



Leaf Area, Competition with Grass, and Clover Cultivar: Key Factors to Successful Overwintering and Fast Regrowth of White Clover (*Trifolium repens* L.) in Spring

ANDREAS LÜSCHER*, BARBARA STÄHELI, RENATE BRAUN and JOSEF NÖSBERGER

Institute of Plant Sciences, Swiss Federal Institute of Technology (ETH), CH-8092 Zurich, Switzerland

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The greater sensitivity of white clover (*Trifolium repens* L.) to low temperature compared with perennial ryegrass (*Lolium perenne* L.) is a major problem in sustaining the relative contributions to yield of the two species in mixed swards. The objectives of this investigation were to examine the dynamics of leaf development of two white clover cultivars, AberHerald and Grasslands Huia, under field conditions, and to determine the significance of leaf area in winter, and of competition by perennial ryegrass, for the overwintering and regrowth of white clover in spring. Undeveloped white clover plants developed 3.8–6.6 new leaves between late autumn and early spring, and stolon dry matter and total non-structural carbohydrates (TNC) content increased by 262 and by 68 % respectively. In contrast, white clover plants that were defoliated frequently during the winter showed a 28 % decrease in stolon dry matter and an 82 % decrease in the content of TNC. Frequent defoliation in winter caused severe reduction in the rates of emergence of nodes (by 60 %) and of buds (by 67 %), and the rate of death of nodes and buds increased by a factor of 10, leading to small stolon systems of individual plants in spring. Competition had similar, but weaker, effects to those of winter defoliation, presumably caused by shading of white clover leaves. AberHerald had a higher cumulative leaf emergence (by 22 %), a higher mean leaf number (by 23 %), a higher stolon DM (by 36 %) and a higher TNC content per plant (by 115 %) than Grasslands Huia. Results demonstrate the crucial positive role of leaf area during winter, the negative effect of grass competition, and the importance of the clover cultivar, for the overwintering and subsequent spring regrowth of white clover.

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Key words: *Trifolium repens* L., white clover, *Lolium perenne* L., perennial ryegrass, leaf development, death rate, stolon, carbohydrates, nodes, buds, competition, defoliation.

INTRODUCTION

Mixed ryegrass/white clover (*Lolium* species/*Trifolium repens* L.) swards play an important role in intensive grassland systems for ruminants in temperate climates. The high yield and forage quality, as well as the contribution of the legume to the nitrogen supply of the sward by symbiotic N₂ fixation (Boller and Nösberger, 1987; Zanetti *et al.*, 1996, 1997), make white clover a valuable forage crop. However, careful management of grass/clover swards is necessary to prevent the dominance of one species, or great seasonal or annual fluctuations in their relative yield contributions.

The maintenance of an adequate proportion of white clover in mixed grass/clover swards is hindered by the susceptibility of white clover to damage in winter and subsequent poor spring growth (Woledge *et al.*, 1990). Limited freezing tolerance, resulting in extensive death of stolons and buds, together with the low growth rate of clover at low temperatures, are now recognized as the most important competitive disadvantages of clover in mixed swards (Collins *et al.*, 1991; Frankow-Lindberg and von Fircks, 1998). In spring, the low growth rate of white clover at low temperatures (5–15 °C) coincides with much higher growth rates of the companion grass during reproductive

growth (Menzi *et al.*, 1991). In addition, the greater height of grasses in spring (Daepf *et al.*, 2001) hinders white clover in competing for light (Blum *et al.*, 2001). Thus, identification of the morphological and physiological characteristics that contribute to improved persistence and growth during winter, as well as an increased competitive ability of white clover at low temperature in spring, are of great importance.

T. repens is a temperate species that is adapted to regions from the Arctic to the subtropics, and from lowlands to the Alps (Jacot *et al.*, 2000a, b), with southern populations being generally frost tender and winter active, while the more frost-hardy northern populations usually survive winter by becoming dormant and unproductive (Eagles and Othman, 1981; Junttila *et al.*, 1990a, b). The wide latitudinal dispersion of white clover populations is accompanied by a conspicuous morphological pattern: southern populations tend to have thick stolons with long petioles and large leaves, whereas northern populations have thin stolons, small leaves and are prostrate (Boller and Nösberger, 1983; Williams, 1987; Aasmo Finne *et al.*, 2000). In cool regions, however, a combination of winter hardiness, fast growth at low temperatures, and high competitive ability in spring is desired.

The spring and annual yields of white clover are closely related to the number of surviving stolons and buds as well as to carbohydrate reserves in autumn and at the beginning of regrowth (Collins *et al.*, 1991; Collins and Rhodes, 1995;

* For correspondence at: Swiss Federal Research Station for Agroecology and Agriculture (FAL), CH-8046 Zurich, Switzerland. Fax +41 (0)1 377 72 01, e-mail andreas.luescher@fal.admin.ch

Frankow-Lindberg and von Fircks, 1998; Frankow-Lindberg, 1999). Furthermore, the rate of metabolism of reserve carbohydrate and the size of the reserve carbohydrate pool have been shown to be directly related to the life span of wintering plants and to the degree of frost hardiness (Junttila *et al.*, 1995; Sagisaka, 1995; Turner and Pollock, 1998). Stolons, and to a lesser extent roots, are the main sites for the accumulation of carbohydrate reserves (Boller and Nösberger, 1983). Corbel *et al.* (1999) showed that partitioning of dry matter to the below-ground tissues is distinctly greater at low temperature, thus showing the importance of maintaining an intact network of live stolons with roots throughout the winter. Therefore, interception of light by clover leaves may play a role in maintaining a sufficiently large carbohydrate pool for continued plant growth and survival during winter. However, information on how leaves contribute to overwintering and spring regrowth under field conditions is not available.

In the field, the functioning of leaves is expected to be adversely affected by (1) low temperature, (2) low irradiance, (3) short photoperiod and (4) shading by the companion grass. Campbell *et al.* (1999) showed that leaf emergence rate, leaf size and stolon extension rate are each affected differently by temperature, and that the growth potential from late winter to early spring appears to be strongly related to the ability to grow at 5 °C. The main aim of the European multisite research programme (Wachendorf *et al.*, 2001a, b) was to investigate the importance of leaf area and stolons, as well as the physiological characteristics of different clover cultivars, for the winter hardiness and regrowth of clover in spring under widely varying environmental conditions. In the three experiments presented here, more detailed measurements, especially on leaf emergence and leaf death, and additional treatments (defoliation, grass competition) to vary the amount and light interception of leaf area, were included to provide information which is complementary to that of the European multisite experiment. The objectives were to investigate the dynamics of leaf development and the importance of leaves and of competition by ryegrass for the successful overwintering of white clover and satisfactory regrowth in spring.

MATERIALS AND METHODS

Experimental site

The experiments were carried out in Eschikon (47°27'N and 8°41'E; 550 m asl) near Zurich, Switzerland, where the annual precipitation is 1100 mm (long-term mean). The soil is classified as a fertile, eutric cambisol with 19 % clay, 40 % silt and 41 % sand; the pH is 6.1 (measured in water). The air temperature (yellow spring thermistors, series 400) was measured at 2 m above the soil surface at a weather station 200 m from the experimental site. Monthly mean temperatures are means of the daily mean temperatures (24 h). Frost days are defined as having a minimum air temperature below 0 °C.

The weather varied considerably between winters during the experimental period (Table 1). In the winter of 1992/93 there were many frost days without snow cover up to the end of January. Compared with the temperature means recorded over the previous 15 years, the second winter (1993/94) was cold, with unusually frequent frosts in November and April, whereas the third winter (1994/95) was rather mild. Comparison of the temperature means recorded at the 12 sites participating in the European multisite experiment shows that Zurich is one of the colder sites, with low autumn and winter temperatures (Wachendorf *et al.*, 2001a).

Experimental design

Three consecutive field experiments were carried out, one during each winter period in 1992/93, 1993/94 and 1994/95. In 1992/93 and 1993/94, the factorial experiments included four treatments repeated 12 or ten times respectively. The four treatments resulted from the combination of two white clover cultivars, AberHerald and Grasslands Huia, grown as spaced plants or in an established perennial ryegrass (*Lolium perenne* L. cultivar Préférence) sward. In 1994/95, eight treatments and ten replicates were established. The treatments resulted from the combination of (1) two clover cultivars, (2) growth as spaced plants or in an established ryegrass sward, and (3) with or without frequent defoliation. Defoliation was carried out seven times between

TABLE 1. Mean monthly air temperature, number of frost days, days with snow cover during the experimental period (1992–95), and long-term mean air temperatures (1978–92)

Month	1992/93			1993/94			1994/95			1978–92
	*Temp. °C	Frost days	Snow days	*Temp. °C	Frost days	Snow days	*Temp. °C	Frost days	Snow days	*Temp. °C
Oct	7.0	2	0	6.9	1	0	7.9	3	0	9.8
Nov	5.6	4	0	0.4	15	0	6.4	0	0	4.2
Dec	–0.1	24	0	3.0	13	5	2.0	18	8	2.5
Jan	2.8	11	0	0.3	20	10	–1.0	27	24	–1.0
Feb	–1.1	25	13	–0.2	25	11	4.7	10	0	0.0
Mar	3.8	17	10	7.7	4	0	3.8	25	9	4.8
Apr	10.0	1	0	6.2	11	0	9.4	5	0	7.0

*air temperature at 2 m.

7 November and 16 March, and all leaves larger than the 0.5 Carlson stage (Carlson, 1966) were removed.

In 1992/93, 48 plots (4.2 m × 1.8 m), including a border of 0.6 m on each side were established, with five white clover plants per plot. In 1993/94, there were two plants in each of 40 plots, and in 1994/95 there were five plants in each plot. The plots measured 1 m × 1 m and were surrounded by a border of 0.2 m.

In 1992/93, white clover plants were sown in 'quick pots' at the end of May 1992. In 1993/94 and 1994/95, white clover plants were grown from cuttings which were sampled in mid-August at Eschikon from the plots of the European multisite experiment (Wachendorf *et al.*, 2001a). Stolon cuttings and seedlings were propagated in the glasshouse (18/13 °C day/night temperatures; photoperiod 16 h) for 1 month. In June 1992 and August 1993 and 1994, plants were transplanted to plots with bare soil (treatment: spaced plants) or to plots with established ryegrass swards (treatment: with competition) at intervals of 0.6 m × 0.6 m. After stolon elongation and nodal rooting, by the end of August 1992, one rooted stolon with ten nodes was excised from each parent plant with its taproot intact, and left in the plot; the parent plants were removed.

In the plots with perennial ryegrass, the grass was sown (20 kg ha⁻¹) at the end of June in 1992 and 1993. In 1994/95, the plots sown with perennial ryegrass in 1993 were used again. The ryegrass was cut three times during the summers of 1992 and 1993 and four times during the summer of 1994 at a height of 5 cm. Plots for spaced plants were cleared of weeds using 1.2% Roundup ([N-(phosphonomethyl)glycine]; Monsanto, St. Louis, USA) 2 weeks before planting the white clover. After planting, plots were weeded by hand throughout the experimental period to prevent competition.

Plots were fertilized in spring with 90 kg P₂O₅ ha⁻¹ and 180 kg K₂O ha⁻¹ applied as Foskal (15% P/30% K; Agrolin, Basel, Switzerland) before the beginning of the experiment. Nitrogen was applied as ammonium-nitrate (27.5% N) after sowing the grass and at the beginning of each regrowth period, resulting in a total annual application of 125 kg ha⁻¹. This is a moderate level of nitrogen fertilization, within the range recommended for grass/clover mixtures under the environmental conditions of the experimental site. From other experiments at the same site it is known that symbiotic N₂ fixation under these conditions contributes 60–90% of the N budget of white clover (Boller and Nösberger, 1987; Zanetti *et al.*, 1996, 1997).

Destructive sampling of white clover

On several occasions during the observation periods from October to April, a set of randomly chosen white clover plants was sampled destructively: four times in 1992/93, twice in 1993/94 and three times in 1994/95. The plants were removed from the soil, washed and divided into leaves and stolons. Clover laminae were counted and their surface measured with an area meter (Li-3000, Li-Cor, Lincoln, Nebraska, USA). Individual leaf size was calculated by dividing the total leaf area of the plants by the number of leaves. After oven drying at 60 °C for 48 h, stolon dry weight was determined.

Non-destructive observations

At the beginning of each winter, one plant per plot was randomly selected for non-destructive observations, while the other plants were reserved for destructive harvests. Eleven observations were made in 1992/93, seven in 1993/94 and three in 1994/95. In 1992/93 and 1993/94, the death or developmental stage of each leaf on the main stolon, according to the Carlson stages (Carlson, 1966), and the formation and loss of marked stolon branches, were monitored. In 1992/93 and 1993/94, leaf production between two observation dates was calculated by adding the Carlson stages of each leaf. In 1994/95, whole plants were used to monitor emergence, death and branching of the nodes. The number of leaves emerging between two observation dates was calculated by adding the increase in the Carlson stages of the youngest leaf and the Carlson stage of a new leaf, if present. Rates of leaf emergence were calculated on a per week basis and were related to the mean temperature of the corresponding period, measured 20 cm above the soil surface (yellow spring thermistors, series 400).

Carbohydrate extraction and analysis

Carbohydrate contents of stolons were analysed in 1994/95 at all three destructive harvests. Aliquots of the oven-dried and milled stolons were taken to determine the contents of water-soluble carbohydrates (WSC) and starch according to the method described by Fischer *et al.* (1997).

Statistical analysis

Statistical analyses were performed using the SAS (Statistical Analysis System, SAS Institute, Cary, North Carolina, USA) computer program. Analyses of variance were carried out separately for each individual harvest as well as overall (over the whole observation period) using the General Linear Model procedure. The experimental design was a complete randomized block. Non-destructive observations were repeated measurements on the same plant and were treated accordingly in the overall analyses, i.e. a split-plot design with the time of observation as the sub-plot factor (Gomez and Gomez, 1984). Thus, the effects of cultivar, competition and defoliation, as described here, were the main plot factors and were tested against the appropriate error term (error A). Linear regression analysis was carried out to calculate the relationship between temperature and the rate of leaf emergence for each treatment. Regression slopes were compared using the Student's *t*-test (Zar, 1984).

RESULTS

Leaf development

AberHerald had, on average, 23% more leaves on the main stolon than Grasslands Huia (cultivar, $P \leq 0.001$; Fig. 1A) in 1992/93 and 1993/94. In general, leaf number was lowest from December to February when AberHerald had 40% more leaves on the main stolon than Grasslands Huia (cultivar, $P \leq 0.05$). Competition with perennial ryegrass

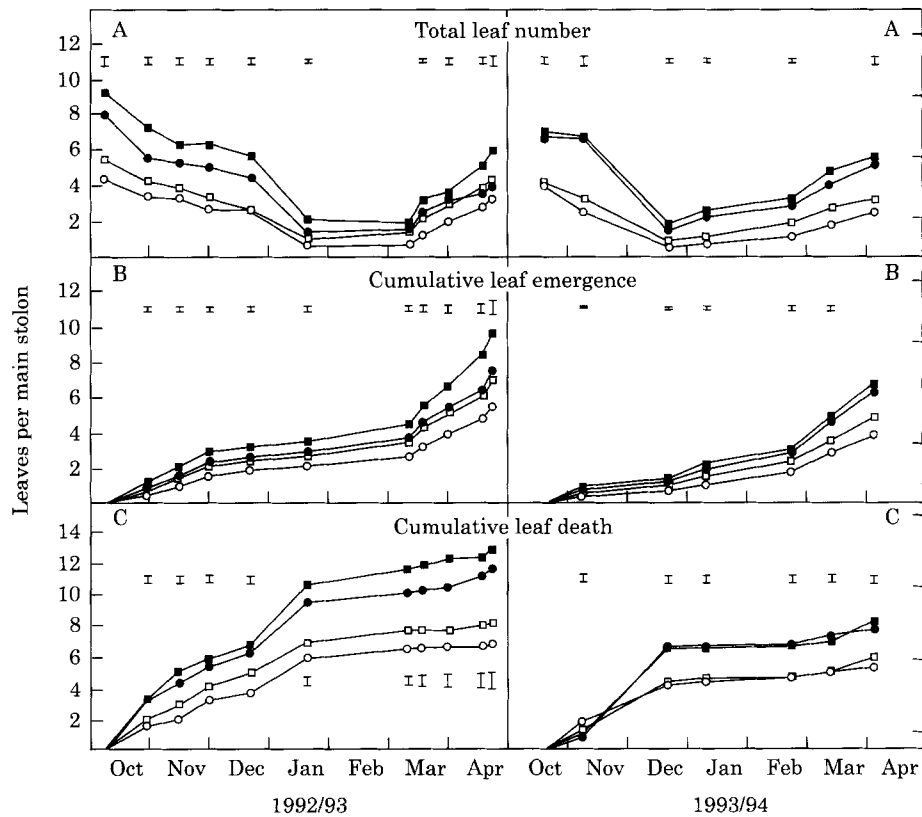


FIG. 1. Effects of competition with perennial ryegrass (*Lolium perenne* L.) on (A) the dynamics of leaf number, (B) cumulative leaf emergence, and (C) cumulative leaf death on the main stolon in two cultivars of white clover (*Trifolium repens* L.). Values are means of 12 (1992/93) and 10 (1993/94) replicates with s.e.m. ■, AberHerald spaced plants; □, AberHerald with competition; ●, Huia spaced plants; ○, Huia with competition.

caused a decrease in the number of leaves by 44 % compared with spaced plants (competition, $P \leq 0.0001$).

New leaves on the main stolon emerged at a low rate throughout the winter, resulting in 3.8–6.6 new leaves by the beginning of April (Fig. 1B). Cumulatively, over the whole observation period, AberHerald produced 22 % more new leaves than Grasslands Huia (cultivar, 92/93 $P \leq 0.0001$; 93/94 $P \leq 0.05$). Competition reduced cumulative leaf emergence by 28 % compared with spaced plants (competition, $P \leq 0.001$). The leaf emergence rate depended strongly on temperature (Fig. 2; $R^2 \geq 0.92$; $P \leq 0.001$), but with a common threshold temperature for leaf emergence of 2.9 °C (2.3–3.3 °C) in all treatments. A comparison of the regression coefficients showed that the leaf emergence rate of AberHerald increased more (by 31 %) per unit temperature increment than that of Grasslands Huia. The increase in the leaf emergence rate per unit temperature increment was 54 % greater for spaced plants than for plants grown with ryegrass.

Leaf death on the main stolon (Fig. 1C) was highest in autumn and winter (up to January in 1992/93, and up to December in 1993/94). The accumulated number of dead leaves tended to be higher for AberHerald than for Grasslands Huia (cultivar, $P \leq 0.05$, 92/93; ns, 93/94). However, calculated as a percentage of leaves present in

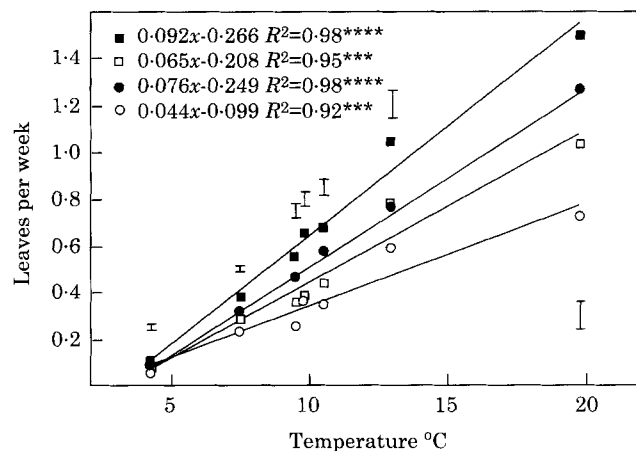


FIG. 2. Effects of competition with perennial ryegrass (*Lolium perenne* L.) on the temperature dependency of the leaf emergence rate on the main stolon in two cultivars of white clover (*Trifolium repens* L.). Leaf emergence rates of the different periods in the years 1992/93 and 1993/94 (Fig. 1) are plotted against the daily mean temperature of the respective periods. Periods with mean temperatures below 3 °C are not represented. Values are means of 12 (1992/93) and 10 (1993/94) replicates with s.e.m. ■, AberHerald spaced plants; □, AberHerald with competition; ●, Huia spaced plants; ○, Huia with competition. *** $P \leq 0.001$; **** $P \leq 0.0001$.

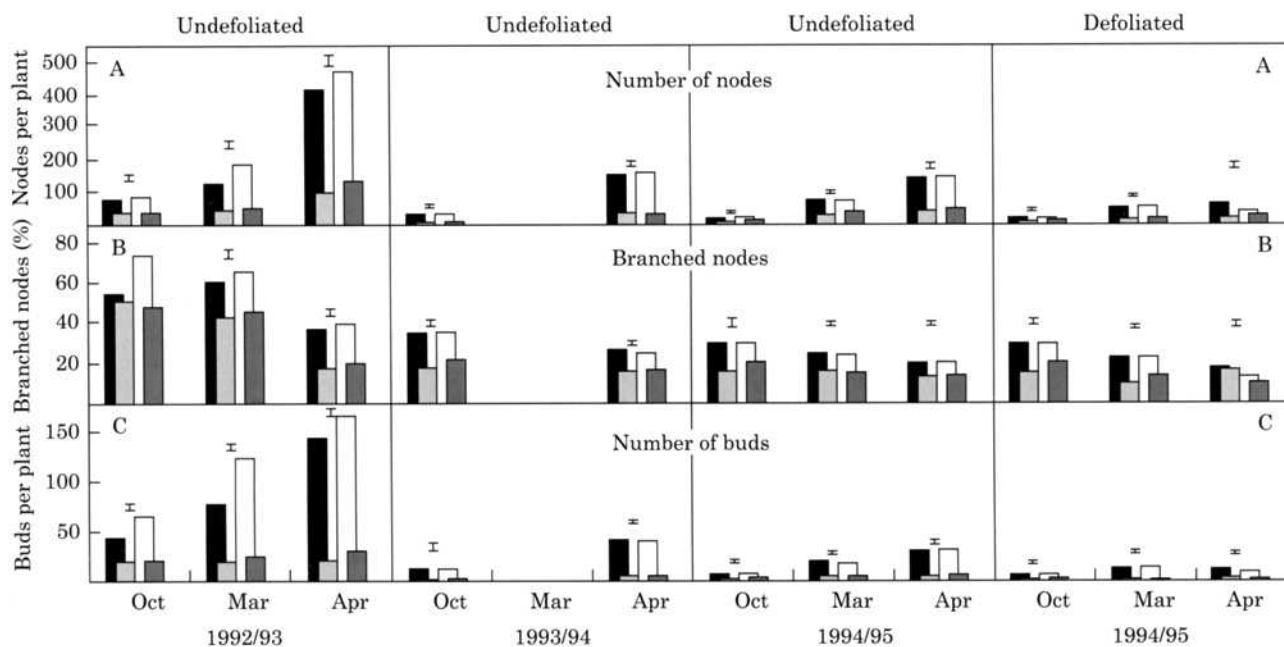


FIG. 3. Effects of competition with perennial ryegrass (*Lolium perenne* L.) and continuous defoliation on the development of (A) node number, (B) node branching and (C) bud number in two cultivars of white clover (*Trifolium repens* L.). Values are means of 12 (1992/93) and 10 (1993/94) replicates with s.e.m. ■, AberHerald spaced plants; □, AberHerald with competition; □, Huia spaced plants; ■, Huia with competition.

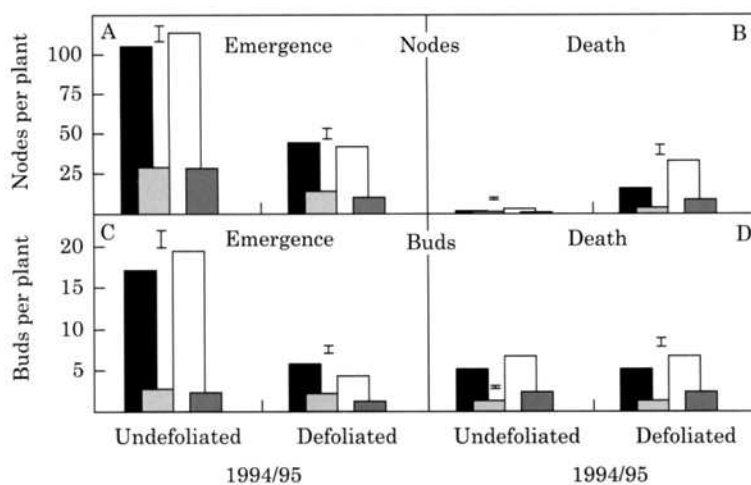


FIG. 4. Effects of competition with perennial ryegrass (*Lolium perenne* L.) and continuous defoliation during winter on (A) cumulative node emergence, (B) node death, (C) bud emergence, and (D) bud death in two cultivars of white clover (*Trifolium repens* L.). Values are means of 10 replicates with s.e.m. ■, AberHerald spaced plants; □, AberHerald with competition; □, Huia spaced plants; ■, Huia with competition.

autumn, each cultivar lost approximately the same proportion of leaves, with a mean of 135% (cultivar, ns). (Values above 100% indicate the death of leaves produced after the first observation in October.) Leaf death, calculated as a percentage of the number of leaves present in October, was lower for spaced plants (130%) than for those grown with ryegrass (145%; competition, $P \leq 0.05$).

Stolon development

In the first experimental year, Grasslands Huia had 31% more buds per plant than AberHerald (cultivar, $P \leq 0.001$).

However, no difference was found between cultivars in 1993/94 or 1994/95 (Fig. 3C). Spaced plants had 340% more buds than plants grown with ryegrass (competition, $P \leq 0.0001$). Frequent defoliation clearly reduced the total number of buds (defoliation, $P \leq 0.0001$) and, in late spring, undefoliated plants had 138% more buds than defoliated plants. Similarly, the total number of nodes (Fig. 3A) differed little between cultivars throughout the observation period (cultivar, ns). Spaced plants had more nodes than plants grown with ryegrass (competition, $P \leq 0.0001$), with the advantage being greater in late spring (247%) than in autumn (114%). This was due to a net

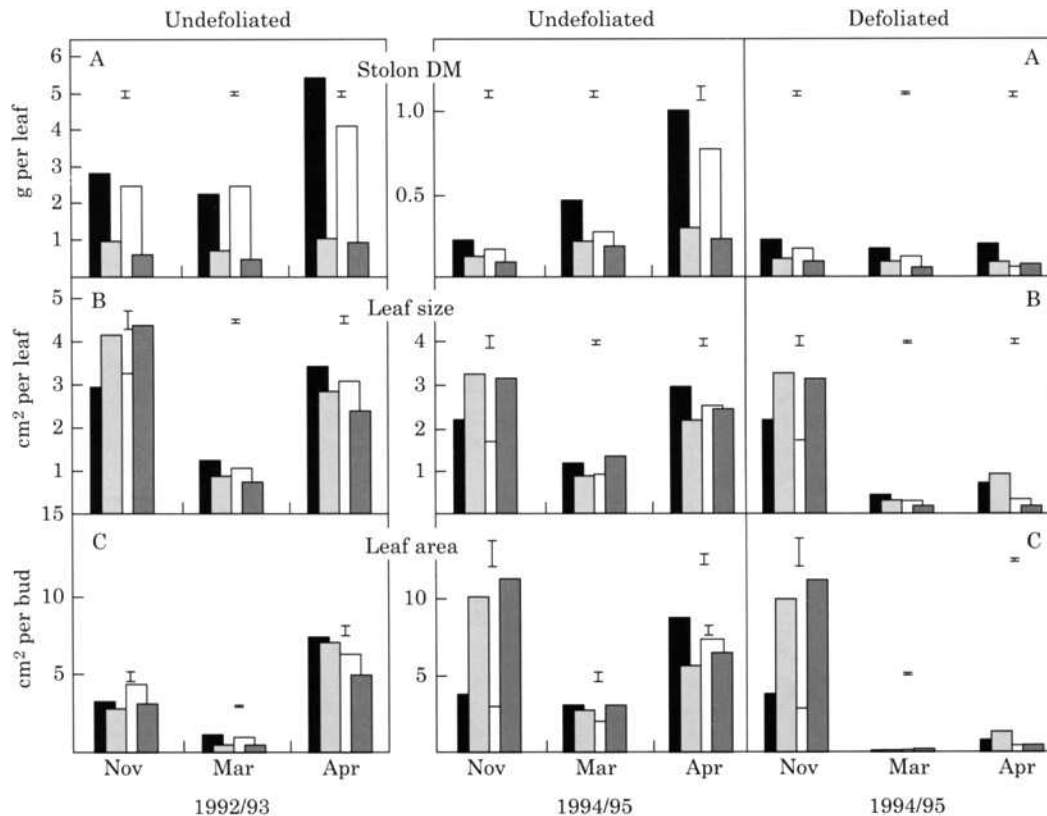


FIG. 5. Effects of competition with perennial ryegrass (*Lolium perenne* L.) and continuous defoliation during winter on the development of (A) stolon dry matter, (B) leaf size, and (C) leaf area per bud in two cultivars of white clover (*Trifolium repens* L.). Values are means of 12 (1992/93) and 10 (1994/95) replicates with s.e.m. ■, AberHerald spaced plants; □, AberHerald with competition; □, Huia spaced plants; ■, Huia with competition.

increase in nodes from autumn to late spring, which was greater (by 321 %) for spaced plants than for clover grown with ryegrass (competition, $P \leq 0.0001$). Averaged over all three years, the net increase in the number of nodes over winter was similar for both cultivars (cultivar, ns). The net increase in the number of nodes of defoliated plants was only 29 % that of undefoliated plants (defoliation, $P \leq 0.0001$).

A change in the number of buds and nodes is the net result of the emergence and death of nodes and buds. The cumulative number of emerged nodes and buds (Fig. 4A and C) was similar for both cultivars (cultivar, ns), but competition significantly depressed node and bud emergence (by 72 and 81 % respectively) (Fig. 4A and C; competition, $P \leq 0.001$). Frequent defoliation reduced node emergence by 60 % and bud emergence by 67 % compared with undefoliated plants. Node and bud death (Fig. 4B and D), calculated as a percentage of the number present in autumn, tended to be higher for Grasslands Huia (by 77 and 38 %) than for AberHerald (cultivar, nodes $P \leq 0.06$; buds, ns). Competition tended to reduce node death (by 43 %) (competition, $P \leq 0.07$), whereas bud death was the same for spaced plants and plants grown in competition with ryegrass (competition, ns). However, defoliation markedly increased both node and bud death when expressed as a percentage of nodes and buds present in autumn; node and bud death was ten-times greater for

frequently defoliated plants than for undefoliated plants (defoliation, $P \leq 0.0001$).

The rate of node branching (Fig. 3B) decreased in all three winters, with the strongest decrease (of 50 %) in 1992/93. Clover cultivar had no effect on the percentage of branched nodes (cultivar, ns), whereas competition with ryegrass clearly caused a decrease in the percentage of branched nodes from 35 % for clover grown as spaced plants to 23 % in mixed swards (competition; $P \leq 0.0001$). Defoliation caused a slight reduction in the rate of branching in early spring (March, $P \leq 0.05$).

Stolon dry matter per plant (DM; Fig. 5A) was 36 % higher for AberHerald than for Grasslands Huia (cultivar, $P \leq 0.0001$), and 170 % higher for spaced plants than for plants grown with ryegrass (competition, $P \leq 0.0001$). From early to late spring, the stolon DM doubled in spaced plants, but it increased by only 40 % in plants grown with ryegrass (competition, $P \leq 0.0001$). From November to April, stolon DM of frequently defoliated plants decreased by 28 %, while undefoliated plants showed a net increase in stolon DM of 84 % by March and of 262 % by April (defoliation, $P \leq 0.0001$).

Leaf area per bud

Individual leaf areas were generally smaller during winter (Fig. 5B), but increased in leaves emerging in spring.

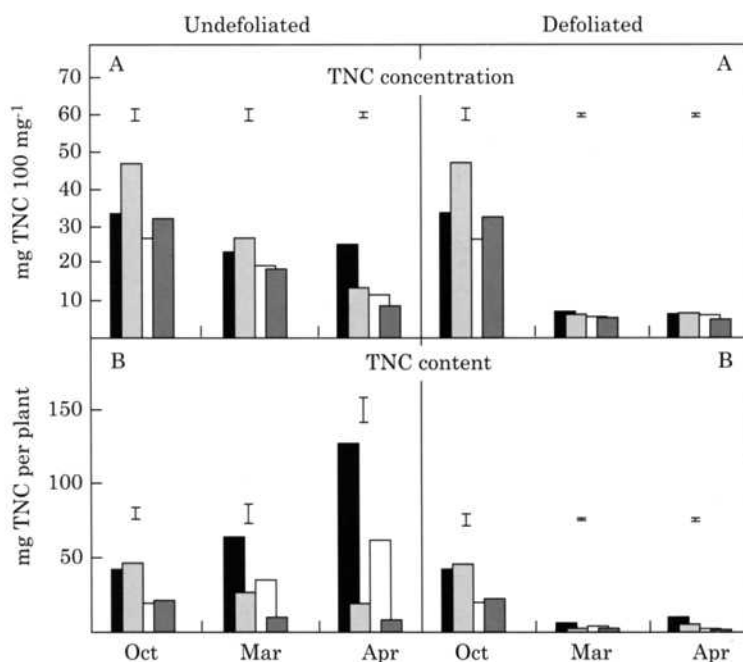


FIG. 6. Effects of competition with perennial ryegrass (*Lolium perenne* L.) and continuous defoliation during winter on the dynamics of (A) stolon TNC concentration, and (B) stolon TNC content in two cultivars of white clover (*Trifolium repens* L.). Values are means of 10 replicates with s.e.m. ■, AberHerald spaced plants; □, AberHerald with competition; ▤, Huia spaced plants; ▥, Huia with competition.

Averaged over the whole observation period, leaves of AberHerald were slightly larger (by 9%) than those of Grasslands Huia (cultivar, ns); this difference had increased to 20% by spring (cultivar, $P \leq 0.01$). In autumn, the leaves of white clover grown with ryegrass tended to be larger (by 39%) than those of spaced plants, although the effect was significant only in 1994/95 (competition, $P \leq 0.05$). In spring, however, leaf size was significantly larger for spaced plants (by 27%) than for those of clover grown with ryegrass (competition, $P \leq 0.0001$). Defoliation reduced leaf size significantly, so that by April the leaf size of previously defoliated plants was only 25% that of undefoliated plants (defoliation, $P \leq 0.0001$).

Averaged over both years, total leaf area per bud (Fig. 5C) was slightly higher (13%) for Grasslands Huia in autumn, whereas it was greater for AberHerald in early (12%) and late (22%) spring, although the difference was significant only in the spring of 1992/93 (cultivar, $P \leq 0.001$). Spaced plants had 23% more leaf area per bud in late spring than plants grown with ryegrass, but the effect was significant only in 1994/95 (competition, $P \leq 0.05$). Defoliation caused a severe reduction in the regrowth from early to late spring when undefoliated plants had ten-times more leaf area per bud than previously defoliated plants (defoliation, $P \leq 0.0001$).

Total non-structural carbohydrates

The concentration of total non-structural carbohydrates (TNC; Fig. 6A) in the stolons was consistently higher (by 40%) for AberHerald than for Grasslands Huia (cultivar, $P \leq 0.0001$) and this effect was even more pronounced when expressed as TNC per plant (Fig. 6B) (115% higher

for AberHerald; cultivar, $P \leq 0.0001$). The TNC content per plant was higher (by 102%) for spaced plants (competition, $P \leq 0.0001$), but the TNC concentration in the stolons was slightly higher (by 13%) for clover grown with ryegrass (competition, $P \leq 0.01$). In addition, the stolon TNC content of spaced plants increased by 85% between early and late spring, whereas plants grown with ryegrass showed a further decrease of 9% in TNC content. During winter, the concentration and content of TNC in stolons of defoliated plants decreased by 82%, whereas TNC concentration decreased by only 59% and TNC content by 68% in stolons of undefoliated plants (defoliation, both $P \leq 0.0001$).

DISCUSSION

Leaf area during winter and grass competition—key factors for overwintering and regrowth in spring

Results of all three experiments demonstrate the importance of the retention of functional leaf area from late autumn to early spring for the successful overwintering and fast regrowth of white clover in spring. This is despite significantly lower total daily photosynthesis in winter compared to that in the growing season, as a result of the shorter photoperiod as well as lower irradiance and temperature (Woledge and Dennis, 1982; Woledge *et al.*, 1989). The emergence of 3.8–6.6 new leaves per main stolon up to the beginning of April, and the net increase in DM and TNC contents of stolons of undefoliated plants, show that leaves did photosynthesize during winter and that plants invested assimilates in growth at this rather cold site. Results obtained from a glasshouse experiment in a more

oceanic climate showed that synthesis and accumulation of starch in white clover stolons occurred during even the coldest periods (Turner and Pollock, 1998).

In contrast, plants that had no leaves during the winter (frequent defoliation from November to mid-March) showed a marked decrease in DM and TNC contents of stolons. Severe defoliation *per se* has been shown to reduce the outgrowth and development of branches (Hay and Newton, 1996; Newton and Hay, 1996). In the experiment presented here, defoliated plants not only showed lower rates of emergence of new nodes and buds, but also had much higher rates of node and bud death during winter, leading to small and simply-structured plants. Small plant size has been shown to be a general characteristic of stressed white clover populations before their collapse in grass/clover mixtures (Fothergill *et al.*, 1997). Thus, these results emphasize the beneficial effect of photosynthetically active leaf area during the winter for the maintenance of a vigorous white clover population in mixed swards.

Maintenance of the stolon network and buds during winter plays an important role in the persistence and yield of clover (Collins *et al.*, 1991; Collins and Rhodes, 1995). The results presented here reveal a distinctly lower rate of spring growth of white clover plants that had no leaves during the winter. It is important to note that this was due not only to a smaller number of buds in spring, but also to a severe reduction in the leaf area generated per bud, as a result of smaller leaves and a lower rate of node emergence (an indicator of leaf emergence) in defoliated plants. This effect, which persisted even after defoliation ceased in mid-March, was stronger than that reported by Sackville Hamilton and Harper (1989), who attributed 3–15% of the variation in leaf emergence to the number of leaves supporting the apex. The importance of leaf area at the beginning of regrowth in spring in the experiment presented here is in line with the results of Baur-Höch *et al.* (1990). In their controlled environment experiment, regrowth depended more on the leaf area remaining after defoliation than on the level of TNC in the stolons. In summary, these results demonstrate the importance of leaf area at the beginning of regrowth in spring for the productivity of white clover. They help to explain why leaves at the end of the winter are important in determining the regrowth of white clover in spring under a wide range of climatic conditions, as observed in the European multisite experiment (Wachendorf *et al.*, 2001b).

Defoliated plants showed a distinct reduction in the content and concentration of TNC by early spring, and the levels remained very low during regrowth in spring. Since the decrease in TNC of defoliated plants over time may represent a loss of TNC through respiration, TNC reserves are an important source of carbohydrates when the leaf area for photosynthesis is limited. In a field experiment in which all the leaves died during winter, the spring yield of white clover was positively related to TNC levels in the stolons in autumn and at the end of winter (Collins and Rhodes, 1995; Frankow-Lindberg and von Fircks, 1998). The marked increase in the death rate of nodes of defoliated plants suggests that when the TNC content is not sufficient for plant maintenance, parts of the stolon system die. Comparable minimal levels of TNC in the stolons (9–15%)

have been found in other experiments (Scheidegger and Nösberger, 1984; Frankow-Lindberg *et al.*, 1997). A positive correlation between the carbohydrate status of stolons and frost hardiness or winter survival has also been observed under other conditions (Rosnes *et al.*, 1993; Collins and Rhodes, 1995; Turner and Pollock, 1998).

Competition with ryegrass affected white clover in a similar manner to defoliation, although the effects were less severe. In comparisons with spaced clover plants, competition caused reductions in stolon DM and TNC contents, and in the rates of emergence of nodes and buds during winter and spring. Woledge *et al.* (1990) explained the effect of ryegrass competition in terms of reduction in the rate of net photosynthesis of clover leaves in lower zones of the canopy where they intercepted less light than ryegrass leaves. It is assumed, therefore, that shading by ryegrass depresses the rate of net photosynthesis of the clover leaf area, which, as discussed above, is a very important characteristic during winter and early spring. This is strongly supported by the observation here that frequent defoliation of white clover has less effect when clover is grown in competition with perennial ryegrass (shaded leaves) than as spaced plants.

When white clover is shaded in a dense sward, the petioles elongate so that the young leaves are in a better position to capture radiation in the upper layers of the canopy (Thompson and Harper, 1988). Single leaves of white clover can reach a height of 40 cm in the sward (Woledge *et al.*, 1992a, b; Blum *et al.*, 2001) but only if the plants invest a large proportion of assimilates in growth of the petioles (Robin *et al.*, 1992). Consequently, the relative growth rate of white clover decreases due to a low leaf area ratio (Woledge, 1988; Davies and Evans, 1990; Soussana *et al.*, 1995). This is a disadvantage when competing with the companion grass, particularly during the reproductive phase in spring when the growth rate of ryegrass is higher and the plants are taller than during the vegetative phase (Menzi *et al.*, 1991; Daepf *et al.*, 2001). The results of the present experiment support this finding, since the decline in stolon TNC concentration of undefoliated plants, grown in competition with ryegrass, continued during March and April.

In contrast to defoliated plants, competition also caused a reduction in the rate of node branching in autumn and, to an even greater extent, in the following spring. The reduced rate of branching was probably caused by a lower red/far red ratio in the lower canopy of mixed swards (Robin *et al.*, 1994a, b; Lötscher and Nösberger, 1997). Competition reduced the quantity and quality of light, thus impairing the functioning of leaves, with the result that the complexity of the stolon structure of individual white clover plants in mixed swards was reduced. These results explain why tiller number, an indicator of the competitive pressure exerted by the companion ryegrass, had an inverse effect on the growth of white clover in the European multisite experiment (Wachendorf *et al.*, 2001b) over a wide range of climatic conditions.

Dynamics of leaf turnover and significance of the cultivar

On the basis of the above results, it is assumed that an increase in leaf number, caused by an increased leaf

emergence at low temperatures, or by lower rates of leaf death during winter, is an important advantage for clover cultivars. However, leaf number alone cannot explain the dynamics of leaf development since only the balance between the rates of leaf emergence and leaf death can provide conclusive information about plant growth. Results show that leaf death was similar for both cultivars in winter and that the highest rates of leaf death occurred at low temperature (around 0 °C) as well as during periods of frost in autumn. The surviving leaves had emerged at low temperature during cold acclimation in autumn and were probably well adapted to freezing temperatures (Collins and Rhodes, 1995). According to Bauer *et al.* (1994), senescence is induced during the cold hardening of leaves of herbaceous plants that were fully developed before hardening, whereas leaves that had developed at low temperature (i.e. under temperatures promoting hardening) showed resistance to frost and photosynthetic activity that was comparable with that of non-hardened leaves. Restoration of photosynthetic capacity and metabolic activity is an important factor in low temperature acclimation of white clover and herbaceous winter annuals (Woledge and Dennis, 1982; Woledge *et al.*, 1989; Hurry *et al.*, 1995). Although leaf emergence is strongly temperature dependent (Haycock, 1981; Eagles and Othman, 1988; Campbell *et al.*, 1999; Corbel *et al.*, 1999), the present results suggest that cultivars that produce leaves at low temperatures are at a considerable advantage compared with cultivars with a higher temperature threshold for leaf emergence.

The number of leaves that emerged during winter, and the increase in the rate of leaf emergence per temperature increment, were clearly higher for AberHerald than for Grasslands Huia, although the temperature threshold (2.9 °C) for leaf emergence was the same for both cultivars and was similar to that reported by Haycock (1981). Similarly, the experiments of Corbel *et al.* (1999) showed a significantly smaller decrease in the rate of leaf emergence of AberHerald compared with Grasslands Huia after chilling at 5 °C. A comparative study with seven white clover cultivars showed that temperature had a stronger effect on plant growth during winter and spring than during the growing season, and that the capacity for growth at 5 °C was important for the success of several white clover populations in the late winter and early spring (Campbell *et al.*, 1999). In the experiment conducted by Campbell *et al.* (1999), Grasslands Huia had the highest rate of leaf emergence at 5 °C of the seven cultivars tested. Thus, the advantage of AberHerald (0.3 leaves per week) over Grasslands Huia (0.1) at 5 °C is remarkable, especially because Grasslands Huia originated in a region with an oceanic climate (Caradus and Woodfield, 1997), conducive to genotypes that grow fast at cool temperatures (< 20 °C) (Haycock, 1981, 1984; Junttila *et al.*, 1990a, b). Although fast growth at low temperature (5–15 °C) is generally associated with low frost tolerance (Eagles and Othman, 1981; Junttila *et al.*, 1990a), the death rates of the leaves, nodes and buds of AberHerald in this experiment did not exceed those of Huia, findings which reflect those of previous reports (Frankow-Lindberg and von Fircks, 1998; Wachendorf *et al.*, 2001a). The results presented here show

that in the European multisite experiment, the significantly greater leaf area per terminal bud of AberHerald compared with Grasslands Huia (Wachendorf *et al.*, 2001a) derived mainly from differences in the rate of leaf emergence.

In addition to more leaves and a higher rate of leaf emergence, individual leaves of AberHerald were slightly larger than those of Grasslands Huia, especially in spring. Leaf size has a large genetic component (Caradus and Christie, 1998; Aasmo Finne *et al.*, 2000) and different organs may have different temperature optima for growth (Haycock, 1981; Campbell *et al.*, 1999). Thus, larger leaves at low temperature may indicate a lower temperature threshold for leaf expansion.

In conclusion, the results clearly demonstrate the importance of leaf area during the winter for a higher growth rate and lower death rate during winter; for the maintenance of a complex stolon structure; and for an increased TNC content and faster regrowth of individual buds in spring. All these characteristics are significant for the maintenance of an appropriate proportion of white clover in grass/clover swards, and for reducing the risk of a collapse of the white clover population. Competition with the companion ryegrass had negative effects on all of the above-mentioned plant characteristics, most probably because clover leaves were shaded. The greater leaf area and higher rate of leaf emergence of AberHerald at temperatures between 5 and 20 °C put it at an advantage compared with Grasslands Huia. These results help to explain the crucial role of leaf area and ryegrass competition (tiller number of ryegrass) as parameters in the model derived from the European multisite experiment (Wachendorf *et al.*, 2001b).

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