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Morphological pattern of development affects the contribution of nitrogen reserves to regrowth of defoliated white clover (*Trifolium repens* L.)

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Abstract

The contribution of nitrogen reserves to regrowth following defoliation was studied in white clover plants (*Trifolium repens* cv. Huia). This was found to be closely linked to the morphological pattern of development of the aerial parts during the same period. Low temperature (6 °C) and short day exposure (8 h photoperiod) were used to induce dwarf development, i.e. to increase branching rate and to enhance new sites of leaf production during a period of regrowth. Treated plants exhibited a large reduction in leaf area and a large increase in leaf pool size for the first 10 d of a subsequent regrowth under standard culture conditions (16 h daylight; 22/18 °C day/night). The contribution of nitrogen from storage compounds in organs remaining after defoliation (sources) to regrowing tissues (sinks) was assessed by ^{15}N pulse–chase labelling during regrowth following shoot removal. The mobilization of nitrogen reserves from storage tissues of regrowing clover was closely linked to the pattern of differentiation of the newly developed organs. It appeared that regrowth was supported less by endogenous N for the first 10 d after defoliation in treated plants, compared with control plants grown continuously in standard conditions. It is assumed that dwarf plants exhibit a lower dependence upon the mobilization of soluble proteins previously accumulated in roots and uncut stolons. The relationship between leaf development rate and N-uptake recovery following defoliation is discussed.

Key words: Defoliation, morphological traits, ^{15}N, N reserves, *Trifolium repens* L.

Introduction

The maintenance of grass/clover associations depends to a large extent on the persistence of an adequate population of the legume in the mixtures. Several studies devoted to improving management interventions have reported that establishment and maintenance states of mixtures were mainly affected by fertilizer N efficiency on both plants (Homes and Maclusky, 1955), overwintering conditions (Woledge *et al*., 1990), grazing patterns specific to animal type (Nolan, 1995), or periodicity and severity of grazing (Leconte and Laissus, 1985).

Even if moderate-to-severe clipping results in an immediate but transient decline in N assimilation in both grass (Ourry *et al*., 1990) and clover (Gordon and Kessler, 1990), the companion grass seems to be a better competitor for soil mineral nitrogen uptake than the legume (Munoz and Weaver, 1999), and may suffer less N deficiency because of a rapid nitrate-induced extension of its root system (Lainé *et al*., 1993). Moreover, the amount of clover harvested from fertilized mixed swards seems not to be significantly dependent upon the level of N application (Laidlaw, 1984). Consequently, when N is provided to mixed swards, the lag period between defoliation and refoliation may be shorter for the grass compared with the legume, and the newly developed leaves may shade the clover stolons and inhibit axillary bud development (Thompson and Harper, 1988; Davies and Evans, 1990). In a similar manner, the disappearance of clover frequently observed in early spring is due to a later spring regrowth compared with the companion grass that is less sensitive to the short days and low temperature conditions in winter (Woledge *et al*., 1990). In relation to these observations, it appears that the first steps in the differentiation of new tissues (sink) during spring or the post-clipping regrowth

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of clover are achieved by the allocation of nutrients from organic reserves previously stored in the remaining (source) organs, i.e. roots and stolons: a quantitative assessment of reserve N rather than C contribution to shoot regrowth has been clearly established (Phillips *et al*., 1983; Marriott and Haystead, 1990; Culvenor and Simpson, 1991). Regarding the source of N used in the production of the new shoots, regrowth of white clover following severe defoliation can be divided into two phases. During the first 6 d, mobilization of N reserves is the major source, but thereafter declines progressively relative to the N acquired externally by N_2 fixation or inorganic ion uptake as the activity of these systems begins to recover (Gordon *et al*., 1990; Corre *et al*., 1996). Soluble proteins are the major N source mobilized from roots and stolons of defoliated white clover, with an average reduction close to 30% occurring within 6 d of regrowth, and protein mobilization has been clearly associated with an increase in the expression of peptidases in roots and stolons (Desjouis *et al*., 1996) and nodules (Gordon and Kessler, 1990) of defoliated clover. Among the soluble proteins mobilized, three low molecular mass polypeptides, referred to as Vegetative Storage Proteins (VSPs), exhibited a pattern of preferential utilization where the amounts declined by up to 80–100% of their initial level during the first week following clipping and accumulation was resumed once N assimilation and photosynthesis had been re-established (Corre *et al*., 1996; Goulas *et al*., 2001). Interestingly, VSPs also accumulated in stolons during the autumn and early winter, and then decreased when growth was resumed during early spring (Bouchart *et al*., 1998; Goulas *et al*., 2001). This suggested that VSPs might supply some of the N required for plant growth when shoot N demand could not be met by N_2 fixation or soil N uptake.

Generalizations regarding the contribution of N reserves to the recovery of white clover after defoliation are complicated on account of the high morphological plasticity associated with this species. This arises from a combination of genetic variation, environmental conditions, and agricultural practices such as periodicity and/or severity of grazing. A less severe defoliation of up to 45–50% removal of the number of leaves increases the rates of leaf emergence and the development of young leaves to maturity (Marriott and Haystead, 1990). Likewise, white clover may survive severe grazing in pure stands since it can develop a dwarf morphology corresponding to a prostrate, small-leaved, short-petioled habit of growth that prevents complete leaf removal (Frame and Newbould, 1986). On the other hand, such a reduction in petiole length would decrease the height of the sward canopy and may lead to a significative shading of the clover laminae growing in a grass/clover association. It has also been shown that rates of leaf appearance and stolon branching are closely related to air and soil temperatures (Davies and Jones, 1992) and light intensity (Davies and Evans, 1990). However, it is generally assumed that clover production is more affected by the number of growing points at the beginning of a growth period rather than by any differences in sward structure or light environment during regrowth.

In a previous work (Bouchart *et al*., 1998), the seasonal pattern of total nitrogen, soluble proteins and VSPs in stolons of *T. repens* L. grown in the field were studied in two contrasting cultivars (the 'giant' cv. Aran and the 'dwarf' cv. Rivendel) varying genetically in their morphological habit of development. In the present paper, the contribution of endogenous N to post-clipping regrowth was investigated in a single genotype of white clover (cv. Grasslands Huia) subjected, before defoliation, to short daylight and low temperature conditions known to affect the morphology of the regrowing aerial parts, i.e. to increase branching rate (Boller and Nösberger, 1983). It is shown that the distribution pattern of stored N within the new leaves after defoliation is dependent upon the initial nitrogen status of the plant and its further morphological development.

Materials and methods

Plant material and growing conditions

White clover seeds (*Trifolium repens* L., cv. Grasslands Huia) were germinated in sand that was moistened daily. Nine seedlings were then transplanted into a 9.0 l culture pot when the primary leaves had developed. Plants were grown hydroponically in a continuously aerated nutrient solution containing 1 mM NH_4NO_3 as the sole nitrogen resource, as described by Kim *et al*. (1991). Plants were grown in winter/spring in a glasshouse (latitude 49°11′ N, longitude 0°22′ W, elevation 41 m), under natural day/night conditions ensuring spontaneous nodulation. During the day, the natural light was supplemented for 16 h with 110 µmol m^{-2} s^{-1} at the height of the canopy, provided by fluorescent 'Phytor' tubes (Claude GTE, Puteaux, France). The thermoperiod was 22±2 °C (day) and 18±2 °C (night). The conditions described above for temperature and light are referred to in the text as 'standard conditions'. Three-month-old plants grown in standard conditions were subjected to a complete defoliation (removal of the laminae and leaf stalks), allowed to regrow for 30 d and labelled with 1 mM $^{15}NH_4{}^{15}NO_3$ for the last 20 d. The labelling period is defined further as regrowth I. At the end of the labelling period, plants were defoliated again and finally allowed to regrow for 25 d (defined further as regrowth II) on a complete medium without ^{15}N. During regrowth I, a set of plants was grown in a controlled environment chamber (6 °C, 8 h photoperiod provided solely by fluorescent tubes), then transferred to the glasshouse under standard conditions for regrowth II. This set is referred in the text as 'treated' plants, compared to the control plants grown continuously under standard conditions. The labelling treatment consisted of a ^{15}N excess of 5% and 1%, respectively, for treated and control plants in order partly to counterbalance the likely reduction in N absorption and assimilation associated with exposure to low temperature. On each date of sampling for N and protein measurements, three pots of nine plants were harvested. Roots and shoots were weighed separately, immediately frozen in liquid nitrogen and then (i) freeze-dried for total N and ^{15}N measurements or (ii) stored (–80 °C) for further analysis of soluble proteins.

Morphological traits description

The average area of the leaves was determined (Area Meter Delta-T Devices, Cambridge, England) on days 0, 12, 18, 27, and 30 of regrowth I, and 0, 3, 6, 10, and 25 of regrowth II. At these dates, the ground area covered by the plants was estimated for each culture pot. The average area of the leaves was obtained from at least 70 measurements on individual fully expanded leaves made up of three leaflets and chosen randomly from nine plants. The mean value (expressed as cm^2 ±SE, n=70) was used to estimate the Leaf Area Index (*LAI*) of three independent pots for which the leaves were previously counted (number of leaves per unit of ground area ±SE, n=3). Results of *LAI* are expressed as cm^2 of laminae per unit of ground area ±SE (n=3). Fifteen main stolons axes (5 stolons×3 culture pots) were marked by a ring at the beginning of regrowth I, and the branching rate was followed on days 0, 3, 12, 18, and 24 of regrowth I, and 0, 6, 10, 20, and 25 of regrowth II. For this purpose, the three types of vegetative points previously defined by Simon *et al*. (1989) were counted as only one. Results are expressed as the number of vegetative points per stolon ±SE (n=15).

Exogenous nitrogen uptake measurements

Net nitrate and ammonium uptake rates were calculated from the linear regression of NH_4^+ and NO_3^- depletions from the nutrient solution on days 0, 1, 2, 3, 4, 5, 7, and 10 of regrowth II (three measurements on each date). Ammonium and nitrate concentrations were determined by colorimetric methods using a continuous-flow autoanalyser (Bran Luebbe, Noderstedt, Germany). The buffered solution containing ammonium reacted with salicylate 8% (w/v) and dichloroisocyanuric acid 0.18% (w/v) with nitroprusside 0.1% (w/v) as the catalyst. NO_3^- in the samples was analysed after reduction with copperized cadmium into NO_2^- before reaction with sulphanilamide 1.2% (w/v) and *N*-(1-naphthyl)ethylenediaminedihydrochloride 0.06% (w/v). Results are expressed as total nitrogen loss in the culture solution per unit of ground area (total g N m^{-2} ±SE, n=3).

Nitrogen isotopic analysis

Determination of ^{15}N content in freeze-dried samples was performed by a continuous flow isotope mass spectrometer (Twenty-twenty, Europa Scientific Ltd, Crewe, UK) linked to a C/N analyser (Roboprep C/N, PDZ Europa Ltd, Crewe, UK). The calculations performed in this study are dependent on various assumptions, many of which are supported by other work and have been summarized previously (Ourry *et al*., 1994). The net change (*dN/dt*) in N content in a plant organ during the regrowth under standard conditions is the difference between nitrogen inflow and outflow from this organ during the time *dt*:

$$dN/dt = N_{inflow} - N_{outflow} = N_{t+dt} - N_t \quad (1)$$

where N_t and N_{t+dt} are the N content at time t and $t+dt$, respectively. Nitrogen inflow derived from N uptake and N_2 fixation during *dt* can be calculated from ^{15}N content as:

$$N_{inflow} = N_{t+dt} \times (1 - E_{t+dt}/E_t) = N_{\text{derived from uptake and fixation}} \quad (2)$$

where E_t and E_{t+dt} are atom% ^{15}N excess in the plant part measured at the time t and $t+dt$, respectively. N outflow from a plant organ, corresponding to endogenous nitrogen mobilization during *dt*, can therefore be calculated from equation 1:

$$N_{outflow} = N_t - N_{t+dt} + N_{inflow} \quad (3)$$

Substitution from equation 2 gives:

$$N_{outflow} = (N_t \times E_t - N_{t+dt} \times E_{t+dt})/E_t \quad (4)$$

If it is assumed that N outflow from all the remaining organs after defoliation is uniformly labelled and that no isotopic discrimination occurs during further distribution to regrowing leaves and petioles, then the amount of N mobilized to these organs is directly proportional to their ^{15}N contents. Natural ^{15}N abundance (A=0.3663%) of atmospheric N_2 was used as a reference for ^{15}N analysis. Results are expressed as % of total N content in the tissues (laminae and petioles) at the time of sampling.

Analysis of soluble proteins

Soluble proteins were extracted from 1 g (sampled randomly from nine plants) frozen (–80 °C) tissues at 4 °C with 7 ml of 50 mM TRIS–HCl buffer (pH 7.5) containing 0.1% (v/v) β-mercaptoethanol, 10 μM leupeptin, 2 mM phenylmethylsulphonylfluoride (PMSF), and 1 mM EDTA. After centrifugation (1200 *g*, 4 °C, 10 min), nucleic acids in the supernatant were precipitated using protamine sulphate (1 mg ml^{-1}) for 15 min at room temperature. After centrifugation (18 000 *g*, 4 °C, 10 min), protein precipitation in the supernatant was achieved using the deoxycholate-trichloracetic acid protocol described by Peterson (1983). After centrifugation (18 000 *g*, 4 °C, 10 min), the pellet was washed with 100% (v/v) acetone and then centrifuged again for 10 min at 18 000 *g* (4 °C). The resulting pellet was air-dried and protein concentration was estimated using the Lowry *et al*. (1951) method. All data are expressed on a dry matter basis as mg of soluble protein g^{-1} DW ±SE (n=3).

Results

Effects of short day and low temperature exposure on plant morphology

Compared with control plants grown continuously in standard conditions, a transient exposure to a shorter photoperiod (8 h) together with a low temperature (6 °C) strongly affected the leaf area attributes of defoliated treated plants (Fig. 1). By the end of regrowth I, treated cultures exhibited a lower leaf area index (*LAI*, Fig. 1A), directly linked to a 35% reduction in the average area of each trifoliate leaf (Fig. 1B), and to a number of leaves two times lower than in control plants (Fig. 1C). Treated and control plants were then defoliated and both allowed to regrow in standard conditions (regrowth II). During the first 10 d of this second regrowth, treated plants developed smaller leaves than control plants (Fig. 1B). Nevertheless, the reduction in the average area of the leaves was largely counterbalanced with a large increase in the number of new leaves during the same period (Fig. 1C). Indeed, treated and control plants exhibited the same *LAI* by day 10 of regrowth II (Fig. 1A).

The differentiation of new growing points was also followed during both regrowth I and II. The branching rate was strongly inhibited by low temperature under shortened days (Fig. 2). The after-effects of previous culture conditions on plant morphology were also noticeable, since defoliated treated plants had the greatest ability to produce new stolons during the subsequent regrowth in standard conditions. This led, by the end of regrowth II, to very densely branched plants compared with control plants. Thus, the large increase in new sites of leaf and stolon

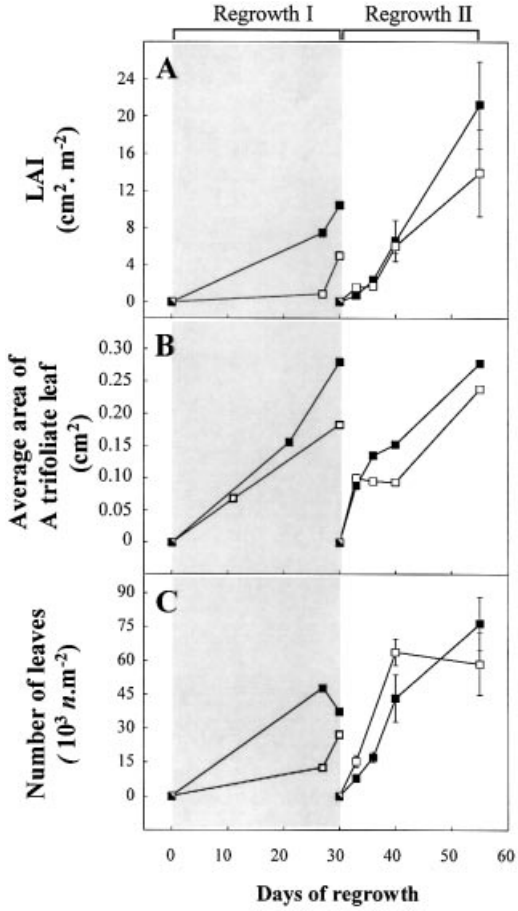

Fig. 1. Changes in leaf area attributes of white clover plants during regrowth following defoliation. (A) Leaf area index (LAI) estimated as cm^2 of laminae per unit of ground area; (B) average area of a trifoliate leaf (cm^2); and (C) number of leaves per ground area during 30 d of regrowth I (shaded area) and 25 d of regrowth II (white area). Closed symbols, control plants; open symbols, treated plants. Vertical bars, when larger than symbols, indicate ±SE with n=3 (A, B) or n=70 (C).

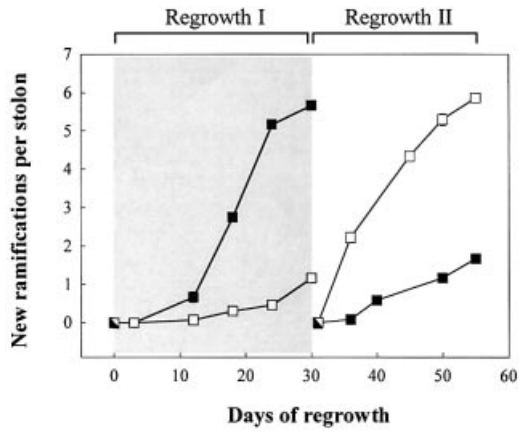

Fig. 2. Branching rate (number of vegetative growing points per stolon) of white clover during regrowths I and II following defoliation. Closed symbols, control plants; open symbols, treated plants. Vertical bars (±SE with n=15) were always less than the symbol size.

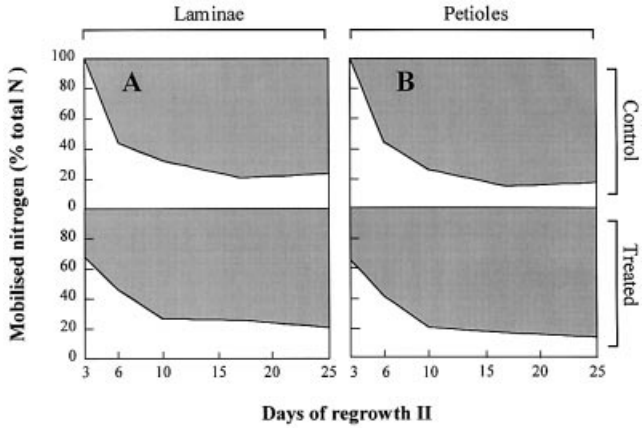

Fig. 3. Origin of nitrogen in regrowing leaves of white clover following defoliation. White background, nitrogen from reserves; grey background, exogenous nitrogen. (A) Laminae; (B) petioles of control or treated plants during 25 d of regrowth II. Results are expressed as % of total N content in the tissue at the time of sampling. Each value is the average of three independent measurements.

production produced, in treated plants, a final morphological status that could be considered as a dwarf phenotype.

Nitrogen sources involved in regrowth

The partitioning of previously accumulated ^{15}N during 25 d of regrowth II on an unlabelled medium was followed in newly developed leaves (laminae and petioles). The amount of total N mobilized was calculated from equation (4) given in the Materials and methods. Regrowth II can be divided into two different periods, depending on N source, but the extent to which endogenous N participated in the differentiation of the new leaves after defoliation depended on the morphological pattern of development of the plant (Fig. 3). During the first 3 d, nitrogen in the regrowing leaves of control plants came exclusively from the mobilization of N reserves, while the differentiation of new organs in treated plants was already supported by the assimilation of exogenous N (inorganic N uptake or N_2 fixation): in control plants, 100% of laminae and petiole nitrogen measured at the time of sampling was derived from reserves by day 3 after defoliation, compared with, respectively, 67% and 65% in treated plants. Later, the contribution to leaf growth of N from reserves decreased in favour of exogenous nitrogen participation that constituted the major nitrogen fraction of the total N pool by the end of regrowth II (i.e. 76% and 83% of total N content at the time of harvest in laminae and petioles, respectively).

Soluble protein contents throughout regrowth

Soluble protein contents (Fig. 4) were examined in the remaining organs (i.e. stolons and roots) of defoliated treated and control plants over the 25 d of a regrowth

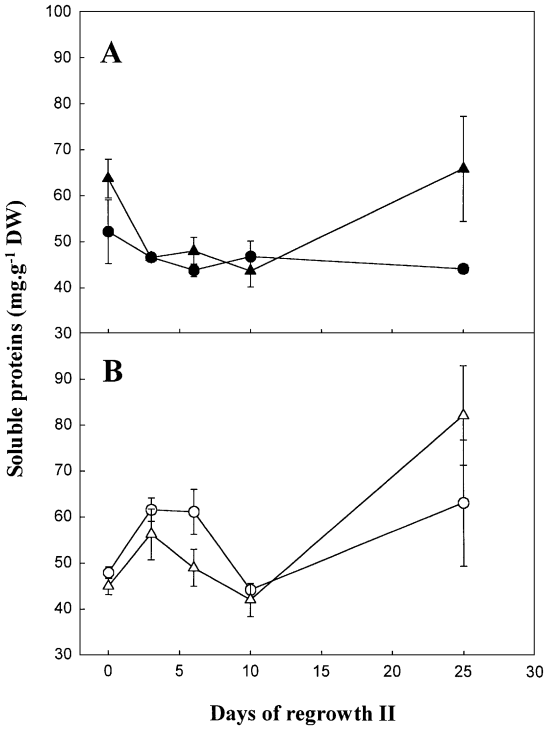

Fig. 4. Soluble protein content (mg g^{-1} DW) in stolons (circles) and roots (triangles) of white clover during 25 d of regrowth II after defoliation. (A) Control plants (closed symbols); (B) treated plants (open symbols). Vertical bars, when larger than symbols, indicate ±SE with n=3.

period in standard conditions (regrowth II). Changes in the soluble protein depended on the type of organ and the previous culture conditions. By the day of defoliation, treated plants exhibited a low protein content compared with control plants. This was associated to a lower nitrogen status since the amounts of N reached approximately 45 and 110 mg N per plant at the start of regrowth II in treated and control plants, respectively (data not shown). A decrease in soluble proteins of 27% and 11%, respectively, in roots and stolons of control plants was noticed during the first 6 d (Fig. 4A). After this date, the soluble protein content increased to about 100% of the value found on the day of defoliation in roots, but remained at a steady-state level in stolons. In treated plants (Fig. 4B), a transient increase in soluble protein occurred in both organs during the first 3 d of regrowth. Nevertheless, a decrease in protein content was noticeable from the third day in roots and the sixth day in stolons. By the end of regrowth II, the protein content in stolons and roots of treated plants exceeded the initial values and was similar to the protein content in control plants (in the range of 50–80 mg g^{-1} DW).

Exogenous nitrogen uptake rates

During the first 2 d of regrowth II (Fig. 5), N uptake from the nutrient solution was clearly higher for treated plants (2.4 g N m^{-2}) than for control plants (1.6 g N m^{-2}).

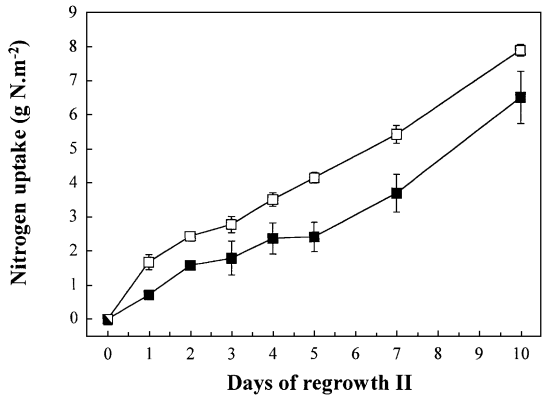

Fig. 5. N uptake per ground area (g N m^{-2}) by white clover plants during the first 10 d of regrowth II following defoliation. Closed symbols, control plants; open symbols, treated plants. Vertical bars, when larger than symbols, indicate ±SE for n=3 replicates.

Between 3 d and 5 d of regrowth, N uptake by control plants slightly slowed down, and from 5 d to 10 d exogenous nitrogen uptake rates remained similar for both sets of plants.

Discussion

Management interventions (periodicity and/or intensity of grazing) on mixed pastures have to take into account the persistence of the legume, partly defined by genetic performance. The 'giant' genotypes of white clover with large laminae and long-petioles are widely assumed to be more persistent in grass/clover associations, but also to be less tolerant to intensive defoliation than the densely-branched 'dwarf' genotypes (Grant and Barthram, 1991; Kang and Brink, 1995). The well-known phenotypic plasticity of the plant seems to be involved in the maintenance of the mixture, since heavy grazing results in the small-leaved prostrate forms of clover (Ryle *et al*., 1989). On a physiological basis, severe defoliation decreases the availability of photoassimilates, NO_3^- and NH_4^+ absorption and assimilation, and N_2 fixation by the nodule during the first days of regrowth (Bouchart *et al*., 1998). Corre *et al*. (1996) suggested that this might occur until shoot regrowth had advanced sufficiently for renewed photosynthesis to support nodule metabolism and the energy requirements for root N uptake. Another explanation has been given by Gordon and Kessler (1990) since the activities of proteins or key enzymes involved in N and C assimilation such as leghaemoglobin, glutamine synthetase, invertase, and even sucrose synthase, recently identified as essential for N_2 fixation (Gordon *et al*., 1999), were strongly repressed in response to defoliation. Nevertheless, mobilization of N and C reserves during a first step should be a prerequisite for efficient shoot regrowth, i.e. for clover persistence, more especially as the legume is grown in mixed pasture with ryegrass, a better

competitor for exogenous N and light (Edwards *et al*., 1996). Ecological and biochemical studies on the responses of clover subjected to severe defoliation (e.g. intensive animal grazing) concluded that persistence of clover in mixed swards was mainly dependent upon (i) its ability to extend the height of the canopy rapidly to prevent competition for light with the companion grass (Davidson and Robson, 1985), and (ii) the mobilization of previously stored organic compounds over the same period, from the remaining tissues to the newly developed organs (Corre *et al*., 1996). In the present glasshouse study, it was shown that a tight relationship exists between these two adaptive responses.

Direct and delayed effects of photoperiod and thermoperiod on shoot morphogenesis

A treatment consisting of both reduced temperature and photoperiod exposure altered the morphological development of the aerial apparatus of clover plants. In white clover, it is believed that the processes which influence number and development of leaves are markedly temperature-dependent (Guckert *et al*., 1983; Simon *et al*., 1989; Guinchard *et al*., 1997), whereas those governing leaf differentiation and size are mainly light-dependent (Boller and Nösberger, 1983; Frame and Newbould, 1986). In this study, the rate of leaf appearance was strongly reduced in response to chilling (6 °C). This is in accordance with previous data demonstrating that the production of phytomass is inhibited in plants subjected to atmospheric temperature around 5–7 °C (Davies and Evans, 1982), and that the mean rate of leaf appearance is closely associated with the temperature in the first 10 cm of soil (Davies and Jones, 1992). Once transplanted under optimal growth conditions, treated plants developed a prostrate morphology characterized by significantly smaller leaves and an increased branching rate. It is generally assumed that this is due to a rapid differentiation of axillary shoot meristems towards new stolon buds (further elongating as branch stolons), as a consequence of modifications in light composition and quantity (Thompson and Harper, 1988) because of a competition for light resulting from new regrowing leaves (Simon *et al*., 1989). A more likely explanation would lie in the chilling conditions which may lead to apical bud breakdown, thus decreasing apical dominance. This hypothesis is supported by field observations since the high mortality of stolons by apical necrosis during the winter (Collins *et al*., 1991; Marriott and Smith, 1992) commonly enhances branching, and promotes the independent existence of individual stolons as the centre of the plant dies (Hay *et al*., 1987). Moreover, a section of stolons including one node and one internode exhibits a high branching rate for buds located at the nearest extremity of the main vegetative point (Davies and Evans, 1990).

Effects of photoperiod and thermoperiod on the N status of the plant

Recent attempts have been made to correlate morphological alterations of the aerial parts and C and N reserve availability and mobilization (Guinchard *et al*., 1997; Corbel *et al*., 1999). According to the authors, overwintering of white clover was favoured by stolon density which seems to be essential to maintain the residual leaf area needed to sustain carbon and nitrogen acquisition during winter. Consequently, spring regrowth was intimately linked to the existence of a 'viable bud bank' (Harper, 1977) which ensured the development of the photosynthetic apparatus. It is also well-established that exposure to low temperatures not only induces energetic metabolism modifications (Graham and Patterson, 1982), but also important changes in the distribution of C assimilates (Guinchard *et al*., 1997) to the detriment of leaf growth, and in favour of stolon and root expansion (Frankow-Lindberg, 1997). In chicory, Améziane *et al*. (1997) have shown that N limitation during vegetative growth delayed the accumulation of a 17 kDa vegetative storage protein in roots. Modifications in source/sink relationships are actually considered as prominent factors in N reserves accumulation (Bouchart *et al*., 1998). Thus, defoliation followed by both reduced temperature and exposure to short days have logically led treated plants to a lower N status compared with control plants grown continuously under standard conditions.

Effects of treatment on nitrogen protein mobilization

During shoot regrowth following defoliation, two physiologically distinct periods are commonly observed in white clover on the basis of the origin of nitrogen found in the regrowing organs (Corre *et al*., 1996; Bouchart *et al*., 1998). Whatever the previous treatment, a decrease in soluble proteins occurs following defoliation. However, a 3 d lag time in protein N utilization was noticed for treated plants. This could be the result of a rapid emission of a very large number of new leaves, which allowed treated plants to dispose of numerous young leaves readily. Since the photosynthetic efficiency seems to be closely related to the leaf age (Woledge, 1977; Woledge *et al*., 1983), treated plants may thus present a functional photosynthetic apparatus during the first steps of regrowth II, as compared to control plants. As a consequence, exogenous C and N assimilation should be quickly restored for treated 'dwarf' plants, which should, therefore, be less dependent on the previously stored organic reserves compared with control 'giant' plants. This hypothesis seems to be supported by the ^{15}N transfers from reserve organs to the new tissues, and by a higher N uptake rate during the first 5 d of regrowth. Later, during a second period, C and N assimilation abilities should not be enough yet to sustain the entire C and N cost of stolon and leaf elongation. This

could explain the delay observed in the utilization of N reserves.

It was also noticed that treated plants exhibited a low protein content associated with a lower nitrogen status, comparable to a deficiency in nitrogen at the start of regrowth. Evidence that N absorption is tightly regulated by N demand has previously been provided by studies where plants are grown at constant N in the nutrient medium, but under differing relative growth rates (Gastal and Saugier, 1989). In a similar manner, changing photoperiod and temperature presumably resulted in changes in the rate of N_2 fixation and NO_3^-/NH_4^+ uptake from identical nutrient solutions (1 mM ammonium nitrate). Once transplanted under standard conditions, 'N-depleted' treated plants exhibited a higher uptake of N compared with 'N-depleted' control plants. It cannot be excluded, therefore, that this enhanced N uptake relieves the negative feedback of N absorption/fixation by N status.

Finally, the overall results confirm that significant amounts of N in regrowing shoots of white clover are derived from mobilized N reserves from the roots and stolons. Nevertheless, the role of endogenous N to sustain growth after defoliation seems to be closely associated with the ability of the plants to recover photosynthesis, i.e. to develop young new leaves rapidly. As a conclusion, it should be noted that the overwintering of white clover is closely related to the existence of previously stored compounds and to its morphological pattern of development, but that a very tight relationship exist between these two adaptive responses.

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