



## Effect of inbreeding on growth of white clover

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**Abstract.** Determining the impact of inbreeding on white clover growth will assist in determining the optimal breeding strategies for future population improvement. A dominant self-fertility allele (*Sf*) at the S locus was used to inbreed white clover to near-homozygosity ( $F=0.99$ ). Inbreeding depression was higher in the glasshouse experiment than was observed under sheep grazing in the field. It was also higher for comparable generations with a 54% reduction in herbage yield of the  $S_1$  generation in the glasshouse compared with 29% yield reduction under grazing. The level of inbreeding depression for herbage yield of the  $S_1$  and  $S_2$  generations observed in the glasshouse were consistent with the theoretical reduction in yield. However in later generations the actual herbage yield was consistently higher than expected. This may be due to heterozygosity being maintained during inbreeding or to the elimination of weaker individuals under higher competition and grazing experienced under field conditions. The degree to which inbreeding depression is overcome by outcrossing (heterosis) in white clover will determine whether hybrids or semi-hybrid cultivars can be successfully developed.

### Introduction

The annual productivity of most temperate pastures is well below their biological potential, and this feed barrier represents a major challenge for forage improvement (Woodfield and Easton 2004). Faster genetic improvement, than the current 1% per annum in major forages such as white clover (*Trifolium repens* L.), is required to meet the demands imposed by the rapid intensification of farming systems internationally. New breeding methods that identify and capture heterosis (hybrid vigour) are required.

Inbreeding depression and hybrid vigour are complementary phenomena; the greater the inbreeding depression, the greater the observed heterosis (Bingham 1983). Most outcrossing species, including white clover carry a high genetic load of deleterious recessive alleles and these are uncovered (and reduced) through inbreeding and selection. Inbreeding depression frequently involves a decrease in herbage yield, fertility and general plant vigour, but inbreeding can also produce inbreds that have superior breeding value to their original progenitors. In diploids and disomic polyploids such as white clover, heterosis should be maximised in the single-cross between inbred parents (Bingham 1983). These inbred parents are generally developed through several generations of selfing or sib-mating in a cross-pollinated species.

White clover has a multi-allelic gametophytic self-incompatibility system based at the S locus, but, self-incompatibility can be overcome in two ways. Firstly, the use of high temperatures has been shown to allow a low frequency of plants to self-pollinate (Douglas and Connolly 1989). Secondly, self-compatible genotypes and a dominant self-compatible allele (*Sf*) were identified at the S locus as early as 1942 (Atwood 1942). Despite the availability of these methods, self-fertility has not been systematically used in population improvement schemes in white clover until recently (Yamada *et al.* 1989, Michaelson-Yeates *et al.* 1997).

This paper presents results on inbreeding depression from a grazed field trial with three generations of inbreeding in eight cultivars and a glasshouse study involving eight generations of inbreeding in three cultivars.

## **Materials and methods**

### Field trials

Eight white clover cultivars (Dutch, Elen, Kersey, Sustain, Crau, Kopu, Irrigation and Haifa) were inbred for three generations by self-pollination, using high temperature (35°C) to overcome self-incompatibility. Seed of each of the resultant inbred generations ( $S_1$ ,  $S_2$  and  $S_3$ ) and their respective non-inbred parental cultivar ( $S_0$ ) were sown in the glasshouse and grown for 12 weeks prior to transplanting. Seedlings were transplanted into perennial ryegrass swards at two locations in Manawatu, New Zealand. Individual plots (1-m rows) contained 10 seedlings planted at 10-cm spacings, with 1 m between neighbouring plots, with each line replicated in four randomised complete blocks. The performance of the inbred lines was monitored for 3 years with herbage dry matter (DM) harvests to 2 cm occurring immediately prior to each grazing. Harvested herbage was separated into white clover and grass to determine dry weight (g) of each component. Plots were rotationally grazed by sheep an average of seven times per year. Plant spread (cm) and stolon growing point density (no.m<sup>-2</sup> using a 100 cm<sup>2</sup> quadrat) were assessed on all plots during the first 2 years.

### Glasshouse experiment

Three cultivars (Huia, Crau and Nomad) were crossed with a white clover genotype containing a dominant self-fertile allele ( $S_f$ ), and the resulting  $F_1$  progeny were backcrossed for three generations to the original cultivar. Different genotypes randomly selected from the original cultivars were used in each backcross. Self-fertile genotypes from the  $BC_3$  generation were self-pollinated for eight generations to generate the lines evaluated in this experiment. Residual seed of each generation, except the  $S_5$  generation for which there was insufficient residual seed, were sown in the glasshouse in September 2004. Individual genotypes were grown in 0.7-litre pots containing 420 g of sand/soil/peat mix. A completely randomised design was used with up to 10 genotypes per generation for each population. The pots were randomised in the glasshouse and re-randomised on a weekly basis to avoid position effects. The plants were harvested for herbage yield on three occasions at 6-weekly intervals in November, December and February. Harvested material was dried and weighed.

## **Results**

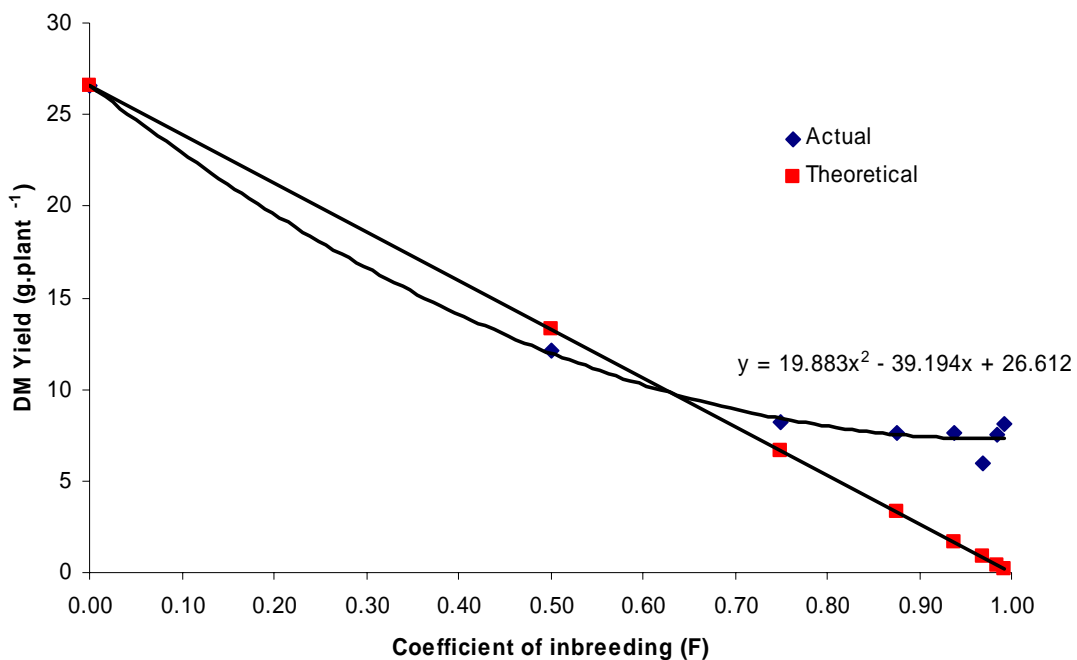
Under grazing there was a 29% decline in herbage yield in the  $S_1$  generation, a further 5% decline in  $S_2$  performance, and a further 18% decline in yield of the  $S_3$  generation (Table 1). These decreases in yield are less than would be expected based on the theoretical decrease in heterozygosity of 50%, 75% and 87.5% in these generations compared to the non-inbred  $S_0$  control. Plant spread and stolon density was generally less affected by inbreeding than herbage yield, with the exception of stolon density in the  $S_2$  generation (Table 1).

Table 1. Mean herbage yield, plant spread and stolon density of eight cultivars during three generations of selfing relative to non-inbred (=100%) control across two locations.

Trait	Generation				LSD <sub>0.05</sub>
	S <sub>0</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	
Herbage Yield	100	71	66	48	15
Plant Spread	100	91	86	72	13
Stolon density	100	82	63	74	19

In the glasshouse experiment there was a 54% decline in herbage yield in the S<sub>1</sub> generation, a further 14% decline in S<sub>2</sub> performance, but the yield of the subsequent generations was very similar to that of the S<sub>2</sub> generation (Figure 1). The impact of inbreeding on herbage yield in the early generations is close to that predicted on the basis of the change in theoretical change in coefficient of inbreeding (F). However, in later generations herbage yield is higher than is expected based on the level of inbreeding (Figure 1).

Figure 1. Actual and theoretical herbage yield of eight inbred generations of white clover compared with the non-inbred control.



### Discussion

The severe inbreeding depression that occurs with self-pollination in white clover emphasises the reasons for the overwhelming use of open-pollinated recurrent selection schemes for population improvement in white clover. The decrease in DM yield of the S<sub>1</sub> generation in the field trials is consistent with that reported in smaller previous studies of inbreeding in white clover (Atwood 1938, Yamada *et al.* 1989). Both authors reported approximately 30% lower yield in S<sub>1</sub> families than their non-inbred controls

which fits well with the 29% yield reduction observed under grazing across eight cultivars.

Stolon density and plant spread exhibited less inbreeding depression in general than was observed for herbage yield. These traits are important contributors to the persistence and herbage yield of white clover. It is apparent, however, that not all traits are equally affected by inbreeding and that the overall yield loss is made up of smaller effects on component traits.

The yield loss in the glasshouse study was more severe than that observed under grazing in the field. This indicates that selection for higher-yielding genotypes in the field plots offset some of the inbreeding depression observed in the glasshouse, where even very weak individuals are maintained and measured throughout the experiment. In the field these weaker genotypes are eliminated through the inability to compete with the combined effects of competition and grazing stresses. This level of yield reduction from selfing results from the fixation of unfavourable alleles at many loci. The rapid reduction in heterozygosity may offset the potential improvement in breeding value targeted through inbreeding. Selfing has been shown to result in more rapid inbreeding depression than sib-mating in white clover (Carnahan 1960, Cope 1978), and a more systematic use of sib-mating should allow for more recombination and selection for favourable alleles during inbreeding.

In maize, a review of inbreeding data found that there was a linear relationship between percentage homozygosity and the performance of quantitative traits (Hallauer and Miranda 1988). Our results for the glasshouse experiment show that the level of inbreeding depression for herbage yield in  $S_1$  and  $S_2$  generations is consistent with the theoretical reduction in yield. However in later generations the actual yield was consistently higher than expected. This may be due to heterozygosity being maintained during inbreeding or may be due to the elimination of weaker individuals.

This study has quantified the magnitude of inbreeding depression and the rate of inbreeding depression across a range of white clover genetic backgrounds. The next stages of this research involve determining whether inbreeding has led to an improvement in the breeding value of the surviving inbreds. Michaelson-Yeates *et al.* (1997) reported high-parent heterosis for herbage yield in approximately half of the crosses between inbred white clover parents that they evaluated. The degree to which inbreeding depression is overcome by outcrossing (heterosis) in white clover will determine the optimal breeding strategies for future population improvement.

Hybrid cultivars are used in maize, tomatoes, onions, sorghum, broccoli, cabbage, carrots, beets and pearl millet production systems but have not been successfully commercialised in any forage crops. The lack of success to date led Brummer (1999) to suggest a semi-hybrid breeding scheme to capture partial heterosis in forage crops. Both hybrid and semi-hybrid breeding schemes require the identification of heterotic groups that can be further enhanced through divergent selection to provide long-term population improvement. Heterotic groups have not been clearly established in white clover, although a survey of the genetic background of successful cultivars such as Crusader (Woodfield *et al.* 2006) in Europe does indicate that Southern European germplasm and Middle-Eastern germplasm constitute potential heterotic groups.

Hybrid and semi-hybrid cultivars offer the potential to significantly increase herbage yield, as well as increasing the uniformity of the final cultivar. This is particularly important given the increasing pressure that white clover is under to be

more productive and fix greater levels of nitrogen in the rapidly intensifying agricultural systems being used in New Zealand (Woodfield and Easton 2004).

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