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The XIX International Grassland Congress took place in São Pedro, São Paulo, Brazil from February 11 through February 21, 2001.

Proceedings published by Fundacao de Estudos Agrarios Luiz de Queiroz

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**PATTERNS OF DEVELOPMENT AND NITROGEN RESERVES MOBILIZATION  
DURING REGROWTH OF DEFOLIATED CLOVER**

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**Abstract**

Contribution of nitrogen reserves to regrowth following defoliation was studied in white clover plants (*Trifolium repens*) according to the morphological pattern of differentiation of the aerial parts during the same period. Low temperature and short day lengths were used as a pre-treatment in order to increase branching and enhance new sites of leaf production during a further 25 d period of regrowth. Pre-treated plants exhibited a large reduction in leaf area largely counterbalanced with a high increase in leaf pool size during the first 10 d of regrowth. The mobilization of nitrogen reserves during regrowth of defoliated clover was intimately linked to the pattern of differentiation of the newly developed organs. It thus appeared that regrowth of pre-treated plants was less supported by endogenous N during the first 10 d as compared to control plants continuously grown in standard conditions. It is assumed that regrowth of dwarf plants is less dependent upon the mobilization rate of soluble proteins previously accumulated in roots and uncut stolons.

**Keywords** : Defoliation, morphological traits, nitrogen, reserves, clover

## **Introduction**

Regrowth of defoliated clover might be divided into two phases in regard to the source of N used in the differentiation of new tissues. Mobilization of endogenous N from roots and stolons sustains the edification of the new shoots during a first 6-day period, but thereafter declines progressively relative to N acquired externally (Gordon et al., 1990 ; Corre et al., 1996). During this first week, it has been shown that three low molecular mass polypeptides (defined as VSP, Vegetative Storage Proteins) have preferential utilization, and are depleted 80 to 100% of their initial value before accumulating again, once N assimilation and photosynthesis were re-established. Nevertheless, studying the contribution of N reserves to regrowth of white clover is complicated because of the high morphological plasticity of the plant linked to genetic performance, environmental conditions, or agricultural practices such as periodicity and/or severity of grazing. In the present paper, we investigated the contribution of endogenous N to post-clipping regrowth of clover grown under controlled conditions known to affect the morphology of the aerial parts, i.e. to increase branching rate (Boller and Nösberger, 1983).

## **Material and Methods**

White clover (cv. Grasslands Huia) was grown hydroponically in a nutrient solution containing 1 mM  $\text{NH}_4\text{NO}_3$  as the sole nitrogen resource. Plants were grown in winter/spring in a glasshouse, under natural day/night conditions ensuring spontaneous nodulation. The natural day light was supplemented for 16 h with  $110 \mu\text{mol m}^{-2} \text{s}^{-1}$  at the height of the canopy, provided by fluorescent phytotubes (Claude). The thermoperiod was  $22 \pm 2^\circ\text{C}$  (day) and  $18 \pm 2^\circ\text{C}$  (night). These conditions are referred to in the text as “standard conditions”. During the first regrowth period (regrowth I) following complete defoliation, a set of plants was grown in a

controlled environment chamber (6°C, 8 h photoperiod solely provided by fluorescent tubes), then transferred to the glasshouse under standard conditions for a second regrowth period (regrowth II). This set is referred in the text as “treated” (regrowth I) or “pre-treated” (regrowth II) culture, as compared to control plants continuously grown under standard conditions. Soluble proteins were analyzed according to Lowry et al. (1951).

## **Results and Discussion**

As compared to control plants continuously grown in standard conditions, a transient exposure to a shorter photoperiod (8 h) associated to a decreased temperature (6°C) slackened the development of the regrowing aerial parts in defoliated treated plants (Fig. 1A-C). At the end of regrowth I, treated plants exhibited a lower leaf dry matter production directly linked to a leaf pool size 5 times less than control plants, and a 35% reduction in averaged leaf area. Treated and control plants were then defoliated and both allowed to regrow in standard conditions (regrowth II). During the first 10 d, pre-treated plants developed smaller leaves than control plants. Nevertheless, reduction in averaged leaf area was largely counterbalanced by a high increase in leaf pool size during the same period.

Branching rate was strongly inhibited by low temperature and exposure to shorter days. After-effects of previous culture conditions on plant morphology were noticeable, since defoliated pre-treated plants had the greatest ability to produce new growing sites during the subsequent regrowth in standard conditions (Fig. 1D). This led, at the end of regrowth II, to a denser stolon branching than observed for control plants. Leaf appearance rate was strongly reduced during chilling exposure (6°C) according to previous data by which the production of phytomass was inhibited for plants subjected to atmospheric temperatures no more than 5-7°C

and the mean rate of leaf appearance was associated with 10 cm soil temperature (Davies and Jones, 1992). Once transplanted under optimal growth conditions, pre-treated plants developed a prostrate morphology characterized by small leaves and an increased branching rate. This was achieved owing to a rapid differentiation of axillary shoot meristems towards new stolon buds, further elongating as branch stolons. A most likely explanation would lay in chilling conditions which may have led to apical bud breakdown and thus decreased apical dominance. This is supported by field observations since high mortality of stolons during winter by apical necrosis (Collins et al., 1991 ; Marriott and Smith, 1992) commonly enhances branching and promotes the independent existence of individual stolons, as the center of the plant dies (Hay et al., 1987). In white clover plants, it is believed that the processes which influence numbers and development of the leaves are markedly temperature-dependent (Simon et al., 1989), whereas those governing differentiation and size are mainly light dependent (Frame and Newbould, 1986). Moreover, it is commonly admitted that large-laminae and long-petioloated “giant” phenotypes of white clover are more persistent in grass/clover pastures but, on the other hand, less tolerant to intensive defoliation than the dense-branched “dwarf” phenotypes resulting from heavy grazing.

Soluble protein concentrations (Fig. 2) were examined in remaining stolons of defoliated pre-treated and control plants over the regrowth II period. Dwarf pre-treated plants seemed to be less dependent on N reserves than giant control plants, even if protein mobilization occurred regardless of previous treatment.

Results suggest that persistence of clover following defoliation is mainly dependent upon (i) its ability to extend rapidly the height of canopy maybe to prevent competition for light with putative companion grasses, and (ii) a mobilization rate of previously stored N compounds from the remaining tissues to newly developed organs over the same period. In the present

glasshouse study, it was shown that a tight relationship might exist between these two adaptative responses.

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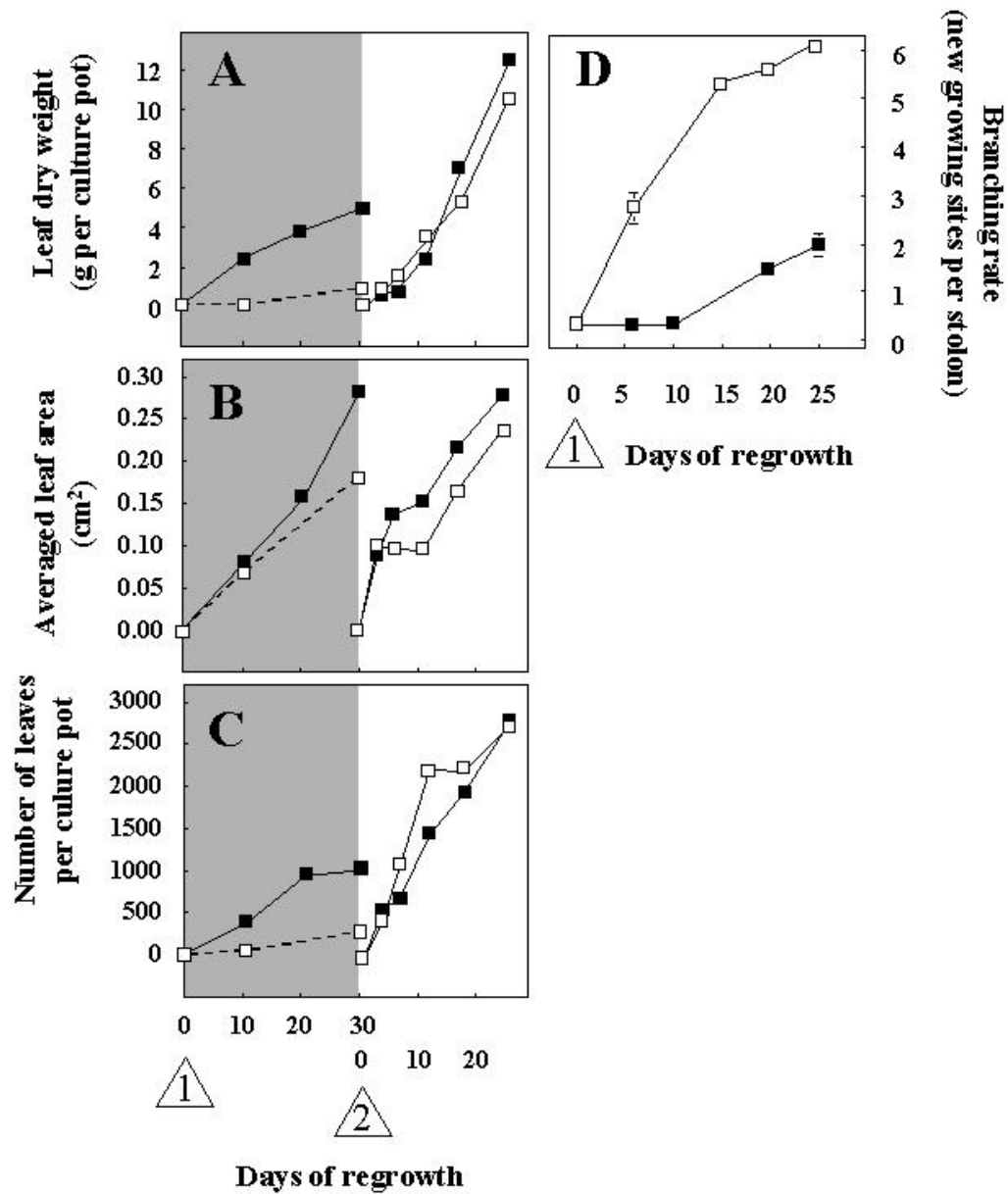
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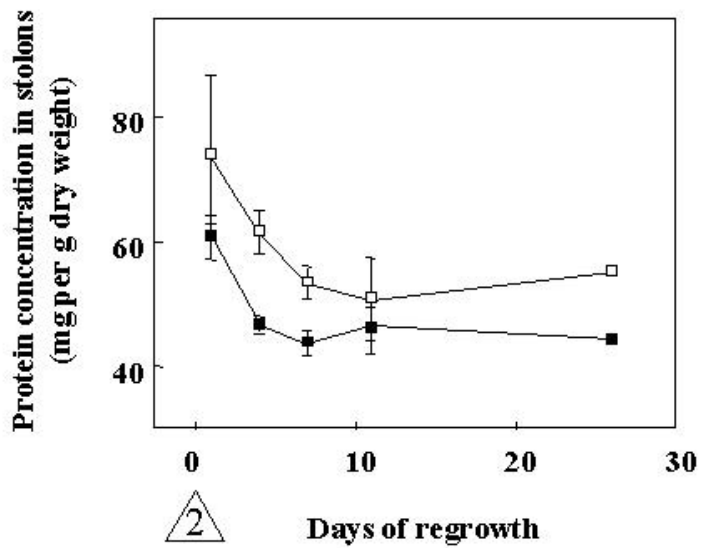
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**Figure 1** - Development of new leaves and shoots following two complete defoliations (numbered arrows) of clover. Grey area, regrowth I ; white area, regrowth II. Dotted line, plants grown under low temperature and short photoperiod ; full lines, plants grown under standard conditions. White symbols, treated or pre-treated plants ; black symbols, control plants (see Material and Methods for complete description).





**Figure 2** - Mobilization of soluble proteins in remaining stolons during post-clipping regrowth of clover in standard conditions. White symbols, pre-treated plants ; black symbols, control plants (see Material and Methods for complete description).