Achieving persistence and productivity in white clover

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Abstract

White clover (Trifolium repens) is a valuable forage and soil fertility resource whose persistence and contribution to production and profitability can be constrained by genetic, farm management, and environmental factors. Here we outline the growth stages of the plant, and factors affecting persistence at the plant and the population level in pasture. Breeding strategies that bring together new germplasm sources within white clover have improved persistence on farm. New experimental lines, including some accessing genetics from related Trifolium species, show advances in forage productivity and persistence in multi-site, mixed sward, trial systems under dairy, sheep and cattle grazing. New germplasm sources and the use of new tools for characterising and selecting superior plant material will enable increased genetic gain for traits including persistence and forage production in white clover and related forage legumes.

Keywords: Lolium perenne, pasture, persistence, stolon density, Trifolium repens, white clover

Introduction

Providing nitrogen (N), increased animal production from high quality feed, and enhanced soil quality are key features white clover (Trifolium repens) offers in well managed, mixed-sward pastures throughout temperate grasslands of New Zealand and the world. For both dairy and meat production, the proportion of white clover in the diet is a major determinant of performance (Woodward et al. 2003), and stock given free choice between white clover and perennial ryegrass (Lolium perenne) will consume 70% of their diet as white clover, with related benefits to production (Cosgrove et al. 2001).

The critical first step in accessing the benefits of white clover in pasture is to establish the legume component successfully. Following a farm survey in the North Island, Brock et al. (2005), emphasised the value of seedbed consolidation as a leading determinant in successfully establishing clover during pasture renewal, with a trebling of clover after the first year based on this factor alone. Researching sowing depths, Milne (G.D. Milne, pers. comm.) showed that 75% of clover seedlings emerged when seed was drilled at 10 mm; dropping to 50% at 25 mm and 0% at 50 mm sowing depth. Black et al. (2006) showed that in well consolidated seedbeds, ryegrass sowing rates of 6-8 kg/ha in autumn and 10-12 kg/ha in spring maximised total pasture yield, and improved clover establishment. These rates contrast with the commercial recommended ryegrass sowing rates of 15-20 kg/ha which lower establishment risk in less well prepared seedbeds.

Once established, white clover faces challenges from biotic factors such as root invading nematodes and clover root weevil (Sitona lepidus), abiotic stress factors such as water stress, and farm management factors including grazing management and farm fertiliser policy, all of which can undermine plant and population persistence of white clover. Understanding the plant growth stages will allow farmers to better manage and realise the value of white clover on-farm. It will also allow plant breeders to better employ the tools of genetic improvement through selective breeding to develop populations into varieties that can better meet these on farm challenges. This paper will focus primarily on plant factors affecting the persistence of white clover in New Zealand pastures, with emphasis on drought tolerance. We outline specific examples where selective breeding has, and may further, improve white clover persistence.

White Clover Plant Development

Understanding the growth stages and complexities of the clonal white clover plant arising from a single seed is linked to understanding persistence of this species on farms. Whether from seed heads in pasture or dung, or via drill or aerial seeding, each new plant from seed has three distinct development stages. The first is a small, slow growing, compact rosette seedling phase, followed by a rapidly expanding and branching large tap-rooted phase, and finally upon the death of the tap-root, fragmentation into smaller plants, i.e., the normal clonal phase observed in mature pasture (Brock & Hay 2001).

The seedling stage has few stolon branches, lasting for up to 3 months after germination. During this time the taproot elongates in preparation for the second stage, which lasts up to 18 months from sowing and is characterised by rapid stolon elongation and active
branching with nodal roots. White clover tends to produce the greatest amount of herbage per unit area in this second stage.

The final stage starts 1 to 2 years after sowing, and leads to the mature clonal set of plantlets. In this stage the taproot dies and stolons become solely reliant on the nodal root network. With time and treading, the large plant fragments into smaller clonal plants with fewer branches (Brock & Tilbrook 2000). In this stage, the plant and clonal plantlets are mobile as they forage for sunlight, moisture and nutrients within the canopy and soil. When clover is fully clonal, the dry matter production oscillates, but in time a general ‘clover decline’ commences (Caradus & Williams 1989). Clonal populations are influenced by paddock history and biotic stress, in particular pests and diseases such as leaf viruses, root-feeding nematodes and insects such as grass grub (Costelytra zealandica) and clover root weevil (Watson et al. 1994; Gerard et al. 2009) which have marked negative affects on clover performance. These growth stages and decline pattern have implications for timing clover renewal for farmers wanting to maximise animal production from clover-rich pastures.

**White Clover Persistence in Pasture**

In a paddock, growth and persistence of individual white clover plants is dynamic and determined by interactions among plant species, soil fertility, pests and pathogens, grazing stock, and seasonal weather conditions. Awareness of the annual and multi-year cyclic nature of clover content, and the annual stolon death and renewal cycle can help determine grazing management practices to maximise value from white clover productivity via self-reseeding and clonal persistence (Brock & Hay 2001).

A primary force in this is the oscillating ‘predator-prey’ interaction between legumes and grasses in mixed swards (Chapman et al. 1996; Schwinning & Parsons 1996). The compatibility of white clover and grass is based on a competitive balance in which clover is favoured at low soil N by its capacity for N fixation, while grass is favoured at high soil N when it has a greater capacity to capture the N for growth. This cyclical interaction related to soil N, clonal growth, and light levels is self-regulatory and explains in large part the clover-grass interaction seen in pastures. This includes cyclical changes in clover content, which should not be confused with increased or decreased persistence *per se* of either ryegrass or white clover.

Once in the mature clonal phase in a pasture, a white clover plant persists as variable-sized plant fragments which exhibit an annual cycle of stolon development and death. During the winter, clover growth slows and

![Figure 1: Increase of stolon density within leaf size class in white clover over time. Data show the relationship between leaf size and stolon density of white clover cultivars with their year of release (triangle symbols: A=Aran (1983), K=Kopu (1985), T=Tahora (1987), C=Challenge (1988), D=Demand (1988), P=Prestige (1990), S=Sustain (1993), and circular symbols: KII=KopuII (1997), B=Bounty (1999), N=Nomad (2000), Tr=Tribute (2000). Data are from a set of observations at AgResearch Lincoln, assessing clover cultivars established within ryegrass swards under sheep grazing.](https://example.com/figure1.png)
Achieving persistence and productivity in white clover (K.H. Widdup and B.A. Barrett)

and appropriate selection criteria is a powerful force to improve the genetic potential of a species. A white clover plant’s genetic potential for persistence is the result of interactions among a suite of traits including plant health, plant morphology (stolon diameter and branching, leaf size and root structure), primary and secondary metabolites relating to stress tolerance and defence, and abiotic stress tolerance mechanisms.

Breeding strategies bringing together new germplasm sources within the species, or between closely related species, offer the opportunity to improve plant performance. This, in tandem with evaluation systems and genetic selection criteria to maximise genetic improvement rates, is a proven approach to increasing persistence in white clover populations.

New Zealand field-based breeding programmes are focused on screening germplasm in multi-site locations reflecting the environmental and management conditions that are encountered on pastoral farms; i.e., clovers growing in association with grass, under sheep and/or cattle grazing and across a range of climatic zones. Each new cultivar to market from the AgResearch Grasslands programme has more than 10 years of evaluation data including a minimum of 3 years on each of five sites in New Zealand. This is sufficient to test for successful transition of plants to the nodal root network in the clonal phase, and tolerance of acute plant health or abiotic stress factors such as drought. Though possible (Campbell et al. 1994), this system is not optimal for assessing long-term success of individual plants in the clonal phase in mature pasture, and experimental and selection systems better suited to efficiently assessing this are needed.

### Improving Persistence via Ecotype Breeding

White clover collected in high stress environments, such as summer-dry areas, soils with low P and pastures under intensive grazing, tend to be small-leaved types characterised by high stolon density, thin stolons, short stolon internodes, prostrate growth habit and a more fibrous nodal root system (Caradus & Williams 1989). These more persistent types have a low harvest index and produce lower leaf and petiole dry matter yield than larger-leaved cultivars characterised by low stolon density, thick stolons, long internodes, more

### Table 1

The legume content (%) of interspecific clover hybrids, white and strawberry clover cultivars grown in a mixed sward during spring and summer over 3 years at AgResearch Lincoln. For the Tr x Tu hybrids, a BC1 on average contains 75% and a BC2 87.5% white clover genetics, respectively. All entries were sown with a novel endophyte perennial ryegrass in March 2008, and managed under rotational grazing by sheep.

<table>
<thead>
<tr>
<th>Interspecific hybrids</th>
<th>2008-09</th>
<th>Legume content %</th>
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<th>2010-11</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spring</td>
<td>Summer</td>
<td>Spring</td>
<td>Summer</td>
</tr>
<tr>
<td>Ta x Tr ISH ‘Dense’</td>
<td>2.8</td>
<td>10.5</td>
<td>10.8</td>
<td>24.1</td>
</tr>
<tr>
<td>Ta x Tr ISH ‘Open’</td>
<td>3.0</td>
<td>10.8</td>
<td>14.8</td>
<td>29.5</td>
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<tr>
<td>Ta x Tr ISH ‘Persistor’</td>
<td>3.5</td>
<td>18.0</td>
<td>20.8</td>
<td>35.3</td>
</tr>
<tr>
<td>Tr x Tu (BC1, small leaf)</td>
<td>9.1</td>
<td>23.0</td>
<td>11.5</td>
<td>22.0</td>
</tr>
<tr>
<td>Tr x Tu (BC1, med leaf)</td>
<td>6.3</td>
<td>17.5</td>
<td>15.5</td>
<td>28.0</td>
</tr>
<tr>
<td>Tr x Tu (BC2, large leaf)</td>
<td>6.2</td>
<td>28.8</td>
<td>20.5</td>
<td>36.2</td>
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<table>
<thead>
<tr>
<th>White clover</th>
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<th></th>
<th>2009-10</th>
<th></th>
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<tr>
<td></td>
<td>Spring</td>
<td>Summer</td>
<td>Spring</td>
<td>Summer</td>
<td>Autumn</td>
<td>Spring</td>
</tr>
<tr>
<td>Lincoln Selection</td>
<td>22.4</td>
<td>52.5</td>
<td>28.8</td>
<td>33.6</td>
<td>41.3</td>
<td>17.0</td>
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<tr>
<td>Tahora</td>
<td>14.0</td>
<td>28.8</td>
<td>16.3</td>
<td>26.7</td>
<td>14.6</td>
<td>8.4</td>
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<tr>
<td>Demand</td>
<td>10.7</td>
<td>38.8</td>
<td>23.3</td>
<td>32.9</td>
<td>42.0</td>
<td>13.8</td>
</tr>
<tr>
<td>Nomad</td>
<td>13.9</td>
<td>33.0</td>
<td>20.3</td>
<td>33.0</td>
<td>27.5</td>
<td>13.1</td>
</tr>
<tr>
<td>Onward Strawberry clover</td>
<td>3.8</td>
<td>4.3</td>
<td>9.0</td>
<td>22.8</td>
<td>26.8</td>
<td>18.6</td>
</tr>
</tbody>
</table>

LSD (5%) 5.5 10.1 6.0 9.7 10.6 6.0 7.1
The forage yield and recovery of white clover via seedling regeneration in a tall fescue-clover pasture following a severe drought in 2001. (Trial was established in autumn 1999).

Figure 2: The forage yield and recovery of white clover via seedling regeneration in a tall fescue-clover pasture following a severe drought in 2001. (Trial was established in autumn 1999).

Improving Yield and Persistence
Over the last 25 years, breeders have worked to improve persistence in conditions that stress plants so that farmers can now more confidently use white clover over a greater range of environments. Farmers, no matter what environment and seasonal conditions they face, benefit not only from persistence but from a greater contribution of clover herbage in their pastures to increase N fixation and animal performance.

There is a recognised trade-off between herbage yield and persistence with an inverse relationship between leaf size, a proxy measure of yield under optimal conditions, and stolon density, a proxy measure of persistence. Forage breeders have worked against this constraint to improve both persistence and herbage yield by combining the features of small-leaved adapted New Zealand ecotypes and large-leaved overseas germplasm into new options for farmers.

The success of this approach is demonstrated by the breakthrough development of ‘Demand’, a cultivar bred for use in southern regions of New Zealand under intensive sheep grazing (Widdup et al. 1989). To achieve this breakthrough, hybrids were developed between persistent ecotypes collected from old Southland pastures and large-leaved Mediterranean-European based germplasm. A further example was the development of ‘Sustain’ where the aim was to break the link between leaf size and stolon density so as to achieve high herbage yields and improved persistence (Caradus et al. 1997).

The strategy of combining locally adapted material with overseas germplasm showing desirable traits has continued into this century, resulting in a shift in the stolon density to leaf size relationship in more recently developed cultivars (Fig. 1). ‘Tribute’, for example, is a medium-leaf sized cultivar which produces considerably greater numbers of stolons/m² compared to the older cultivar of comparable leaf size, ‘Challenge’. The data suggest that leaf size and stolon density have not been de-coupled in white clover, but that breeders have increased the stolon density of white clover across leaf size classes, which has led to improved persistence of new medium- and large-leaved cultivars suitable for a range of environmental conditions.

Improving Drought Tolerance in White Clover
Summer moisture is the greatest non-genetic determinant of white clover persistence in many cases. Knowles et al. (2003) and Dodd et al. (2001) demonstrated the direct relationship between severity of drought and the loss of white clover from pasture. Modified grazing management can offer protection under some dry circumstances. Brock (1988) demonstrated the greatly increased grass tiller density (>14 000 tillers/m²) under...
spring set-stocked grazing compared to 3-5000 tillers/ m² under rotational grazing. Grass tillers in set-stocked pastures almost completely covered the soil and provided protection to surface clover stolons. During a severe summer drought, clover content fell from 12% to 3% in the rotationally grazed swards due to high stolon mortality whereas clover content increased from 9% to 16% in set-stocked managed pastures which also showed little loss of stolon material.

There is scope for genetic improvement of drought tolerance in white clover. Greater stolon growing point densities, deeper nodal roots and a higher root-to-shoot ratio have been identified as important morphological traits that can be used to breed white clover with better persistence under short term droughts (Brock 1988; Woodfield & Caradus 1987). Related research has shown extensive genetic variation in white clover for root morphology (Caradus & Woodfield 1998) and that many of the root traits have moderate to high heritability (Woodfield and Caradus 1990; Jahufer et al. 2008), indicating likely responses to selection. ‘Nomad’ was selected from ecotypes that showed thicker nodal roots and higher root-to-shoot ratio compared with other clovers with comparable leaf size. However, there have not been the physiological studies to demonstrate how much greater drought tolerance and persistence ‘Nomad’ displays compared to other cultivars.

Sustained droughts in recent years have created major farm management challenges for New Zealand farmers and industry. However, the sector may benefit in the long term through access to improved plant genetics identified in these weather events. Droughts and other plant stress events are valuable for forage plant breeders, allowing them to identify lines with key traits including persistence and recovery from drought. For example, the 2008 Waikato summer drought allowed the AgResearch Grasslands team to identify, in an ongoing dairy grazed plant breeding trial, a new large-leaved line ‘GC216’ with drought recovery 55-113% better than the recovery scores for the current large-leaved cultivars Emerald, Kopu II and Aran. Combined with the line’s pre-drought performance being 42-53% better than these three cultivars in the same trial and strong performance in multi-site trials, this fortuitous drought and resulting observations has led to the advancement of this line to the variety certification stage in preparation for marketing. This selection shows how, in the absence of adequate resources to run controlled moisture and heat stress trials across a plant breeding programme, multi-site testing and periodic drought can lead to valuable ad hoc discoveries of populations showing valuable characteristics contributing to persistence in white clover.

Further marked gains in drought tolerance of white clover may be achieved by inter-specific hybridisation with related Trifolium species. Some of these species have dramatically improved drought tolerance compared to white clover, having evolved and adapted in dry environments. Current work is focused on developing inter-specific hybrids between white clover and these related species so as to incorporate new genetic variation for drought stress tolerance into white clover (Williams et al. 2010). T. ambiguum (caucasian clover) and T. uniforum both produce deep taproots and in the case of T. ambiguum, an extensive rhizome system. Both characters are desirable for improved drought tolerance.

Breeding populations have been developed by the AgResearch Grasslands genetic resources team by crossing these species with white clover (Williams et al. 2010). These have undergone field screening and selection in summer-dry trial systems (Widdup et al. 2003). A recent trial sown at AgResearch Lincoln in March 2008 compared new selections of the T. ambiguum x T. repens (Ta x Tr) and T. repens x T. uniforum (Tr x Tu) interspecific hybrids with a group of small-leaved white clover cultivars (Table 1).

All white clover cultivars had significantly better growth than the inter-specific hybrids and strawberry clover until the second summer (December 2009), when all entries had similar legume content in the pasture plots. By the second autumn (March 2010), the Ta x Tr ‘Persistor’ inter-specific hybrid was significantly better than all other entries in the trial, and this advantage continued into the third year when it was significantly better than all but the white clover experimental line ‘Lincoln Selection’ and the ‘Onward’ strawberry clover line, for which there was a non-significant advantage for the hybrid (Table 1). These Ta x Tr hybrids produce deeper tap and nodal roots compared to white clover (Williams & Hussain 2008) and it is likely that this trait is improving the performance of the Ta x Tr ‘Persistor’ selection over the dry summer months in Canterbury. It is important to add that the Ta x Tr hybrids have been through a number of selection cycles and agronomically useful material is beginning to show. In contrast, the Tr x Tu inter-specific hybrids have only recently been developed and as yet are not showing an advantage over white clover in Lincoln.

These data suggest a seed mixture of a white clover and an inter-specific hybrid should be tested to see if the combination of good production in early years from white clover and a substantial lift in persistence from the inter-specific hybrid can be achieved under grazing.

**Using Buried Seed to Achieve Population Persistence**

Under extreme dry conditions, recovery through regeneration from buried seed becomes an important
population persistence mechanism (Archer & Robinson 1989). In the east coast regions of New Zealand, regular summer droughts and clover recovery through plant and animal-mediated seed dispersal and regeneration may be an important and preferred mechanism of consistently achieving good clover content in the diet.

Evidence for this comes from a clover germplasm screening trial at Lincoln where white clover collapsed in a tall fescue sward following a dry period in the third summer (Brock et al. 2003). Subsequent recovery in clover content was shown to be from clover seedling emergence from buried seed during autumn and spring, rather than surviving stolon pieces, resulting in a marked resurgence of clover herbage in the autumn of the fourth year (Fig. 2). Such rejuvenation of clover in dryland pastures may not be a regular event but it is possible following severe droughts and in open pasture conditions. MacFarlane & Sheath (1984) developed the profuse flowering cultivar ‘Prop’ to aid persistence through higher seed set and seedling rejuvenation in dry environments. Seed set is one essential trait, but other features such as strong seedling vigour and appropriate grazing management to ensure seedling survival need further research. Current breeding programmes have specific selection for seed yield, making seed-based population persistence a viable option with all new cultivars for achieving good white clover content following drought and in summer-dry country.

Conclusion

Plant breeders today have access to proven systems for genetic improvement, augmented with new components including marker-aided breeding, enhanced trait assessment systems, and new germplasm. These systems, if sufficiently resourced, will provide continuous and potentially dramatic changes in the genetic potential for persistence in new varieties of white clover.

However, plant breeding is only one step in realising the value of white clover on farm. It is essential that farmers aiming to realise value from clover, use the proven but sometimes neglected principles and practices of mixed sward pasture establishment and management. Improved plant genetics will never be a replacement for proper attention to pasture establishment and grazing management, including periodic seed borne renewal of clover in pastures where appropriate. It is also important to recognise a boundary to the scope for improving plant genetics for drought adaptation in white clover, in that other legume species (for example lucerne or annual clovers) better fill the niches on the dry and hot margins of white clover’s adaptive range.

A key challenge in the future is in systematically addressing and improving the performance of white clover under chronic moisture limitation, as limits to irrigation and changes in rainfall and temperature patterns are likely to increase their impact on grazing systems in New Zealand. This focus, combined with breeding for general adaptation and resilience or resistance to other biotic and abiotic limitations, will allow breeding to increase the persistence of white clover populations. This in turn will enable an improved proportion of legume in the sward, with linked contributions to animal performance and farm profitability.

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