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The effect of interspecific hybridisation with *Trifolium uniflorum* on key white clover characteristics

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ABSTRACT

A study was conducted to determine the effects of hybridisation with a wild relative, *Trifolium uniflorum*, on key morphological characteristics and N fixation of white clover (*Trifolium repens*). Overall, over 19 months under the conditions of the field environment used, dry matter (DM) production of the backcross 1 (BC_1) generation was reduced compared with white clover, and did not increase with a second generation of backcrossing. The general morphological type of BC_1 hybrids had a smaller leaf lamina area, shorter internodes and a more prostrate growth habit compared with white clover, which is likely to contribute to reduced DM production. Generally, lateral spread did not differ among BC_1, backcross 2 (BC_2) and white clover, except in spring when white clover had a wider spread than the hybrids. Hybridisation did not affect the proportion of shoot N derived from fixation, or shoot %N content. Fungal disease and virus symptoms were lower in the *T. uniflorum* parent than in white clover. This did not affect virus symptoms in the hybrids, and fungal disease scores were higher in BC_1 than in the white clover parent. However, some hybrid families were more superior than others at maintaining (or improving) characteristics of their specific white clover parental cultivars, such as DM production, stolon density, growth habit, and resistance to shoot fungal diseases and viruses. This suggests it should be possible to develop elite hybrid breeding populations by recurrent selection of superior hybrid genotypes.

1. Introduction

White clover (*Trifolium repens* L.) is a major legume in temperate agriculture. The value of white clover to pastoral agriculture lies in its productivity, nutritive value, and the ability to fix atmospheric N (Ulyatt, 1981; Caradus et al., 1996). However, its potential as a significant legume species is constrained by a number of limitations including requirements for high soil fertility and high soil moisture, susceptibility to various pests and diseases, and a relatively small root system (Jahufer et al., 2012). Interspecific hybridisation of white clover with other species within the genus *Trifolium* has been used as a means of introducing traits outside its existing genetic variation (Hussain et al., 1997; Williams et al., 2006). *T. uniflorum* L., a close, wild relative from the Mediterranean region, was first hybridised with white clover over 50 years ago (Pandey, 1957). Nichols (2012) reported increased drought resistance and improved growth at low external P supplies for *T. repens* × *T. uniflorum* backcross 1 (BC_1) hybrids. While some limitations of white clover may be addressed through interspecific hybridisation, it is important that key white clover traits are not adversely affected in the process. For example, effects on seed production could be anticipated, but these can be overcome (Naeem, 2013). In general, it can be expected that traits in the hybrids will be intermediate to the *T. uniflorum* and white clover parents, becoming more like white clover with successive backcross generations. This is reported in the limited information in the literature (Pandey, 1957; Gibson et al., 1971; Pandey et al., 1987), but little data has thus far been presented.

White clover is a highly heterozygous and outcrossing species, which leads to genetic variation through recombination of genes (E.G. Williams, 1987; W.M. Williams, 1987). This variation can be harnessed through breeding to develop cultivars adapted to particular environments and agronomic systems. The stoloniferous nature of white clover is an important characteristic that enhances its ability to spread and persist vegetatively in the sward.

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Table 1
Soil test (0–75 mm) results for the experimental site at establishment. me = milli equivalents. Numbers in parentheses are results in New Zealand MAF (Ministry of Agriculture and Fisheries) Quick Test units, to allow comparison of K, Ca, Mg and Na with [a]target values for New Zealand sheep and beef farms (sedimentary soils) (Fert Research, 2009).

pH	Olsen P (mg l^{-1})	SO_4-S (mg kg^{-1})	K (me 100 g^{-1})	Ca (me 100 g^{-1})	Mg (me 100 g^{-1})	Na (me 100 g^{-1})
6.1	39	7	1.18 (23)	9.5 (11)	1.13 (24)	0.17 (7)

[a] pH = 5.8–6.0; Olsen P = 20–30; SO_4-S = 10–12; K = 5–8 MAF units; Mg = 8–10 MAF units.

Morphological characteristics such as leaf size, stolon diameter, stolon density, and internode length contribute to the performance of white clover and are often the basis of plant breeding selections (W.M. Williams, 1987; Caradus and Woodfield, 1997).

The transfer of fixed N from white clover to grasses in grazed swards has been measured using the ^{15}N dilution method (Ledgard, 1991), with above- and below-ground transfer estimated at 60 and 70 kg N ha^{-1} $year^{-1}$ respectively, accounting for 50% of grass N. In the laboratory, two strains of N fixing *Rhizobium* from *T. uniflorum* were not effective on white clover, but rhizobia from white clover were not tested on *T. uniflorum* (Yates et al., 2003; Howieson et al., 2005). However, we have observed healthy nodules in *T. repens* × *T. uniflorum* backcross hybrids grown without targeted inoculation, suggesting that hybridisation does not compromise nitrogen fixation.

The effect of hybridisation with *T. uniflorum* on key characteristics of white clover has not been quantified. The objective of this study was to measure important white clover traits, which ultimately impact productivity, in a range of BC_1 and backcross 2 (BC_2) hybrids under field conditions, and to compare these to commercial white clover cultivars. In particular, there is no published data on the performance of hybrids in the field, and little information is published about nodulation in *T. uniflorum* and how this may affect N fixation of *T. repens* × *T. uniflorum* backcross hybrids.

2. Materials and methods

2.1. Experimental area

The experiment was conducted from November 2008 to May 2010 on the AgResearch farm, Lincoln, New Zealand (43°37′38.17″ S, 172°28′10.2″ E). The soil type was a Wakanui silt loam (Cox, 1978) (Udic Ustochrept, USDA soil taxonomy). Soil samples taken at the beginning of the experiment showed that fertility conditions were not limiting for growth (Table 1), and no fertiliser was added at establishment. The soil cores were taken to 75 mm, the standard depth for soil sampling in New Zealand pastures (McLaren and Cameron, 1996).

2.2. Plant material

Plant material was grouped into five clover types – *T. uniflorum*, BC_1, BC_2, white clover and red clover (*T. pratense* L.). There were 27 clover entries, including 10 BC_1 and six BC_2 families, two *T. uniflorum* accessions, eight white clover cultivars representing a range of morphologies, and one red clover cultivar ('Sensation') as a standard control (Table 2). Seedlings were established in a glasshouse in September 2008. Seed scarified with sandpaper was germinated on damp filter paper in Petri dishes and transplanted into 40 mm × 40 mm × 120 mm root trainers containing a sand/peat potting mix. Seedlings were not inoculated because of the free availability of rhizobia in pasture soils.

2.3. Experimental design

The experiment was positioned within the paddock to avoid high stock traffic areas such as gateways and shelter belts. A split plot design was used, with five replicates (blocks) and four sub-plots within each replicate, for a total of 540 experimental plants. The four sub-plots allowed for harvesting of plants over time to study tap root life span, which will be reported elsewhere. Each sub-plot contained 30 plants in a 5 × 6 randomised design. All 27 entries

Table 2
T. uniflorum accessions, BC_1 and BC_2 families, and white clover and red clover cultivars used in the experiment. OP = open pollinated; cv = cultivar.

Entry number	Clover type	Description	White clover cultivar leaf size
1	*T. uniflorum*	AZ4382[a] OP	
2	*T. uniflorum*	AZ4383[a] OP	
3	BC_1	Aran × 900-3	
4	BC_1	Barblanca × 82-3	
5	BC_1	Crusader × 80-2	
6	BC_1	Crusader × 900-4	
7	BC_1	Crusader × 902-11	
8	BC_1	Kopu II × 900-4	
9	BC_1	Kopu II × 80-2	
10	BC_1	Sustain × 82-3	
11	BC_1	Tribute × 900-4	
12	BC_1	Trophy × 902-6	
13	BC_2	Crusader × (Crusader × 900-5)	
14	BC_2	Kopu II × (Kopu II × 902-1)	
15	BC_2	902-1-OP-4 × Trophy	
16	BC_2	(Crusader × 902-1) OP	
17	BC_2	Durana × (Crusader × 902-1)	
18	BC_2	Durana × (Kopu II × 902-4)	
19	White clover	cv. 'Crusader'	Medium
20	White clover	cv. 'Grasslands Kopu II'	Large
21	White clover	cv. 'Grasslands Sustain'	Medium–large
22	White clover	cv. 'Grasslands Tahora'	Small
23	White clover	cv. 'Grasslands Tribute'	Medium–large
24	White clover	cv. 'Trophy'	Medium–large
25	White clover	cv. 'Aran'	Large
26	White clover	cv. 'Barblanca'	Large
27	Red clover	cv. 'Grasslands Sensation'	

[a] Accession number, Margot Forde Forage Germplasm Centre (Palmerston North, New Zealand). AZ4382 is of Greek origin and AZ4383 is of Turkish origin.