicum* was productive whereas for winter cold environments (i.e. Ballantrae and Hokonui) 'Nangeela' was best adapted (Chapman *et al.*, 1986) which contrasts with previous data (Smetham, 1968).

**Box 12 Cold tolerance**

Sub clover cultivars can tolerate winter temperatures as low as – 4°C (Frame, 2005) but there is little information about the frost-cold tolerance and subsequent regrowth of the three different subspecies or the newly registered Australian cultivars. Frost during seedling and flowering phases is an issue in southern New Zealand, which requires further assessment. Cold tolerance is not an existing priority for the Australian breeding programs and is one of the reasons of unsuccessful use of Australian cultivars in European regions (Section 2.3).

# 8 Defoliation (grazing management)

General guidelines for grazing sub clover are available for New Zealand. Practical advice is that the longer the sub clover has to establish and develop canopy and roots in autumn, the greater the production in spring.

### 8.1.1 Defoliation during the vegetative phase

A range of 21-35 days after emergence was previously suggested to start grazing after autumn establishment and early winter growth (Silsbury & Fukai 1977 cited in (Silsbury & Fukai 1977 cited in Smetham, 2003a)). The period supposedly would allow leaf area to build up, promote herbage yield and adequate annual reseeding (Smetham, 2003b). This recommendation was reviewed in detail in studies considering the relationship of the environment (mainly temperature) with the growth stages of sub clover (Section 4.1).

After establishment subterranean clover seedlings are likely to support grazing after six leaves are produced. The number of days to reach this stage depends on location and time of autumn rainfall, but is constant at 434 °Cd. As an example, in warm areas such as Napier if the rainfall occurs on 1st of May, the 6 leaf stage is achieved in 39 days. But in cooler areas (such as Alexandra) seedlings grow slowly and are prone to frost damage; the surviving seedlings will have 6 leaves after 102 days (Moot *et al.*, 2003).

To date, the general and practical recommendation for farmers is that the first grazing during spring should be lax (for instance over 2000 kg DM/ha) and autumn defoliation should not occur if pasture mass is under 1000 kg/ha (20 mm height) (Beef&Lamb, 2007). When mixed with cocksfoot grass, the ideal grazing intervals found to favour sub clover plants were 21-28 days (Roux, 2012).

### 8.1.2 Defoliation and the flowering phase

To ensure adequate seedling regeneration in subsequent years sub clover plants should not be grazed below 1600 kg DM/ha (LAI 1) during the flowering and seed maturation period (Smetham and Dear, 2003b).

Ates *et al.* (2013) observed that when pastures were grazed until early December the sub clover seedling population in the following autumn decreased by approximately 50% when compared with pastures which were closed in spring (mid-October). Continuous or heavy grazing decreased the number of leaves, runners, inflorescences and burrs and consequently compromised seed production and seedling regeneration (Smetham and Dear, 2003a). Sheath and MacFarlane (1990) reported that under continuous sheep grazing successful reseeding of sub clovers was ultimately determined by the growth habit. The more prostate 'Tallarook', 'Mt Barker' and 'Howard' regenerated more rapidly under the heavy grazing management.

Collins (1978) reported the time of flowering was unaffected by defoliation but pure swards which received frequent defoliation at the start of flowering had a faster rate of inflorescence production than the uncut controls. Similarly, Ru and Fortune (1999) reported that grazing early in spring resulted in an increase in the number of subterranean clover growing points (and secondary branching) possibly due to greater incidence of light (Smetham and Dear, 2003a). This could lead to more sites for flower and burr production, and increased seed production if combined with an early closing date (Ates *et al.*, 2013).

However, grazing after the first flower appearance and during the flowering period decreased seed yield, even though grazing or cutting height was adjusted to avoid removal of flowers

(Collins, 1978; Collins *et al.*, 1983). Archer (1990) recorded a 75% reduction in seed yield to 310 kg/ha when plants were regularly defoliated every 2 weeks to 3 cm, mainly due to the removal of the inflorescences.

### 8.1.3 Defoliation and burr burial

The effect of defoliation was observed by Collins *et al.* (1983) on the number of burrs produced and the pattern of burial. They tested regular defoliation regimes with weekly cuts from 35 days after sowing until the commencement of flowering, until 50% flowering and until the end of the flowering. All regimes increased burr burial by approximately 90% for 'Seaton Park', 'Yarloop' and 'Midland B' when compared with the uncut control.

Archer (1990) tested 'Nungarin', 'Seaton Park' and 'Woogenellup' and also observed that defoliation increased burr burial from 77% to 92%. Burr burial ability is a desirable trait for pasture regeneration and persistence. Nichols *et al.* (2013) ranked the cultivars according to their burr ability. 'Seaton Park' and 'Yarloop' are rated as 7 and 6 (strong burial) respectively. However, it was noted that this attribute may change with management, for example, defoliation regime and potentially soil type.

# 9 Research focus in Australia

Long term research and development, and industry investments have made Australia the modern reference in sub clover management and production in the Southern Hemisphere. Nevertheless there are issues and opportunities for sub clover improvement in Australia.

The reduction of out of season germination ("false break") is a research priority in Australia. Ecotypes with increased seed hardness are being tested to minimise risk associated with "false breaks". In addition, hardseededness is a desirable trait to maintain a dense seed bank particularly in areas with unreliable spring rainfall.

The deep acid sand soils and the high levels of salinity require plants with deep root system. Plants with deep root systems can obtain moisture from depth and have delayed senescence. There is currently a strong focus on breeding and genetics for tolerance to root rot caused by fungi and red legged earth mite.

In addition, herbicide tolerance/sensitivity and increased ability to fix nitrogen are other areas that have been researched in Australia. Practical issues also revolve around seed harvest. The soil erosion due to vacuum harvesters and the challenges around burr burial ability which is highly desirable in grazing systems but not in a seed production mechanical harvest situation.

# 10 Research Focus in New Zealand

In New Zealand sub clover research have been neglected since the main research efforts during the 80's and 90's. Possible reasons for that are:

- The priority given to other perennial pasture legumes (white and red clover and lucerne).
- The limited access to annual clover seeds and dependence on Australia for supply.
- The lack of serious engagement from the industry and production sectors.
- The tradition and lack of understanding of the sub clover in contrast the more common white clover based pastures. Overgrazing for example, which leads to poor regeneration and persistence of sub clover. Basic recognition of the cultivars is also important.
- Relatively little information on integrating sub clover as a legume component in the overall dryland farm system and in combination with as cultivar mixes and mixed with other species.
- Insufficient data of sub clover performance for the diverse New Zealand microclimates and soils (e.g. cold/driest areas of South Island) and longer term investigations beyond a May sowing date.
- Limited infrastructure, resources and support for new sub clover adopters (e.g. few registered products designed for weed management in sub clover).

Since sub clover is not (or was not) considered a “relevant” or economically interesting legume in New Zealand it is natural that there is a lack of current literature compared with white clover. But this scenario may change as land use, environmental awareness and market pressure force dryland farmers to look for alternatives to maximise production and reduce inputs (e.g. nitrogen fertilizer). Sub clover has a place to aid innovation for more sustainable farming in New Zealand dryland systems.

In contrast with Australia, in New Zealand there is insufficient information to draw solid recommendations for growers to add sub clover in their farming systems. Research is required how genotypes (mainly the newly released) respond to a variety of management practices (i.e. mixed cultivars or species) and edaphic-climatic scenarios (e.g. low South Island winter temperatures). It is also necessary to develop technical support for sub clover pastures (e.g. seeds compatible with temperate cool areas, herbicides, successful establishment and skilled management in dry pasture systems).

# 11 Final remarks

The main research opportunities identified through this review were:

- Hydrothermal requirements for sub clover seed germination across cultivars.
- Influence of spring sowing of sub clover on flowering patterns, yield and seed production.
- Photothermal time requirements for the reproductive phases and seed maturity of sub clover.
- Weed management and sub clover cultivars sensitivity to herbicides.
- Sub clover genotype adaptability and interactions with rhizobium strains adapted to low P soil conditions.
- The genetics controlling seed hardness and its relationship with different field growing conditions and management practices.
- Selection for sub clover cold/frost tolerance, mainly for the temperate cool areas in New Zealand.

- The relationship between flowering date and productivity and the potential to combine sub clover cultivars with different flowering times and hardseed ratings to create resilience in the sub clover seed bank.

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