Effects of white clover cultivar and companion grass on winter survival of seedlings in autumn-sown swards

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Research Note

Effects of white clover cultivar and companion grass on winter survival of seedlings in autumn-sown swards

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Abstract

The aim was to study the effects of white clover cultivar and combinations with perennial ryegrass cultivars on seedling establishment in autumn-sown swards and on winter survival of seedlings. Large-leaved white clover cv. Alice and small-leaved white clover cv. Gwenda, and an erect and a prostrate perennial ryegrass cultivar were sown in autumn in pure stands and as four binary grass-clover mixtures. Mixtures of white clover cv. Huia and Aberherald with perennial ryegrass were also sown. Companion grasses had no significant impact on the establishment of white clover. The number of seedlings of white clover cv. Alice in mixtures (335 m^{-2}) was higher than cv. Gwenda (183 m^{-2}) and pure swards had similar white clover population densities as mixed swards. White clover cv. Huia tended to have more seedlings than Aberherald (355 and 205 m^{-2} respectively). No stolons were produced prior to a severe winter, because of the late sowing date. Winter survival of clover seedlings was 0·56 in mixtures and 0·69 in pure stands, irrespective of white clover or companion grass cultivar. Stolon development of white clover in autumn is often considered essential for overwintering survival and spring growth. In this study, there was considerable survival of the non-stoloniferous tap-rooted seedlings of all four clover cultivars despite a severe winter.

Keywords: stolon, perennial ryegrass, grass and white clover mixtures, seedling establishment, cultivars

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Introduction

White clover (*Trifolium repens* L.) is usually grown in cultivated pastures with companion grasses, mainly perennial ryegrass (*Lolium perenne* L.). Reliable establishment of white clover is crucial for a wider adoption of white clover-based swards. Spring sowing generally results in higher white clover yields and contents during the first harvest year than autumn sowing (e.g. Younie *et al.*, 1985). However, in practice, grasslands are often resown in autumn and white clover may be vulnerable to winter damage in these circumstances (Younie, 1998). Therefore, it is relevant to investigate if white clover cultivars that are frequently used in northern Europe, such as Alice and Gwenda, are tolerant to late sowing. Another cultivar (Aberherald) has been selected for higher winter survival and increased spring growth (Wachendorf *et al.*, 2001).

There are indications (e.g. Evans *et al.*, 1985) that white clover performance may be influenced by the growth habit of companion grass cultivars. Interaction between grass and clover cultivars at the seedling stage (Collins *et al.*, 1996) might explain the performance of specific mixtures. In most agronomic studies the white clover content is generally estimated visually or assessed when the established sward is cut rather than during establishment. Consequently, there is a lack of detailed information during the seedling stage. The aim of this work was to study the effect of white clover cultivars in combinations with grass cultivars on white clover establishment and winter survival of white clover seedlings in autumn-sown perennial ryegrass–white clover mixtures.

Materials and methods

Experimental site, design and management

Four experiments were established on former arable land on a sandy soil at Wageningen, The Netherlands (51°58′N/5°40′E, 7 ma.s.l.) on 2 October 1995. The sandy soil layer had a thickness of 0·6 m and had been put on top of a clay soil layer in 1971. Soil analyses (0–25 cm) revealed that the soil contained 2·5% organic matter and the pH (KCl) was 4·8. Prior to sowing, the soil was limed, and N, P and K were each applied at 25 kg ha^{-1}.

Certified seeds from the diploid perennial ryegrass [*L. perenne*; cv. Heraut (H) (prostrate) and Barlet (B) (erect)] and the white clover [*T. repens*; cv. Alice (A) (large-leaved) and Gwenda (G) (small-leaved)], were used. Mixtures are henceforth referred to by the first letters of their component cultivar names. Heraut and Barlet are both diploid and have similar heading dates (30 May and 1 June respectively). In recommended list trials both are persistent (8); winter hardiness and rate of spring development are slightly higher in Heraut (7) than in Barlet (6·5) on a scale from 1 (poor) to 10 (excellent). The yield potential of both cultivars is similar. White clover seed was sown at 4 kg ha^{-1} and perennial ryegrass seed at 25 kg ha^{-1} in all experiments by hand.

In Experiment 1, four mixtures were sown (HA, BA, HG and BG). In Experiment 2, the two white clover cultivars (A, G) were sown and in Experiment 3, perennial ryegrass monocultures (H and B). Experiment 4, comprising eight plots, was sown with two mixtures, consisting of the diploid perennial ryegrass cv. Preference with medium-leaved white clover cv. Huia and Aberherald. Mixtures are abbreviated as PHu and PAh. This experiment was part of a larger experiment on overwintering and spring growth of white clover (Wachendorf *et al.*, 2001). The heading date of Preference is 4 June, its relative assessment marks are persistence (8), winter hardiness (7·5) and rate of spring development (6). Its yield potential is 0·04 lower than that of Barlet and Heraut.

Data for growth habit were obtained from official cultivar descriptions based on DUS (Distinctness, Uniformity and Stability) tests for cultivar registration; Barlet and Preference are erect (4) and Heraut is prostrate (6) (D. J. A. Klein Geltink, personal communication).

Phosphate and potash fertilizers were applied in March according to requirements as indicated by recommendations based on soil analyses (Elgersma *et al.*, 2000). All experiments had a randomized complete design with four replications in Experiments 1 and 4 (mixtures), two replications in Experiment 2 (white clover) and six replications in Experiment 3 (perennial ryegrass). Weather data were obtained from the Haarweg meteorological station, located 500 m from the experimental site.

Seedling measurements

Three soil cores (0·25 dm^2, 10 cm depth) were taken from each plot 8 weeks after sowing on 28 November 1995. The number of plants of perennial ryegrass, white clover, and unsown grasses and herbs were recorded. The soil was carefully washed off the seedlings so as to retain as much root material as possible. In randomly taken intact perennial ryegrass plants (fifty-nine in Experiments 1 and 3, and twenty-one in Experiment 4), tillers were counted, and shoot height and root depth were measured. In nineteen clover seedlings from Experiments 1 and 2 and six from Experiment 4, the number of trifoliate leaves, exceeding growth stage 0·8 (Carlson, 1966), root depth (cm) and the number of nodules were determined.

On 7 May, two cores of 1 dm^2 and 10 cm depth were taken from each plot, core size conforming to a common protocol described by Wachendorf *et al.* (2001). Tiller numbers of perennial ryegrass, white clover seedlings and other plants were determined.

Seedling survival

On 25 November 1995, about 8 weeks after sowing, permanent quadrats were established in Experiments 1, 2 and 4. In each plot, ten quadrats of 1 dm^2 were marked and the number of white clover seedlings was recorded in each quadrat. On 29 February and 27 March 1996, the number of white clover plants was assessed in the same quadrats to determine seedling survival. Data were averaged for each quadrat, and mean quadrat values were averaged for each plot.

Statistical analyses

GENSTAT 5 software (GENSTAT, 1987) was used for all analyses. The plot was the experimental unit. Standard analysis of variance (ANOVA) was performed to test for significance between mean values of the treatments in each experiment. Data were transformed to satisfy the requirement for constant variance in ANOVA, where necessary.

Within each experiment, mean values for the individual treatments were compared at the 0·05 probability level based on the *F*-test of ANOVA. For comparisons among experiments, differences between mean values were analysed using Student's *t*-test.

Results and discussion

Establishment and seedling characteristics

During October 1995 the weather was mild. Many seeds of unsown species germinated, mainly *Spergula arvensis* L. and annual meadowgrass (*Poa annua* L.). Mean plant population densities 8 weeks after sowing are presented in Table 1.

The establishment of the experiments was successful; a target of 150 white clover plants per m^2 3 months

Table 1 Mean number of seedlings (numbers per m^2, s.e.m.) sampled 8 weeks after sowing, on 28 November 1995.

	Experiment 1 (mixture)	Experiment 2 (pure clover)	Experiment 3 (pure grass)	Experiment 4 (mixture)
Trifolium repens	270 (80)	200* (3)	–	250 (17)
Lolium perenne	1300 (300)	–	730 (70)	867 (2)
Poa annua	970 (210)	970 (100)	1400 (270)	110 (4)
Spergula arvensis	820 (100)	700 (30)	1130 (130)	1400 (133)
Other species	570 (100)	200* (4)	100 (100)	67 (67)

*In two cases there was a significant cultivar effect ($P \leq 0{\cdot}001$); in pure stands the number of white clover seedlings per m^2 was higher in cv. Alice (270) than in cv. Gwenda (130) and there were more seedlings of other unsown species in plots with cv. Alice (330) than with cv. Gwenda (70).

after sowing has been proposed as an establishment goal (Haggar *et al.*, 1985). Differences in white clover population density between treatments within an experiment were restricted to Experiment 2, where Alice had a greater density than Gwenda (270 and 130 m^{-2} respectively), despite plots with Alice containing more plants of 'other species'. Otherwise, seedling numbers in any category did not differ between treatments within an experiment. Although there was a tendency for population density of sown species to be lower in monoculture than in mixture, as this comparison was across experiments, the difference may not be real. The population density of *P. annua* was much lower in Experiment 4 than in the other experiments.

The number of white clover seedlings in the ten 1 dm^2 quadrats 8 weeks after sowing is shown for each plot in Table 2. There was a wide range of seedling numbers per quadrat (0–13). The standard errors associated with seedling numbers per m^2 illustrate the large variability among the quadrats in each plot. The number of quadrats without any white clover seedlings ranged from none to four of ten measured.

There were differences among the four mixtures of Experiment 1 (Table 2), which were associated with white clover cultivar. In November 1995, the number of white clover plants was higher ($P = 0{\cdot}054$) in mixtures with Alice (335) than with Gwenda (183), but there was no effect of companion perennial ryegrass cultivar. In the pure white clover swards, the same trend was observed although the difference between seedling numbers in Alice (335) and Gwenda (190) was not significant. Although in Experiment 4 the difference was not significant, the number of Huia white clover plants per m^2 (355) tended to be higher than that of Aberherald (205). For all experiments, the values found in the quadrats (Table 2) were comparable with those found in the core samples (Table 1).

Apart from cultivar effects, establishment and seedling vigour can also be affected by differences in seed quality. Quality is affected by the environment in which the seed is produced, and also by storage conditions and duration. However, because basic seed of Alice, Gwenda and Huia, which had been produced and stored under similar conditions, was used, it was assumed that the seed quality of those cultivars was not an important factor in this study. Characteristics of seedlings were similar for pure stands and mixtures in all experiments. In Experiments 1 and 3, randomly taken grass seedlings had mean values of 3·1 tillers per plant, a shoot height (s.e.m.) of 6·9 (0·6) cm and a maximum root depth of 7·0 (0·5) cm in mixtures. In Experiments 1 and 2, fifteen of nineteen white clover seedlings had nodules (2·3 on average, ranging from 1 to 4) and had on average 1·9 trifoliate leaves and a root depth of 5·8 (0·3) cm. The absence of an interaction between the contrasting growth habits of perennial ryegrass cultivars and white clover is in contrast with the findings of Collins *et al.* (1996).

In Experiment 4, there were no significant differences between the two mixtures containing white clover cv. Huia and Aberherald in the numbers of sown or unsown species (Table 1), grass seedling tiller number, shoot height or root depth (Table 2). On average, grass seedlings had 3·4 tillers per plant, a shoot height of 6·7 (0·1) cm and a maximum root depth of 6·8 (0·2) cm in mixtures, which is comparable with the values found in Experiment 1. White clover seedlings had on average 2·4 trifoliate leaves and their root depth was 5·5 (0·1) cm, but unfortunately only a very small sample was examined. The three Huia seedlings were not nodulated, whereas the three Aberherald seedlings had 1–3 nodules.

Weather conditions during winter

Frosts started in December and there was a severe period of frost with some snow cover until mid-January. A second frost period without snow occurred from mid-January until late February. The minimum air temperature in the canopy (at 10 cm height) was frequently below zero. The minimum soil temperature under grass was below zero from 26 January until 17 February; the lowest recorded soil temperature during this period was −1·8°C at −5 cm and −0·9°C at −10 cm (data not shown). As the maximum soil temperatures at

Table 2 Number of white clover plants (m^{-2}) in autumn (25 November 1995) and spring (29 February 1996) (both based on ten 1 dm^2 quadrats per plot) and proportion of seedlings that survived (based on quadrats marked in November that contained white clover).

	Cultivar combinations				Level of significance	s.e.d.	Mean
	HA*	BA	HG	BG			
Experiment 1							
Number of seedlings							
Autumn	305	365	195	170	0·054	44·2	259
Spring	200	275	75	105	*	30·1	164
Proportion of seedlings that survived	0·56	0·67	0·41	0·61	NS	0·149	0·56
	PHu		PAh				
Experiment 4							
Number of seedlings							
Autumn	355		205		NS	84·7	280
Spring	175		120		NS	35·2	148
Proportion of seedlings that survived	48		52		NS	5·1	0·50
	Cultivar						
	A		G				
Experiment 2							
Number of seedlings							
Autumn	335		190		NS	75·0	262
Spring	270		160		NS	19·8	215
Proportion of seedlings that survived	0·79		0·59		NS	4·1	0·69

Perennial ryegrass cv. H, Heraut; B, Barlet; White clover cv. A, Alice; G, Gwenda; PHu and PAh, perennial ryegrass cv. Preference with medium-leaved white clover cv. Huia and Aberherald respectively; NS, not significant.
*$P \leq 0{\cdot}05$.

−10 cm were also below zero from 31 January until 10 February, entire grass and clover seedlings including their roots, were frozen during this period.

Effects of white clover and perennial ryegrass cultivar on seedling density after a frost period

In February 1996, in Experiment 1 all eighty marked quadrats were intact, but in Experiment 2 five of forty and in Experiment 4 six of eighty marked quadrats had to be replaced as a result of disturbance of the tags. Both the November and February estimates of seedling numbers presented in Table 2 are thus based on ten quadrats per plot.

In February, a significant difference in seedling numbers per m^2 was found in Experiment 1 between the four mixtures (Table 2) and there were significantly ($P < 0{\cdot}01$) more white clover plants per m^2 in mixtures with Alice (238) than with Gwenda (90). The effect of perennial ryegrass cultivar on white clover seedling number was not significant ($P = 0{\cdot}087$), but there was a tendency for a higher white clover density with Barlet (190) than with Heraut (138). There was no grass × clover cultivar interactions. In Experiments 2 and 4, there were no differences between treatments.

White clover seedling survival

The assessment of winter survival is based on white clover plants in originally marked quadrats. Hence, the values presented do not correspond with the spring population density as a proportion of the autumn population density data described earlier. More than 5000 white clover seedlings were examined to measure winter survival. There were no significant differences in winter survival among white clover cultivars in any of the experiments (Table 2). On average, survival in the mixtures was 0·56 in Experiment 1, 0·50 in Experiment 4 and 0·69 in pure stands. Changes in numbers of seedlings between 29 February and 27 March were negligible (not shown). On 6 May an attempt was made to count the white clover seedlings again, but this proved difficult because of the tall, weedy canopy. Counts in three mixtures (twenty-five quadrats) showed a decreased number in five quadrats compared with March, but a higher number in thirteen quadrats, which must have been caused by spring-germinated white clover seeds. Winter survival of Huia and Aberherald was similar, despite the fact that Aberherald has been selected for higher winter survival combined with growth at low temperatures (increased spring growth) in Wales.

It is widely believed that well-developed stolons in autumn are considered beneficial to white clover growth in the following year (Frame and Newbould, 1986; Patterson *et al.*, 1995). For ley systems, Harris *et al.* (1983) warned that in colder latitudes the period after harvesting winter cereals might be too short for satisfactory plant development before winter onset. However, in the late-sown white clover in this study, no stolons had developed before winter; in fact stolons only started to develop in May in Experiments 1, 2 and 4. Despite the severe winter with two frost periods with very low temperatures, followed by a very dry, cool spring, the survival of the small non-stoloniferous seedlings was sufficient and subsequent establishment of the swards was satisfactory (Elgersma *et al.*, 2000). Possibly the tap roots of the seedlings contained enough carbohydrate reserves, which are stored in stolons in more mature white clover plants, but this was not tested. There were no significant differences between mixtures, i.e. no effects of perennial ryegrass or white clover cultivar on establishment, seedling morphology or seedling survival.

Factors other than climate are known to damage white clover plants over winter, e.g. slugs (*Deroceras reticulatum*), waterlogging, competition from weeds, or *Sitona* weevil. However, in this study none of these was important.

Conclusion

Although there was an indication that swards with the erect perennial ryegrass cultivar Barlet had a higher white clover population density in late winter than those with the prostrate cutivar Heraut, generally the perennial ryegrass cultivars did not affect white clover establishment. Despite differences in white clover seedling population density between cultivars sown in autumn and, while all white clover cultivars suffered a decline in plant numbers over winter, population density was sufficiently high for white clover in all swards to establish satisfactorily. Following autumn-sowing, the winter survival of non-stoloniferous clover seedlings was 0·56 in mixtures and 0·69 in pure stands, irrespective of white clover or companion perennial ryegrass cultivar.

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