Morphological Compatibility of White Clover and Perennial Ryegrass Cultivars Grown under Two Nitrate Levels in Flowing Solution Culture

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Received: 4 April 2003 Accepted: 24 April 2003

The effects of nitrate (NO$_3^-$) supply on shoot morphology, vertical distribution of shoot and root biomass and total nitrogen (N) acquisition by two perennial ryegrass (Lolium perenne L.) cultivars (AberElan and Preference) and two white clover (Trifolium repens L.) cultivars (Grasslands Huia and AberHerald) were studied in flowing nutrient culture. Cultivars were grown from seed as monocultures and the clovers inoculated with Rhizobium. The 6-week measurement period began on day 34 (grasses) and day 56 (clovers) when the NO$_3^-$ supply was adjusted to either 2 mmol m$^{-3}$ (low nitrogen, LN) or 50 mmol m$^{-3}$ (high nitrogen, HN). These treatments were subsequently maintained automatically. Plants were harvested at intervals to measure their morphology and N content. Cultivars of both species differed significantly in several aspects of their response to NO$_3^-$ supply. In the grasses, the LN treatment increased the root : shoot ratio of AberElan but did not affect the distribution of root length in the root profile. In contrast, this treatment changed the root distribution of Preference compared with HN, resulting in a larger proportion of root length being distributed further down the root profile. The morphology of white clover Grasslands Huia was for the most part unaffected by the level of NO$_3^-$ supply. In contrast, AberHerald exhibited different growth strategies, with LN plants increasing their stolon weight per unit length at the expense of leaf production, leaf area and stolon length, whereas HN plants showed reduced stolon thickness, greater leaf area production and stolon length per plant. Cultivars with different morphological/physiological strategies in response to NO$_3^-$ supply may be of value in the construction of ‘compatible mixtures’ aimed at reducing oscillations in sward clover content by extending the range of conditions that allow balanced coexistence of species to occur.

**Key words:** Lolium perenne L., perennial ryegrass, Trifolium repens L., white clover, nitrate supply, morphology, compatibility.

**INTRODUCTION**

In temperate climates, associations of perennial ryegrass (Lolium perenne L.) and white clover (Trifolium repens L.) form the basis of low input, sustainable grassland production. It is considered that perennial ryegrass (grass) is the most suitable companion species for white clover (clover) (Camlin, 1981; Harris, 1987). However, individual genotypes of white clover can exhibit significantly different growth responses when grown with different grasses (Turkington, 1989) and the agronomic performance of grass/clover mixtures depends significantly on the ‘compatibility’ of the component genotypes. Compatibility is defined in this context as the ability of the two species to grow together and produce high herbage yields, with a sward clover content sufficient to optimize the benefits of its nitrogen fixation and superior forage quality (Collins and Rhodes, 1989). Varietal and genotypic differences in compatibility result from a complex of interacting spatial and temporal factors (Collins and Rhodes, 1990), including environmental conditions affecting plant development, morphology and fitness.

Amongst environmental factors, competition for nitrogen (N) has long been recognized as being of paramount importance in determining the balance between grasses and clovers in mixtures (Donald, 1963; Haynes, 1980; Schwinning and Parsons, 1996b). Field and glasshouse studies (Collins et al., 1996a) carried out to investigate interactions between grass/clover mixtures, using the clover cultivars AberHerald and Grasslands Huia and the grass cultivars Preference and AberElan (identified in the latter study by its IGER accession code, Ba10761), showed that the magnitude of the effects of the companion grass cultivar on clover seedlings depended strongly on the level of N supply. Thus, under low N conditions, large differences were observed in clover seedling size and development when grown with different companion grasses, with AberElan exerting a strong negative influence on associated clover plants. Higher rates of N application were found to reduce clover seedling size and developmental complexity irrespective of the companion grass cultivar. Consequently, high N conditions suppressed not only differences between the clover cultivars themselves, but also the differential...
impact of the companion grass cultivars on the clovers. This result suggests that grass/clover compatibility is likely to assume greater agronomic significance in temperate pastures as the trend towards reducing fertilizer inputs of N continues.

The simple morphological and physiological traits measured by Collins et al. (1996a) did not provide information that could explain these compatibility effects; in fact, canopy light interception data and total root weights obtained in that experiment suggested that AberElan should be less aggressive than Preference towards associated clover plants. The present experiment was therefore carried out to determine whether the differential effects of N level on the compatibility and performance of grass and clover cultivars observed in the field and glasshouse (Collins et al., 1996a) could be explained by intrinsic differences between cultivars in shoot and root morphology, and/or differences in N acquisition in response to nitrate (NO$_3^-$) supply. To avoid confounding effects, the cultivars were grown as monocultures under two contrasting and closely controlled levels of NO$_3^-$ supply in a flowing solution culture (FSC) system. The FSC system allowed plants of each species to develop in the absence of interspecific competition and under optimal levels of nutrients other than NO$_3^-$, thus providing an environment in which their growth potential could be expressed. The use of FSC facilitated the detailed measurement of root system architecture, which was identified by Collins et al. (1996a) as a factor likely to be highly significant in grass/clover interactions, but which is prohibitively difficult to carry out in field conditions. The system also allowed comparisons to be made of grass canopy structure using a stratified clip procedure (Rhodes and Collins, 1993). It was anticipated that growth responses and characteristics of NO$_3^-$ uptake, which can be accurately measured in FSC, could then be related to the behaviour of these cultivars under field conditions.

MATERIALS AND METHODS

Plant culture

Seeds of two cultivars of white clover (AberHerald and Grasslands Huia) and two of perennial ryegrass (Preference and AberElan) were sown into culture units of an FSC system in a glasshouse. The FSC system provided automatic control of concentrations of NO$_3^-$, NH$_4^+$, K$^+$ and H$^+$ in solution (Clement et al., 1974; Hatch et al., 1986; Wild et al., 1987). Two culture units were allocated per cultivar, each containing 200 L of nutrient solution recirculating through 24 × 1 L vessels. Each vessel held either six (clover) or 12 (grass) plants following emergence and thinning of the seedlings. Because of differences in seedling growth rates between the species, clover was sown 22 d before the grass. This ensured that the clover and grass plants were of similar size at the start of the treatment period.

The initial composition of nutrient solutions in the clover units was (mmol m$^{-3}$): NO$_3^-$, 143; K$^+$, 154; Ca$^{2+}$, 286; SO$_4^{2-}$, 333; Mg$^{2+}$, 50; H$_2$PO$_4^-$, 25; Fe$^{2+}$, 5-4, with micronutrients at half the concentrations used by Clement et al. (1978). For ryegrass it was (mmol m$^{-3}$): NO$_3^-$, 250; K$^+$, 250; Ca$^{2+}$, 344; SO$_4^{2-}$, 424; Mg$^{2+}$, 100; H$_2$PO$_4^-$, 50; Fe$^{2+}$, 5-4, with micronutrient concentrations as used by Clement et al. (1978). Until the imposition of the NO$_3^-$ supply treatments, plants were allowed to deplete these nutrients on weekly cycles, at the end of which all culture units were replenished with fresh solution. The differences in nutrient solution composition between species reflected (a) the lower P requirement of clover and (b) the desirability of maintaining lower NO$_3^-$ concentrations to promote nodulation in clover. Solution pH (6·0 ± 0·5) was maintained by automatic titration of Ca(OH)$_2$ or H$_2$SO$_4$ as required throughout the experiment. Plants were grown under natural late summer/autumn light conditions until day 23 after sowing, and then supplemented between 0800 and 1800 h by artificial illumination (Phillips SONT 400 W high-pressure sodium lamps, one per culture unit) providing 250 μmol m$^{-2}$ s$^{-1}$ PAR (photosynthetically active radiation) at plant height. Air temperature was kept at 20/15 °C (day/night) throughout the experiment and the temperature of the nutrient solution was allowed to vary diurnally between 18 and 20 °C in all tanks. Clover plants were inoculated on day 41 with a standard mixture of Rhizobium strains (IGER strain nos 5, 509 and 501) known to support nitrogen fixation in a range of clover cultivars. Nodules were observed on the roots 7 d later.

Table 1. Harvesting dates and intervals for perennial grass and white clover harvests

<table>
<thead>
<tr>
<th>Harvest no.</th>
<th>Perennial ryegrass</th>
<th>White clover</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Harvest date</td>
<td>Duration (d)</td>
</tr>
<tr>
<td>H1</td>
<td>30 Sep.</td>
<td>Start</td>
</tr>
<tr>
<td>H2</td>
<td>5 Nov.</td>
<td>6</td>
</tr>
<tr>
<td>H3</td>
<td>12 Nov.</td>
<td>13</td>
</tr>
<tr>
<td>Defoliation</td>
<td>harvest 15 Nov.</td>
<td></td>
</tr>
<tr>
<td>H4</td>
<td>26 Nov.</td>
<td>27</td>
</tr>
<tr>
<td>H5</td>
<td>9–10 Dec.</td>
<td>41</td>
</tr>
</tbody>
</table>

**Treatments**

The treatment period began on days 34 (grass) and 56 (clover) after sowing, and consisted of either high (HN) or low (LN) levels of NO$_3^-$ supply, maintained automatically...
over 6 weeks as concentrations of 50 and 2 mmol m\(^{-3}\) NO\(_3^-\), respectively. Previous studies investigating plant growth in FSC have used concentrations of 20 mmol m\(^{-3}\) NO\(_3^-\) for white clover (e.g. Abberton et al., 1998) and 10 mmol m\(^{-3}\) NO\(_3^-\) for perennial ryegrass (e.g. Wilkins et al., 1997) as standard treatments. There was one culture unit of each cultivar per treatment. Treatment concentrations were achieved initially by allowing NO\(_3^-\) to deplete by plant uptake over several hours. Thereafter, the automatic nutrient control system maintained the concentrations on the basis of supplying Ca(NO\(_3\))\(_2\) every 27 min at a rate equal to the measured depletion. Likewise, the concentration of K\(^+\) was achieved initially by allowing NO\(_3^-\) to deplete by plant uptake for each treatment. Treatment concentrations were maintained automatically at 20 mmol m\(^{-3}\) in all culture units by addition of K\(_2\)SO\(_4\). All other nutrients were re-supplied in fixed proportion to that of NO\(_3^-\).

**Plant harvests and morphological measurements**

Plants were harvested at intervals (Table 1) throughout the treatment period. At the start of the experiment two vessels were harvested from each culture unit (H1). Two subsequent harvests, each involving the removal of four vessels per culture unit, were carried out at weekly intervals (H2 and H3). Immediately following the third harvest (days 15 and 16 of treatments), all remaining plants were defoliated to a height of 4 cm above the root–shoot junction. A further harvest was carried out 12 d later (H4), followed by a 14-d interval before the final harvest (H5) (41 and 43 d after treatments started for clover and grass, respectively). The harvesting protocol for grass plants was as follows: first, tiller numbers per vessel were counted and then each vessel was laid out horizontally over a 2.5 cm grid. The plants were cut at their junction with the vessel and the leaves were extended to their full length on the grid. Maximum leaf length was recorded and the leaf canopy was then sectioned along the grid markings into horizontal strata at 2.5 cm intervals. The leaf area (Delta-T Devices, Cambridge, UK) and dry weight (oven dried at 80°C at 25 cm intervals) of herbage from each stratum were measured. A similar stratification procedure was performed on the root system and the dry weight of root material in each stratum measured. Root length per stratum was estimated by taking subsamples and measuring the length of root using a root length measurement system (Delta-T Devices).

A similar procedure was used for white clover plants, with measurement of the number of fully expanded leaves, total expanded leaf area, mean petiole length of the fully expanded leaves, total stolon length and number of growing points per plant. All fractions were oven dried and the dry weights obtained. Clover root systems were stratified and sectioned as described for grass, but subsamples were also taken from each stratum for determination of nodule numbers and dry weights.

Statistical comparisons of the above data were made using means and standard errors based on per plant values obtained from individual vessels. Replication was achieved in the sense that more than one vessel per treatment was measured at each harvest. At each harvest destructive measurements were made on different vessels, rather than successive measurements on the same experimental units, thus minimizing the likelihood of the occurrence of serial correlations among data from different harvests (Collins et al., 1996b).

**NO\(_3^-\) uptake and N\(_2\) fixation**

Net uptake of NO\(_3^-\) per plant was calculated on a daily basis from the automatic delivery of nutrient solutions to maintain the set point concentrations in the LN and HN treatments. Mean inflows (I) of NO\(_3^-\) (uptake rate per unit root length) were calculated using a modified version of the formula given by Williams (1948):

\[
I = \frac{[(U_2 - U_1)/(t_2 - t_1)] \times (\ln L_2 - \ln L_1)/(L_2 - L_1)] \text{ pmol s}^{-1}\text{cm}^{-1}
\]

where \(L_2\) and \(L_1\) are total root lengths per plant (cm), respectively, at times \(t_2\) and \(t_1\) (s), corresponding with cumulative NO\(_3^-\) uptake (pmol per plant), \(U_2 - U_1\).

Nitrogen fixation per plant was calculated by subtracting the measured uptake of NO\(_3^-\) from the total N content of the plant as follows: after grinding the oven-dried samples to pass through a 0.5 mm sieve, total N in all plant fractions was measured using a continuous flow isotope mass spectrometer (20/20, Europa Scientific Ltd, Crewe, UK) linked to a C/N analyser (Roboprep CN; Europa Scientific Ltd). The total N content per plant was calculated as the sum of the products of (%N in dry weight of fraction \(\times\) fraction dry weight) for each plant fraction. The cumulative nitrogen fixation per plant between each harvest was calculated as the increment in the total N content of the plant after subtracting the corresponding increment in NO\(_3^-\) uptake.

The above parameters were calculated on a per culture unit basis, and the culture units themselves were not replicated. However, the large number of plants in each culture unit, combined with the automatic monitoring and maintenance of NO\(_3^-\) levels, means that results from the different treatments can be compared with confidence.

**RESULTS**

**Grass morphology in response to NO\(_3^-\) supply**

In terms of overall morphology (Fig. 1) the dominant effect on grass plants was that of N treatment, with the cultivar effect being of smaller magnitude. Leaf area and leaf dry weight responded in a similar way, with HN plants producing more leaf by H3 (just prior to defoliation). The slightly higher leaf dry weight and leaf area of Preference was reflected in its higher dry matter production from the defoliation harvest on 15 November (Table 2). In both cultivars the trend for greater production of leaf area and weight under HN was maintained in the last two harvests. Again, there were only small differences between the cultivars in these quantities (Fig. 1A and B). In contrast, under the LN regime, a strong cultivar effect became evident after the defoliation harvest, and by the final harvest (H5) Preference had produced 33 % higher total leaf area and 10 % greater leaf dry weight per plant than AberElan.

Total root length and dry weight were greater in the LN compared with the HN treatment in both cultivars (Fig. 1C
and D), and the magnitude of the differences increased over time. The shift in the allocation of resources from leaf to root under the LN treatment is demonstrated by the higher values of root : shoot ratio from H2 onwards (Fig. 1F). In summary, the effect of NO$_3^-$ supply on the grass plants was mainly to alter the allocation pattern of dry weight between roots and shoots, with little impact on total plant dry weights (Fig. 1E).

The stratified sampling technique enabled detailed descriptions to be made of the vertical distribution of leaf area in the grasses, and results are presented for H5 (Fig. 2). In the HN treatment there was little difference between the cultivars in terms of either total leaf area or its distribution (Fig. 2A). Under the LN treatment, however, the difference in total plant leaf area between the cultivars at H5 was clearly demonstrated (Fig. 2B), and the larger leaf area of Preference was evident throughout the area profile. There was no difference between the cultivars in the pattern of distribution of leaf area in the canopy under LN.

Under the HN treatment the two grass cultivars showed similar profiles of root length distribution over the 6 weeks of treatment. Profiles obtained at H5 are shown in Fig. 3A. However, under the LN treatment, differences between the root profiles of the two cultivars emerged over time and were significant by H5 (Fig. 3B). The shape of the profile of root distribution for AberElan differed in the N treatments, and under LN there was a pronounced emphasis on the production of root length in the upper sections of the root
system. There were also differences in the actual length of root produced in each stratum under the N treatments. In contrast, the root profile of LN–Preference differed in shape as well as in length from that of HN–Preference, with a high proportion of root length under LN conditions occurring a depth of 15–20 cm. Comparison of the root length distribution patterns of AberElan and Preference under LN showed that the 10–15 cm stratum contained 34 and 19 %, whilst the 15–20 cm stratum contained 31 and 48 %, respectively, of their total root system lengths.

Clover morphology in response to NO\textsubscript{3}– supply

The morphological characteristics of the two white clover cultivars are shown in Fig. 4. Until defoliation, AberHerald had more leaves per plant than Grasslands Huia in the LN treatment (Fig. 4B), although mean individual leaf area (Fig. 4A) and petiole length (Fig. 4C) were similar in the two cultivars. However, under the HN regime differences between the cultivars in leaf number, leaf area and petiole length were insignificant and this was reflected in the similar herbage yields measured at the time of defoliation (Table 2). After H4 (1 week after defoliation) both cultivars started to increase the leaf area per plant (Fig. 4D). However, there was an interaction between cultivar and N treatment such that the rate of increase in leaf area was substantially lower in AberHerald under LN. As a result, by H5 the leaf area per plant of AberHerald in LN was only 43 % of that of AberHerald in HN, whilst in Grasslands Huia little difference could be detected between treatments. Neither cultivar nor NO\textsubscript{3}– supply affected petiole length in the early part of the experiment (Fig. 4C), but, following defoliation, LN–AberHerald produced shorter petioles than the other three treatments.

Differences between cultivars and N treatments in stolon attributes also emerged or intensified following defoliation. LN–AberHerald had the lowest stolon length per plant by H5, whereas HN–AberHerald and Grasslands Huia under both levels of NO\textsubscript{3}– supply had similar values (Fig. 4G). The pattern was similar for the total number of buds (‘growing points’) per plant (Fig. 4E). Stolon weight per unit length (an indirect measure of stolon diameter) was greater in AberHerald than Grasslands Huia from the start of the treatments (Fig. 4F) and, after defoliation, there appeared to be an effect of NO\textsubscript{3}– supply on AberHerald but not on Grasslands Huia. By the final harvest, LN–AberHerald had the thickest stolons (31, 81 and 102 % thicker than HN–AberHerald, LN–Grasslands Huia and HN–Grasslands

<table>
<thead>
<tr>
<th>Species</th>
<th>Cultivar</th>
<th>NO\textsubscript{3} treatment</th>
<th>Herbage d. wt (g plant\textsuperscript{−1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perennial ryegrass</td>
<td>AberElan</td>
<td>LN</td>
<td>0.52 (±0.017)</td>
</tr>
<tr>
<td></td>
<td>AberElan</td>
<td>HN</td>
<td>0.61 (±0.023)</td>
</tr>
<tr>
<td></td>
<td>Preference</td>
<td>LN</td>
<td>0.56 (±0.012)</td>
</tr>
<tr>
<td></td>
<td>Preference</td>
<td>HN</td>
<td>0.64 (±0.027)</td>
</tr>
<tr>
<td>White clover</td>
<td>AberHerald</td>
<td>LN</td>
<td>0.71 (±0.046)</td>
</tr>
<tr>
<td></td>
<td>AberHerald</td>
<td>HN</td>
<td>0.58 (±0.052)</td>
</tr>
<tr>
<td></td>
<td>Grasslands Huia</td>
<td>LN</td>
<td>0.55 (±0.046)</td>
</tr>
<tr>
<td></td>
<td>Grasslands Huia</td>
<td>HN</td>
<td>0.54 (±0.036)</td>
</tr>
</tbody>
</table>

Plants were cut at a height of 4 cm above the shoot/root junction. Values (±s.e.) for each treatment are derived from 14 culture vessels, each containing six plants for clover or 12 plants for grasses.

FIG. 2. Leaf area (cm\textsuperscript{2}) measured within 2.5 cm strata up the leaf profile on perennial ryegrass plants growing in flowing nutrient solutions. Values are means (± s.e.) expressed on a per plant basis for cultivars Preference (filled circles) and AberElan (open circles) at H5 under HN (A) and LN (B) treatments. Measurements were made on four replicate vessels per cultivar, each containing 12 plants.
Huia, respectively). Total root length per plant was unaffected by either N treatment or cultivar prior to defoliation (Fig. 4H). However, following defoliation there was a check on root growth in all plants. LN-Grasslands Huia showed a large increase in root growth after H4, and by the final harvest had the longest root system. Root length in AberHerald under LN was slower to recover after defoliation. Under the HN treatment the differences in root system lengths between the two cultivars were insignificant throughout the experiment.

In the HN treatment, the root length distributions of the two cultivars measured at H5 (Fig. 5A) were similar. The greatest root length was found between 0 and 10 cm, with a peak distribution in the 2.5–5 cm stratum. In contrast, there was a marked difference between the root length profiles of the two cultivars under the LN regime (Fig. 5B), and Grasslands Huia produced a greater root length than AberHerald in all strata to a depth of 20 cm.

Prior to defoliation, nodule numbers per plant were higher in the LN compared with the HN treatment in both cultivars (Fig. 6). Defoliation resulted in a rapid decline in numbers on LN plants, particularly in LN-AberHerald, so that, by H4, all treatments and cultivars had similar nodule numbers. However, the differences between LN and HN plants were restored by H5, and nodules began to develop down the whole of the root profile in both cultivars under LN (data not shown). In the HN treatment there was no immediate reduction in nodule number following defoliation and all nodule formation was restricted to the 0–12.5 cm zone.

**NO$_3^-$ uptake and nitrogen fixation**

The LN regime substantially reduced the total uptake of NO$_3^-$ by both species compared with the corresponding HN plants (Table 3). This reduction was greatest both absolutely and proportionately in the two clover cultivars, and more so for AberHerald than for Grasslands Huia. Both clover cultivars, but particularly Grasslands Huia, were able to compensate for the low uptake of NO$_3^-$ imposed under the LN regime through the process of nitrogen fixation. The degree of inhibition of nitrogen fixation under the HN treatment was similar in both clover cultivars, with fixation contributing only 15–16 % of the total acquisition of N by these plants over the treatment period, compared with 58–63 % by the LN plants (Table 3). Although the data for the total N acquired (through fixation and NO$_3^-$ uptake) by the two species (Table 3) refer to slightly different time intervals, it is evident that the total acquisition of N by LN clover plants far exceeded that achieved by LN grass plants. Total acquisition of N was also very much greater by clover than by grass under the HN treatment.

The time-courses for NO$_3^-$ uptake per plant throughout the treatment period (Fig. 7) showed that the differences between LN and HN clover plants were greater than those between LN and HN grass plants prior to defoliation and during the first 10 d of recovery. However, the divergence between LN and HN grass plants increased towards the end of the experiment. The greatest difference between the two clover cultivars in NO$_3^-$ uptake occurred following defoliation under the LN regime, when rates of uptake by AberHerald remained very low compared with those in Grasslands Huia. In contrast, uptake rates by the two grass cultivars differed under both N treatments, with those for LN-AberElan being consistently lower than those of LN-Preference, both before and after defoliation. The rates for HN-AberElan also fell progressively behind those of HN-Preference following defoliation.

Estimated mean inflows of NO$_3^-$ (pmol s$^{-1}$ cm$^{-1}$ root length) over the entire root system (Table 4) provide a measure of ‘specific uptake’. As expected, inflows were generally higher under the HN than LN regimes for both species, and initially (days 0–6) higher for grass than for clover. Subsequently, inflows were similar for the two species under HN and tended to decrease with time. Inflows under the LN regime also tended to decrease with time in both species. With respect to the cultivar differences in daily rates of NO$_3^-$ uptake per plant described above, it would appear that the lower rates observed in LN-AberHerald compared with LN-Grasslands Huia after defoliation were attributable not only to the lower total root length of the former cultivar but also to its lower inflows (days 28–43). In the case of differences in daily NO$_3^-$ uptake between LN–

![Fig. 3. Root length (cm) measured within 2.5 cm strata down the root profile on perennial ryegrass plants growing in flowing nutrient solutions. Values are means (± s.e.) expressed on a per plant basis for cultivars Preference (closed circles) and AberElan (open circles) at H5 under HN (A) and LN (B) treatments. Measurements were made on four replicate vessels per cultivar, each containing 12 plants.](image-url)
AberElan and LN-Preference, both before and after defoliation the inflows were always slightly lower in the former cultivar and differed by the greatest margin between days 6 and 13.

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![Graphs showing morphology of two white clover cultivars (AberHerald, closed squares; Grasslands Huia, open squares) under two levels of NO₃⁻ supply (HN, dashed lines; LN, continuous lines) in flowing nutrient solutions. The vertical arrow denotes the time of the defoliation harvest. Values are treatment means (± s.e.) of measurements on four replicate vessels per cultivar at each harvest and are expressed on a per plant basis. Each vessel contained six plants.](image-url)
DISCUSSION

Mechanisms of coexistence operating in grass/clover mixtures have received intense study in recent years (Thornley et al., 1995; Schwinning and Parsons, 1996a, b) in an effort to explain the fluctuations in sward clover content observed in long-term studies (Tyson et al., 1990; Fothergill et al., 1996). The majority of models proposed to interpret these fluctuations have a common key element of a trade-off between grass and clover, based on competition for nitrogen. Comparison of results obtained in mixtures and monocultures of the two species at different levels of N exemplify the complexity of the interactions between them. Thus, in grass/clover mixtures under conditions of low N, it has been shown that clover has a comparatively high relative growth rate (Woldeger, 1988) and can compete effectively with grass by increasing nitrogen fixation. The results obtained in the present experiment show that total N acquisition (nitrogen fixation plus NO$_3^-$ uptake) by clover plants growing in monoculture in the LN treatment was considerably higher than that by the grasses. This was particularly evident in Grasslands Huia, whose high nitrogen fixation rate under LN contributed to a total N acquisition that was more than double that of the grasses. However, there was a large difference under LN between the clover cultivars in terms of both NO$_3^-$ uptake and the extent of stimulation of fixation. Measured over the duration of the experiment, AberHerald’s total NO$_3^-$ uptake under LN was markedly lower than that of Grasslands Huia. Its nitrogen fixation rate was also lower in absolute terms than that of Grasslands Huia, although their rates were similar when expressed as a proportion of total N acquisition. It is difficult to compare rates of nitrogen fixation in this experiment directly with those found in other studies carried out in FSC (e.g. Michaelson-Yeates et al., 1998; Abberton et al., 2000, 2001), as levels of NO$_3^-$ supply and plant size differ. However, the variation in the proportional contribution by nitrogen fixation to the total N economy of the clover plants observed in response to NO$_3^-$ supply in the present study was of the same order of magnitude as that reported elsewhere (Macduff and Dhanoa, 1990; Macduff et al., 1996).

Under conditions of high N, perennial ryegrass demonstrates a distinct competitive advantage over white clover when the species are grown together (Davidson and Robson, 1985). However, when grown as monocultures under controlled high N conditions, as in the present study, the ability of clover to sustain high rates of NO$_3^-$ uptake and for uptake to recover immediately following defoliation is clearly greater than that of grass. It appears, then, that the competitive superiority of grass over clover may not be dependent on a greater ability to take up NO$_3^-$ from mixtures in high N environments. In such conditions, grasses seem to be capable of translating their NO$_3^-$ uptake into morphological results that exert a negative effect on adjacent clover plants, for example by altering the quality...
and quantity of light that infiltrates through the canopy (Thompson and Harper, 1988).

In terms of daily NO\textsubscript{3}\textsuperscript{-} uptake per plant, the results of this experiment indicated a difference between the grass cultivars, with AberElan tending towards lower rates under both N treatments. This appears to have had an effect on leaf growth which, under LN, was considerably reduced in AberElan compared with Preference. The stratified clip data from canopies in the LN treatment revealed no difference between the cultivars in the distribution of leaf area, and suggested that a greater quantity of light would penetrate to the base of AberElan canopies. These results might be expected to favour clover growth with AberElan in conditions of low N, if competition for light were of overriding importance. The question then arises of the mechanism through which AberElan exerts its negative influence on clover growth at low N (Collins et al., 1996a).

It has been known for some time that grass canopy structure affects the abundance of clover within swards (e.g. Rhodes and Harris, 1979; Gooding et al., 1996), possibly via the ‘light altering’ mechanism proposed above, but little information exists on the additional effect of contrasting grass root architectures on grass/clover relationships. The detailed analysis of root length distribution carried out in this experiment revealed significant differences between the grass cultivars under the LN treatment. Under this treatment, a large proportion of the root system of AberElan plants was located in the 10–15 cm root stratum, whereas the bulk of Preference root length was further down, in the 15–20 cm stratum. This study, in accordance with the findings of many researchers (e.g. Caradus, 1977), demonstrated that the vast majority of clover roots existed in the upper levels of the root profile (0–10 cm). Close physical proximity of grass and clover roots clearly increases the level of interspecific interaction. Jackman and Mouat (1972) suggested that, because of contrasting root architectures, where clover and grass roots intermingle, those of the clover will always be in direct competition with those of the grass, whereas grass roots will encounter much less competition from clover. Thus, intertwining root systems, in addition to enhancing the transfer of nitrogen between adjacent clover and grass plants, will also inevitably increase competition for other resources, e.g. phosphate. In contrast, root systems that are complementary in architecture may reduce the level of interspecific interaction and could lead to a more stable coexistence of grass and clover. Thus, the combination of either of the two clovers used in the present study with the grass cultivar Preference, under low N conditions, would lead to a greater spatial separation of the root systems of the two species and a reduction in direct interference. This ‘Lock and Key’ form of congruous root architecture could explain the superior performance of clover cultivars in low

### Table 3

<table>
<thead>
<tr>
<th>White clover</th>
<th>Perennial ryegrass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huia</td>
<td>Aberherald</td>
</tr>
<tr>
<td>LN</td>
<td>LN</td>
</tr>
<tr>
<td>Total NO\textsubscript{3}\textsuperscript{-} uptake (mmol N plant\textsuperscript{-1})</td>
<td>4.4</td>
</tr>
<tr>
<td>Total N\textsubscript{2} fixation (mmol)</td>
<td>6.1</td>
</tr>
<tr>
<td>Total N acquired (mmol N plant\textsuperscript{-1})</td>
<td>0.5</td>
</tr>
<tr>
<td>Fraction of total N acquired by N\textsubscript{2} fixation (%)</td>
<td>58</td>
</tr>
</tbody>
</table>

NO\textsubscript{3}\textsuperscript{-} uptake was measured automatically in a single culture unit of each treatment.

### Table 4

<table>
<thead>
<tr>
<th>White clover</th>
<th>Perennial ryegrass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huia</td>
<td>Aberherald</td>
</tr>
<tr>
<td>Days</td>
<td>LN</td>
</tr>
<tr>
<td>0–7</td>
<td>2.1</td>
</tr>
<tr>
<td>7–14</td>
<td>2.5</td>
</tr>
<tr>
<td>14–28</td>
<td>1.7</td>
</tr>
<tr>
<td>28–43</td>
<td>1.5</td>
</tr>
</tbody>
</table>

NO\textsubscript{3}\textsuperscript{-} uptake was measured automatically in a single culture unit of each treatment.
The clover cultivars also demonstrated contrasting morphological strategies under high and low N conditions. Thus, the morphology of Grasslands Huia plants was mainly unaffected by the N treatments used in this experiment, and the growth and structure of the plant were similar whether N was supplied externally or came from internal nitrogen fixation. Conversely, AberHerald demonstrated very different growth strategies under high and low NO_3^- supply. Initially, AberHerald produced large plants under the low N treatment. However, after defoliation, AberHerald plants in this treatment responded with a massive reduction in nodule number and a large increase in stolon weight per unit length at the expense of leaf production, leaf area and stolon length. In contrast, under conditions of high NO_3^- supply, defoliation caused a temporary check in stolon weight per unit length but a much greater subsequent production of leaf area and stolon length per plant. These contrasting strategies can be thought of as a low N ‘obstinate’ strategy and a high N ‘effusive’ strategy, and may be explained in part by the different origins of the two cultivars. Grasslands Huia was bred for improved yield and persistency under grazing from germplasm collected in moderately fertile pastures in Hawkes Bay and North Canterbury in New Zealand (Caradus, 1986), whereas AberHerald was bred from germplasm collected in highly fertilized grass/clover swards in the Züricher Oberland of Switzerland. Plants respond to changes in environmental conditions by allocating growth resources to the organ that is capable of alleviating the limitations imposed by new conditions (Iwasa and Roughgarden, 1984). White clover is known to exhibit a considerable degree of clonal integration between parent and branch stolons (Kemball and Marshall, 1995), which would facilitate the allocation of resources to allow escape from unfavourable local circumstances. Thus, a cultivar that is capable of expressing both ‘obstinate’ and ‘effusive’ tactics for survival is likely to improve its chances of persisting in a heterogeneous sward. The responses of AberHerald to increased NO_3^- supply would fit the hypothesis that an adapted cultivar or population would be capable of consolidating its occupancy of favourable habitat patches and increasing its probability of escape from unfavourable ones, e.g. urine patches (Hutchings et al., 1997). N cycling between plants and the soil allows species in a mixture to establish a soil N level at which competitive advantages are balanced and coexistence operates. However, grazing animals interfere with these processes by accumulating N from across the pasture and concentrating the returns in restricted areas in the form of urine and faeces. This process creates a patchy environment in which the contributions of plant species to the sward fluctuate. Indeed, much of the local spatial heterogeneity in plant yield may be caused by spatial heterogeneity in the availability of soil resources (Snaydon, 1962). The results of the present experiment indicate that reduced competition from grass is not the only factor enabling a clover genotype or cultivar to survive and thrive in a low N patch, and that some cultivars of clover (e.g. AberHerald) are able to respond morphologically to low levels of N supply in such a way that movement away from this environment is restricted.

Given that two species can coexist in habitats in which each species is limited by a different resource (Tilman, 1986), perhaps coexistence can also occur if the cultivars or species demonstrate different morphological/physiological responses to a single factor such as NO_3^- supply. If this is the case, then cultivars of grass and clover that offer different strategies under varying levels of NO_3^- supply could be used in ‘compatible mixtures’. In this way, oscillations in the contribution of species to sward productivity could be damped to such an extent that field-scale fluctuations in yield balance would be kept to a minimum. The ability of genotypes within cultivars to react to the environment by employing different growth strategies derives from phenotypic plasticity enabling morphological adjustment to occur when new neighbourhoods are encountered. This is a mechanism whereby the range of environmental conditions that a single genotype can tolerate is
expanded, and it represents a means of assuring survival in an environment that varies over time (Turkington et al., 1994). Cultivars containing genotypes that exhibit adaptive growth strategies are likely to be at an advantage in a fluctuating environment and, if both species in an association show this flexible response to N level, then the combination may offer another route to the control of population oscillations by extending the range of conditions that allow balanced coexistence to occur.

Caution must be exercised over the use of information derived from young plants in controlled environments to predict mature plant performance in the field. This is particularly pertinent in the case of white clover, in which seedling root systems and those of mature plants differ due to the presence of a tap root for the first year or so of the plant’s life. In addition, although the use of hydropnic systems permits root architecture to be analysed relatively easily, it is not possible at present to state unequivocally that root systems growing in soil would exhibit the same morphology. It is clear, however, that measurements of root length and its distribution shed more light on root functioning than do simple root mass data (Newman et al., 1989), and the incorporation of analyses of root architecture into field-based studies of species/cultivar compatibility seems desirable. The screening of genotypes in controlled conditions of solution culture appears to be at least a useful initial approach in the selection of compatible grass/clover combinations, based on the principle of grass/clover response to N, producing complementary morphologies.

ACKNOWLEDGEMENTS

We thank Ms Simone Jones, Ms Wendy Thornley, Mr Simon Mee and Mr Neil Raistrick for technical assistance. S.P. was funded by the EU COST Programme (Action 814) on a short scientific mission. M.F. and R.P. were funded by Defra.

LITERATURE CITED


