INTRODUCTION OF VEGETATIVE AND REPRODUCTIVE CHARACTERS INTO TRIFOLIUM REPENS BY INTERSPECIFIC HYBRIDISATION

A.H. Marshall, T.P.T. Michaelson-Yeates, M. Meredith and D.H.Hides
Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, Dyfed SY23 3 EB, UK

ABSTRACT
Interspecific hybridization between white clover (*Trifolium repens* L.) and ball clover (*T.nigrescens*) and Caucasian clover (*T.ambiguum*) is a means of improving the reproductive growth and persistency of *T.repens*. F1 hybrids between *T.repens* and *T.nigrescens* were produced without ovule culture and confirmed as true hybrids by chromosome counts, isoenzyme analysis and leaf markers. Their growth habit was generally intermediate between the two parents. After backcrossing to *T.repens* the BC1 and BC2 hybrids had more of the characteristics associated with *T.repens* though they retained some of the greater reproductive growth of *T.nigrescens*. Hybrids between *T.repens* and *T.ambiguum* were produced using ovule culture. Two F1 seedlings were produced with a morphology intermediate to the two parents. 35 BC1 seedlings were produced with a similar morphology to the F1 but with less of the *T.ambiguum* growth habit. The BC2 population showed greater variability in the expression of characters from Caucasian clover and contained only 10% of plants with the combined stoloniferous and rhizomatous habit. The implications for improving the growth of *T.repens* by interspecific hybridization are discussed.

KEYWORDS
Interspecific hybridization, white clover, *T.nigrescens*, *T.ambiguum*, plant growth

INTRODUCTION
White clover (*Trifolium repens* L.) (2n=4x=32) is an important component of temperate pastures. In a mixture with grasses it provides herbage of a high nutritive value and contributes a considerable quantity of nitrogen to the sward. Breeding programmes of *T.repens* seek to improve agronomic characteristics such as dry matter production and persistency and seed yield potential. Where variation does not exist within *T.repens*, important traits can be introduced by interspecific hybridization. Within the *Trifolium* genus, there are more than 250 species and a number of these can produce occasional hybrids with *T.repens*. The annual species, *T.nigrescens* (2n=2x=16) has important reproductive characteristics of potential benefit to *T.repens* which has variable seed yields. Caucasian clover, *T.ambiguum* (2n=4x=32) has a rhizomatous growth habit, superior persistency and resistance to viruses that infect *T.repens*. Both species are invaluable as a source of novel germplasm if the genes controlling these characters could be transferred into *T.repens*. This paper describes the production of hybrids between *T.repens* and these species and the consequences of hybridization on growth of *T.repens*.

METHODS
***T.repens*/*T.nigrescens* hybrids.** Individuals from three populations of *T.nigrescens* were crossed to a large number of *T.repens* genotypes from widely differing populations. With *T.nigrescens* as the pollen parent, most crosses produced some seed. No seed was produced in any of the reciprocal crosses. Embryo rescue techniques were unnecessary as seeds developed *in situ*. There was considerable variation in cross-compatibility, but two interspecific combinations of accessions gave consistently high seed set (1.6 seeds/pod), one of which formed the base for development of further material. Five hand crosses were produced between Ac3715, a high yielding variety of *T.repens* and Ah1524, a population of *T.nigrescens* from Portugal. The development of the F1 and BC1 generation has been described previously (Marshall et al., 1995). These hybrids were confirmed by chromosome counts, isoenzymes and leaf markers. Morphological characterisation was undertaken on plants of each of the parental lines and hybrids grown in a controlled environment in which the day/night temperature was maintained at 20/15C. After 13 weeks growth, the number of inflorescences per plant, primary stolons, and the number of reproductive (those bearing inflorescences) and non-reproductive (not bearing inflorescences) stolons were measured.

***T.repens*/ *T.ambiguum* hybrids.** Plants of *T.ambiguum* (2n=4x=32) were obtained from a wild population (Ah1256) collected in Turkey. The *T.repens* (2n=4x=32) genotype used was derived from crosses between cv.S184 and a collection from Switzerland Ac3782. Details of the development of the F1, BC1 and BC2 generations have been described elsewhere (Meredith et al., 1995). This included chromosome counts, isoenzyme analysis, leaf markers, fluorescence in situ hybridisation (FISH) and morphological characterisation.

RESULTS AND DISCUSSION
Species hybridisation is a means of extending the range of heritable variation, which can be exploited by the plant breeder. Brewbaker and Keim (1953) were among the first to suggest interspecific hybridization of *Trifolium* spp. as a means of improving some of the vegetative and reproductive characteristics of *T.repens*. In this study, using *T.repens* as the female parent, specific cross-combinations with *T.nigrescens* produced for the first time, large numbers of hybrids without resorting to artificial techniques. In addition a backcross generation to *T.repens* (BC_1) has also been produced, confirmed as a true backcross by chromosome counts, isoenzyme analysis and leaf markers (Marshall et al., 1995).

T.nigrescens is a profuse flowering annual species and the objective was to introduce some of its reproductive characteristics into *T.repens*. At harvest, *T.repens* had more primary stolons /plant than *T.nigrescens* and both hybrids, and *T.nigrescens* had fewer than all plants except the F1's (Table 1). *T.repens* also had more vegetative stolons and a higher proportion of vegetative stolons than *T.nigrescens* and the F1 hybrids: the difference between *T.nigrescens* and the F1 however was not significant. *T.nigrescens* had fewest reproductive stolons but the highest % reproductive stolons whilst *T.repens* had a lower proportion of reproductive stolons than *T.nigrescens* than all hybrids. Overall, *T.nigrescens* produced more inflorescences per plant than the hybrids, which were found to be similar, while *T.repens* produced fewest. Although the BC1's had fewer vegetative stolons than *T.repens*, it should be possible to combine characteristics which will ensure persistency similar to *T.repens* whilst increasing reproductive growth. Those characteristics associated with the annual species *T.nigrescens* (profuse flowering, absence of nodal roots) are dominant over the same characters found in *T.repens*. Further breeding is necessary to determine the extent to which vegetative growth can be maintained whilst improving reproductive growth.

The production of *T.repens* x *T.ambiguum* hybrids necessitated the use of ovule culture (Meredith et al., 1995), based on the procedure described by Yamada et al., (1989). This resulted in the production of two hybrid seedlings, confirmed as hybrids by scoring for genetic markers at the PGI/2 locus. The morphology of the F1 hybrids was

intermediate to the two parents. The majority of the BC1 plants had 48 chromosomes, being produced from the fertilization of unreduced female gametes of the F1 hybrid and *T.repens* pollen. Regular meiosis in the BC1 hybrids resulted in the production of balanced gametes containing the full complement of *T.repens* chromosomes and the polyhaploid set of *T.ambiguum* chromosomes. There was no difficulty in establishing a substantial population of BC2 hybrids. The meiotic behaviour of the BC1 hybrids indicated that it should be possible to transfer genes from *T.ambiguum* to *T.repens*. In terms of morphological characters, the BC1 hybrids were similar to the F1 hybrid but the *T.ambiguum* characters were less pronounced than in the F1. The mean of the ratio of the length to width of the leaflet was closer to that of *T.repens* than *T.ambiguum*. Although all the BC1's produced stolons there was some evidence of rhizomatous growth since some of the shoots originated from growing points below the soil surface. In the BC2, leaf shape was more variable and varied from the values of the F1 hybrid to *T.repens*. Only 24 out of 236 BC2 plants showed signs of the development of a rhizome. Further studies are being carried out to examine the morphology of the BC3 hybrids and the effect of the rhizomatous character on plant development and persistency.

This work has shown that traits can be introduced into *T.repens* by interspecific hybridisation with both *T.nigrescens* and *T.ambiguum*. Further work is necessary to assess the products of further backcrossing and the effect of these traits on the growth and development of *T.repens*.

REFERENCES

Brewbaker, J.L. and W.F. Keim. 1953. A fertile interspecific hybrid in *Trifolium* (4n *T.repens* L. x 4n *T.nigrescens* Viv.). Am. Nat. **87**: 323-326.

Marshall, A.H., T.P.T. Michaelson-Yeates, P. Aluka and M. Meredith. 1995. Reproductive characters of interspecific hybrids between *Trifolium repens* L. and *T.nigrescens* Viv. Heredity **74**: 136-145.

Meredith, M.R., T.P.T. Michaelson-Yeates, H.J. Ougham and H. Thomas. 1995. *Trifolium ambiguum* as a source of variation in the breeding of white clover. Euphytica **82**: 185-191.

Yamada, T., H. Fukuoka and S. Higuchi. 1989. Interspecific hybridisation of tetraploid Kura clover (*Trifolium ambiguum* M. Bieb.) and white clover (*T.repens* L.) using ovule culture. J. Jap. Soc. Grassland Sci. **35**: 180-185.

Table 1
Vegetative and reproductive stolons and inflorescences per plant of *Trifolium* species and hhybrids

	Stolons /plant			
Species/hybrid	Primary	Vegetative No. (% of total)	Reproductive No. (% of total)	Inflorescences/ plant
T.repens	18.5	9.4 (50.8)	9.1 (49.2)	26.5
F1 hybrid	10.5	2.5 (23.8)	8.0 (26.2)	83.9
BC1 hybrid	14.4	5.6 (38.9)	8.8 (61.1)	80.9
T.nigrescens	7.5	0 (0)	7.5 (100.0)	265.4
LSD (p=0.05)	5.9	6.0 24.0	4.4 24.0	38.0