White clover vegetative persistence under pressure: sharpening New Zealand's competitive edge

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Abstract

White clover is relied upon as a cornerstone of the international competiveness of New Zealand's pastoral sector, despite its vegetative persistence and yield being constrained by pests and diseases. The species' vulnerability has been highlighted by the clover root weevil (*Sitona lepidus*) incursion, and the impact of increased residual pest and pathogen loadings under minimum tillage techniques. Plant breeding with a focus on plant health has made improvements in white clover performance, and there is scope to develop varieties that ensure a more resilient legume component in pastures. An exemplar outcome from this approach is improved vegetative persistence and dry matter yield under nematode and clover root weevil pressure, as shown in field trials of elite breeding material. An enhanced, unified plant breeding and plant health approach can increase the performance and vegetative persistence of white clover. Trait selections to increase resilience and dry matter yield include optimum root architecture; single and multi-gene resistance or tolerance mechanisms; multi-pest defence compounds; and symbiont:clover combinations enabling induced pest and disease resistance. Cost effective and timely plant health assays for plant breeding are essential. Improved breeding strategies will create value on-farm via perennial forage legumes that perform better under pressure from pests.

Keywords: white clover, plant breeding, plant genetics, nematode, pest resistance, pest tolerance

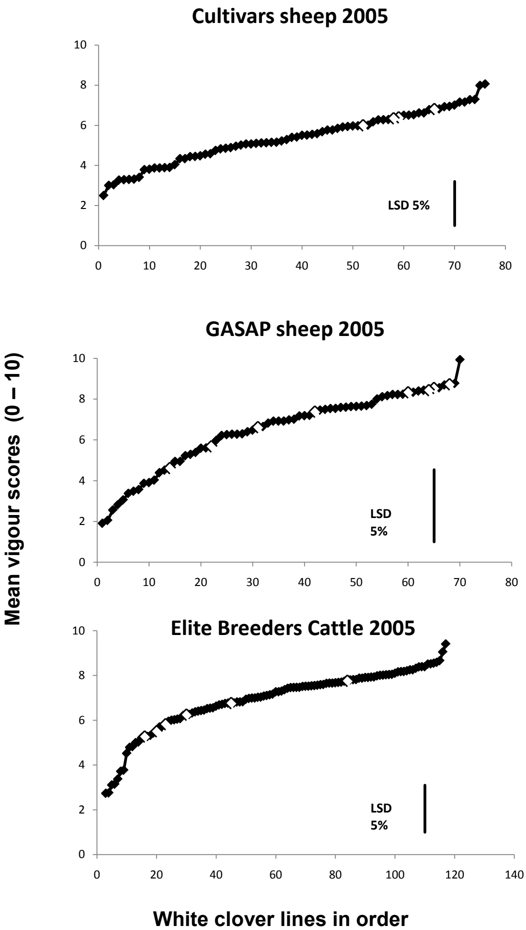
Introduction

White clover (*Trifolium repens*) is relied upon as a key to the international competitiveness of New Zealand's pastoral industries, with proven benefits for milk and meat production, and renewable transformation of atmospheric N into plant available forms (Caradus *et al.* 1996 and references therein). Currently there is strong interest in the economics and techniques of pasture renewal (e.g. Pasture Renewal Charitable Trust 2011). For that value proposition to work, legumes must persist and perform for the expected life of the renewed pasture, which may be over 20 years (Daly *et al.* 1999).

Before it can persist, white clover requires careful management at establishment (Brock & Kane 2003) when it is reliant on a small seed's energy reserve and developing taproot. As this taproot dies after 12 to 18 months of growth, the plant depends on the secondary or nodal root system for water and nutrient uptake. Individual stolons of the plant also die, with only 10% of them surviving for more than 1 year (Chapman 1983). This has led to white clover being referred to as a vegetative annual (Sheath & Hays 1989), whose ability to persist over time is reliant on ensuring the replacement rate of growing points in the stolon network matches or exceeds the rate of growing point loss.

The portion of clover in a mature pasture depends on stolon survival, replacement rate and vigour, all of which are sensitive to competition and predation by the other species in the system. Clover yield will wax and wane in a cyclical manner through legume and grass predator – prey interactions (Schwinning & Parsons 1996a; Schwinning & Parsons 1996b). Feeding damage to clover roots by pests may accelerate this cycling by increasing the levels of N in the root zone (Ayres *et al.* 2007) thereby increasing the level of N available to grasses (Murray & Clements 1998; Murray & Hatch 1994). The arrival of clover root weevil (CRW) into New Zealand systems provided a good example of how a species-specific pest feeding on roots can shift the clover: grass competitive balance. A survey of 417 5-year and older pastures in 1997/98 showed infested pastures predominantly in the 0-10% clover content range whereas uninfected pastures were mainly in the 10-20% range (Gerard *et al.* 2004). Conversely, removal of clover pests from established swards with insecticide for periods of up to 5 years is associated with increased clover content compared to untreated pasture, with the differences increasing with time (Mowat & Shakeel 1988 and references therein). In a major trial across 16 sites in the Waikato/Bay of Plenty, Watson *et al.* (1985) obtained an average increase in annual total herbage yield of 13% through the use of insecticide. The greatest change in production came from clover with a 40% increase in above-ground dry matter yield.

Figure 1 Yield of white clover in three Manawatu trials established in 2005. Each point is a 3-year mean of visual estimates on a 0 – 10 scale. The white points represent lines from the nematode tolerant/resistant breeding programme and the black points are other breeders' lines and cultivar controls.



Higher fertiliser N use and stocking rates in recent years has challenged clover, creating demand for improved white clover content, particularly to lift production in dairy swards (Brock & Hay 2001; Brock & Kane 2003; Woodfield & Caradus 1996). Plant breeders have improved the competitive ability of white clover in these conditions by enhancing cool season growth, stolon growth and density within a leaf size class, and growth under higher levels of soil N (Woodfield & Clark 2009). Improving the resilience to pests and diseases is another worthy target. White clover is compromised from time of sowing onwards, owing to its vulnerability to the residual pest and pathogen loadings especially under minimum tillage techniques. While direct effects on clover survival are seen during pest outbreaks, the chronic effects of pest and disease complexes on long-term clover persistence and performance in mature pastures are largely unrecognised (Brock *et al.* 1989). Pest damage and root and stolon rot are highly correlated (James *et al.* 1980), and subsequent root loss and the stresses induced also increase the plant's vulnerability to abiotic stress factors such as drought and nutrient shortages.

New Zealand farmers have few economic options for managing pests. Genetic resistance is an effective management tool that is constantly "on guard" against pests even before the farmer is aware of its presence. New Zealand white clover cultivars would benefit from improved resilience against the complex of above- and below-ground invertebrate pests and diseases present in our pasture ecosystems. In this paper, we present how a unified plant breeding and plant health approach can increase the yield and vegetative persistence of white clover and suggest attributes and systems that should be included in breeding programmes.

An example of improving forage legume performance using plant breeding and plant health research.

In New Zealand pasture, white clover roots are heavily predated by several nematode species, principally clover root-knot nematode (*Meloidogyne trifoliophila*), clover cyst nematode (*Heterodera trifolii*) and lesion nematodes (*Pratylenchus* spp.). These species dramatically reduce plant vigour, increase the rate of stolon death, and compromise vegetative persistence (Watson & Mercer 2000). Breeding lines from two long-term concurrent breeding programmes which involved recurrent crossing and selection, one for resistance under known nematode pressure in a glasshouse and the other for tolerance to continuous high nematode loadings in the field, have been tested in a series of grazed field trials and shown to perform as well or better than cultivar controls (Mercer *et al.* 2008). More importantly, in the presence of CRW, a nematode-tolerant line was the only white clover line under both dairy and sheep/beef grazing to make the top ten list of 62 white and red clover lines or cultivars evaluated in 2002-05 in the upper North Island (Crush *et al.* 2005).

The breeding worth of these nematode resistant and tolerant lines was tested further in three Manawatu trials established in 2005. White clover lines were planted as seedlings in plots in a grass sward cleared of resident clovers by a selective herbicide. Trial size ranged from 70 to 120 breeding lines and commercial cultivars. They were grazed by sheep (two trials) or cattle (one trial) and clover vigour was scored before grazing. High population densities of CRW in the Manawatu were removing white clover from some pastures and causing white clover trials to be finished earlier than before the CRW's arrival (John Ford pers. comm.). Observations were that the CRW predation pressure was much greater than the nematode stress load.

The scores averaged over the trial life showed that at two of the three sites, nematode resistant and tolerant lines were among the better performing lines and cultivars (Fig. 1). However, because pest abundance was not measured, it is not possible to confirm that the difference in performance of these lines at the three sites was caused by varying levels of biotic pressure. Nevertheless, these trial results suggest that by including a key root pathogen and plant stressor as an integral component of the plant breeding process, a shift in clover resilience in the face of a generic pest load can be achieved. Therefore, to further improve clover, screening and selection should include known pressures from one or more key root pests and disease groups that are chronic in our pastures. Materials from these trials are being incorporated into commercial breeding populations with ongoing testing for pathogen resistance, in order to make them available to farmers.

Plant Defence Compounds

Plants synthesise a range of secondary metabolites, most of which are induced in response to infection or herbivory. Some have direct effects on the herbivores (toxins, anti-feedants, growth regulators) while others are indirect, e.g., alerting natural enemies to the presence of the herbivore. The best characterised system in white clover herbage is the cyanogenesis system which provides protection from slugs but can increase vulnerability to insects (Raffaelli & Mordue 1990). This trait can be quickly and cost effectively assayed for in breeding populations, and is routinely tested in plant breeding programmes to improve and characterise populations.

Comparatively little work has been done internationally on how plant roots achieve protection from herbivory (Hunter 2001), with the *Trifolium* genus receiving very little attention.

While modern New Zealand white clover cultivars are generally susceptible to root herbivores, defence mechanisms have been found in other perennial *Trifolium* species. For example, the isoflavanoid formononetin is associated with broad-spectrum protection in clovers (Wang *et al.* 1998; Wang *et al.* 1999) but can cause infertility in ruminants (McDonald 1995). Root herbivory by CRW has been shown to induce different levels of formononetin in root and/or shoots of three red clover (*T. pratense*) breeding lines (Gerard *et al.* 2005). Not only did this experiment show that it is possible to identify red clovers with high formononetin and other associated defence compounds in the roots, it reinforced how valuable it is to expose genetic lines to biotic pressures from the initial stages of screening. Otherwise, genes associated with expression of plant health secondary metabolites may be lost and unexpected plant persistence or animal health problems could arise when the new cultivar is used in regions with high pest pressures. This red clover example raises the possibility that in white clover germplasm or related species, there may be undiscovered inducible defence mechanisms. Another source of defence responses may come from genetic modification as molecular biologists are making gains in understanding plants defence mechanisms and developing novel genetic solutions (Iakimova *et al.* 2005).

Symbionts

Clover species have a suite of bacterial and fungal symbionts. Like root defence compounds, the impact of symbiont interactions in plant health has rarely been investigated, although arbuscular mycorrhizae may help control root herbivores (Hunter 2001). Indeed, Cooper & Grandison (1986) found that mycorrhizal inoculation increased white clover resistance to infection by root-knot nematode. Considerable research was done in New Zealand in the 1970s and 1980s on the potential of mycorrhizal fungi to improve the efficiency of white clover P utilisation, with the general conclusion that the benefits only occur at low levels of soil P (Brock *et al.* 1989). The genetic control of the mycorrhizal association appears to be with the plant as symbiosis was observed more in white clover land races and wild varieties than in improved cultivars. For example, seven of the 15 white clover genotypes selected to follow up for symbiotic potentials after initial screening were from Prestige, a cultivar selected from ecotypes persisting in old Northland pasture (Crush & Caradus 1996). This cultivar also proved to be the best of the commercial cultivars when clover germplasm was evaluated for CRW tolerance (Crush 2005). These mycorrhizae are invariably present in New Zealand soils so selection of clover genotypes that best make use of these ubiquitous beneficial fungi should facilitate the development of more persistent clovers.

Acquired Resistance through Viruses

Viruses reduce plant yield, compromise longevity of plants, increase susceptibility to root pathogens, and decrease nitrogen fixation (Barnett & Diachun 1985). Development of virus resistance is a longstanding aim of some plant breeding programmes, including genetic modification approaches (Ludlow et al. 2009). However, there has been little work in clover to test the ability of viruses to provide an acquired resistance or tolerance to other pests or pathogens. Recent findings show that some viruses may have a net benefit to white clover, by reducing insect predation. Virus infection apparently induced resistance against grass grub with larvae having greater mortality (20% vs. 0%, $P< 0.01$), and lower weight gains (6.4 mg vs. 19 mg, $P<0.001$) when fed identical amounts of roots from virus-infected clovers compared to virus-free clovers in a recent laboratory trial (P.J. Gerard, unpublished data). In addition, in a 2010 pot trial run under prolonged hot conditions, virus-infected white clover produced 81% more foliar dry matter in February and 56% more in March (pooled data, $P<0.05$) than virus-free plants from the same population (P.J. Gerard, unpublished data). The ability of virus infection to improve drought tolerance by increasing the levels of osmotic regulators and antioxidants has been reported for other plant species (e.g., Xu *et al.* 2008 and references therein). Viruses are ubiquitous in mature New Zealand pastures, with a gradient of increasing diversity from south to north (Denny & Guy 2009). A better understanding of clover - virus interactions, including plant costs and benefits in variable field conditions, may offer an opportunity to select for clovers with the capacity for acquired resistance from asymptomatic viral strains which thereby contribute to resilience in the field.

Root Morphology

A substantially wider range of root morphology exists in white clover germplasm collections than is seen in commercial cultivars. A laboratory experiment comparing CRW larval damage on contrasting genotypes (short thick roots vs. long fine roots) showed that while there was no difference between the amount of root severed, the long fine-rooted genotype maintained a significantly larger root system in terms of root length and number (Care *et al.* 2000). At any level of pest density, a clover with a large finely divided root system with many growing points should be less impaired by herbivory and have better persistence than a clover with comparatively fewer coarse roots. Similarly, a clover with many small nodules will be more likely to continue fixing N in the presence of a pest such as CRW than one with fewer large nodules.

Regional Ecosystem Fit

Considerable effort goes into developing a forage cultivar so an equally determined effort should be made to ensure the plant has traits that provide a net benefit and allow the plant to persist in the pasture ecosystem (Parsons *et al.* 2010). There is a metabolic load on plants to maintain tolerance or resistance mechanisms. In the absence of pest pressure, these plants may perform less well compared to other elite types. Therefore, a clover that persists well under the severe pest challenges found in the Waikato and Bay of Plenty regions, for example, may perform relatively poorly in regions with lower pest loads. Forage plant breeders do undertake multi-site population testing under grazing in Northland, Waikato, Hawkes Bay, Manawatu, Canterbury and Southland in addition to international testing. However, as the levels of biotic pressures at the sites are rarely measured in typical breeding strategies, selection for resistance to most pests and diseases is *ad hoc* and could be improved with targeted pest or pathogen inoculation, identification and enhancement of sites with regular high levels of pathogens.

A recent successful example of *ad hoc* resistance from clover breeding strategies became apparent following a survey of clover virus incidence in North Island pastures in 2010 (P.J. Gerard & P.L. Guy, unpublished data). The results suggested that 'Kopu II' and 'Aran' may be resistant to alfalfa mosaic virus and red clover necrotic mosaic virus, which was confirmed in subsequent glasshouse tests (Paul Guy, Otago University, pers. comm.). Aran germplasm contributed to the development of Kopu II, and the progeny were screened for absence of foliar diseases as spaced plants at Lincoln (Woodfield *et al.* 2001). Both viruses are above average in incidence in mature pastures at Lincoln, especially when compared to Southland (Denny & Guy 2009), Waikato and Bay of Plenty pastures (P.J. Gerard & P.L. Guy, unpublished data). Therefore, Lincoln was a better site for selection against these viruses compared to the other regions listed. It is probable that this virus resistance, inherited from Aran, contributes to the observed vigor and widespread use of Kopu II in dairy pastures.

More Effective Breeding Strategies

Whether the target is a single pest, a disease complex, symbiont associations, plant morphology or complex chemistry; new varieties from an applied breeding programme are the connection between plant breeders and the farmer. In order to deliver plants with inbuilt genetic resilience against pests and pathogens it is necessary to develop and employ cost effective, timely and efficient laboratory, glasshouse and field tests that can be applied in plant breeding populations. These may include bioassays, metabolomic assays, and marker assays and need to be implemented at early stages of the breeding programme. Their utility will be determined by genetic variation or heritability within the breeding populations for the traits under selection and the degree to which they predict genetic merit.

Conclusions

Plant breeders have developed forage legume populations with increased resilience under attack from plant pests and diseases. Further incorporating a plant health focus into plant breeding can improve the forage legume performance and vegetative persistence that farmers and industry are seeking. The best way to incorporate persistence traits is to challenge elite breeding material with the biotic pressures likely to occur during the intended life of a New Zealand pasture, and select for superior genetics.

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