Seasonal Fluctuations of Vegetative Storage Proteins and Starch Concentrations in Stolons of Trifolium Repens L.

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SEASONAL FLUCTUATIONS OF VEGETATIVE STORAGE PROTEINS AND STARCH CONCENTRATIONS IN STOLONS OF TRIFOLIUM *REPENS* L.

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ABSTRACT
The seasonal pattern of nitrogen, starch and vegetative storage protein (VSP) concentrations was studied in the stolons of two *Trifolium repens* L. genotypes (cv Aran and Rivendel). Maximum concentrations of starch were found in summer months; its hydrolysis occurred in winter, at the time, where VSP and nitrogen were accumulated. The decrease of nitrogen and VSP concentrations occurred during spring, and an inverse relationship was found between VSP concentrations in stolons and mean temperatures. The causal implications of starch and VSP availability on spring regrowth potential are discussed in relation with regulatory mechanism inducing VSP synthesis.

KEYWORDS
White clover, Vegetative storage proteins, Starch, Seasonal variations.

INTRODUCTION
White clover (*Trifolium repens* L.) is a major perennial forage legume which is subjected to grazing and cutting. After defoliation, it is well established that nitrogen and carbon reserves contribute to shoot regrowth. Corre *et al.* (1996) demonstrated using ^{15}N labelling that the endogenous nitrogen of stolons and roots of white clover were mobilized to support leaves regrowth; moreover, a 17 kD protein matching the criteria used to define a vegetative storage protein (VSP) as stated by Staswick (1994) was identified in stolons and roots.

Seasonal fluctuations of nitrogenous and carbohydrate compounds are strongly associated with overwintering strategy. The VSP contribute to shoot regrowth, then to survival and spring growth for perennial species like in lucerne (Hendershot and Volenec, 1993) or for many trees (Stépien *et al.*, 1994). In the present study, the seasonal patterns of major storage forms of nitrogen and carbon in stolons i.e. VSP and starch was studied in white clover. Putative factors which may induce the VSP accumulation are also discussed.

MATERIALS AND METHODS
Plant culture. Two contrasted genotypes of white clover (geant, cv Aran and dwarf, cv Rivendel) were sown in September 1993 at the INRA experimental station of Le Vieux Pin (France, 48° 44'N, 0°09'E), harvested 5 fold in 1994 (2/5, 23/5,16/6, 1/8, 3/10) and 4 fold in 1995 (22/5, 12/6, 3/7, 28/8) and received 100kg.ha^{-1} P_2O_5 and 200 kg.ha^{-1} K_2O in March 1994 and 1995. The plants were sampled September 26, December 2 1994, January 16, February 28, March 31, May 2, May 19, July 3, July 31, and August 28 1995. They were freeze-dried, ground to a fine powder and kept at -80°C until analysis.

Chemical analysis. All analytical methods used have been previously described in Corre *et al.* (1996). Briefly, the total nitrogen and starch concentrations of samples were determined with a C/N analyser (Roboprep CN, Europa Scientific Ltd, UK) and using an enzymatic method (Boerhinger combination kit N°207748, Boerhinger Mannheim GmbH), respectively. For each harvest, triplicate samples were pooled and the soluble proteins extracted. Soluble proteins from a constant amount (400µg DW) of stolon dry weight, were submitted to SDS-page electrophoretic analysis.

RESULTS AND DISCUSSION
Seasonal variations in nitrogen and vegetative storage protein concentrations in stolons. Maximum nitrogen concentrations in stolons were found for both genotypes during winter time (January and February, Fig.1A). Spring growth in March, was concomitant with a large decrease of nitrogen concentration of about 34% and 37% of values found in January for Rivendel and Aran genotypes, respectively. Another maximum nitrogen concentration was found later in spring (May, Fig.1A) followed by another decline. Electrophoretic analysis of stolon soluble proteins given in Figure 2 showed that the 17 kD polypeptide was one of the most prominent protein and furthermore was subjected to a seasonal pattern of accumulation/mobilization. The image analysis of this electrophoresis (Fig. 1B), shows that VSP was found at very large concentrations during winter months as found for nitrogen concentration (Fig.1A). Although, VSP was largely and apparently hydrolyzed in March, there was no further accumulation in spring as found for nitrogen.

The electrophoretic analysis of VSP showed two phases: one which is characterized by the accumulation of VSP in fall and early winter, and another during which the degradation of these nitrogenous storage compounds occurred during early spring. This accumulation and utilization of nitrogen compounds is similar to those reported by Cyr and Bewley (1990) in chicory and dandelion and by Hendershot and Volenec (1993) in lucerne.

Seasonal variations in starch. The starch content (fig. 1C) increased rapidly in summer and the maximum of accumulation was observed in September: from 65 in July to 316 in September and from 92 to 194 mg.g^{-1}DW for Rivendel and Aran respectively. Then, the amount of starch decreased during early winter for both genotypes. Concomitantly, an increase of soluble sugars (sucrose, glucose), resulting probably from starch hydrolysis was observed (data not shown).

Witt and Sauter (1994) reported in poplar wood that the starch was converted in soluble sugars at the beginning of the dormant season. This degradation accompanied by an accumulation of soluble carbohydrates can suggest a role in respiration maintenance, or in supplying ATP and carbon skeletons for the formation of nitrogenous reserves (Cyr and Bewley, 1990) or as a protectant against freezing (Graham and Patterson, 1982).

In early spring, when the storage compounds were used, the starch was already partly hydrolyzed, when changes in nitrogen and VSP concentrations began. For example, high nitrogen concentrations were found in late spring (May, June, Fig.1A) when VSP showed their lowest concentrations. These results demonstrate that nitrogen concentration alone may not be used as a criteria to evaluate nitrogenous storage, and furthermore that qualitative changes in soluble proteins occurred. A highly significant ($r=0.83$, $p<0.0001$) inverse linear relationship was found between VSP and mean temperature for both genotypes, suggesting that their accumulation was induced by low temperatures, and its degradation stimulated by increased mean temperature.

It can be hypothesized that the low temperatures in autumn and winter (Fig.1B) changed the source/sink status for carbon and nitrogen within the plant ; the decrease of the shoot growth reduced its sink strength allowing a higher accumulation of storage compounds such

as VSP in vegetative tissues (Staswick, 1994). The source/sink interactions appear like the first effect of cold temperatures and winter conditions, but other factors can induce the accumulation of VSP. The starch degradation enzymes are activated in autumn (Witt and Sauter, 1994), consequently starch concentrations decrease, allowing an increase of soluble sugar content. Therefore, Mason *et al.* (1992) reported the coregulation of soybean vegetative storage protein gene expression by methyl jasmonate and soluble sugars. Then, in fall and winter, the conditions which induce the VSP accumulation in clover may be combined as starch hydrolysis precede VSP accumulation.

Overall results, show that accumulation/hydrolysis pattern of storage compounds (starch, VSP) in clover are submitted to complex interactions. Further studies are needed to reevaluate the exact causal role of starch and VSP on spring growth ability, and the putative relationships in regulatory process involved in their respective metabolic pathways.

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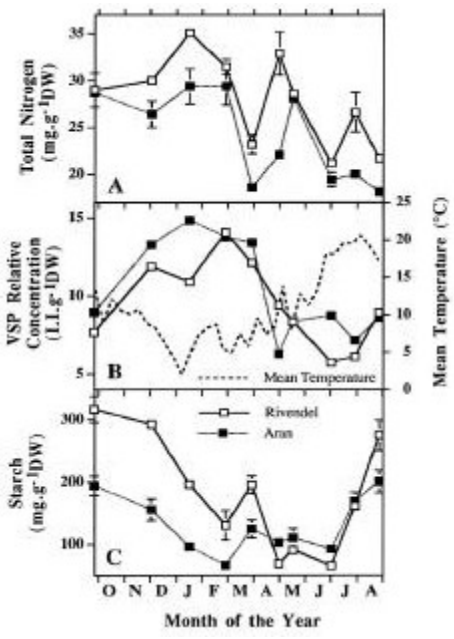

Figure 1
Seasonal variations in cv Rivendel and cv Aran stolons of total nitrogen (A: mg.g-1DW), VSP relative concentration (B: relative integrated intensity obtained from image analysis of SDS-page given in Fig. 2) and starch concentration patter (C: mg.g-1DW). Mean temperatures are presented in (B). For (A) and (C), vertical bars, when larger than symbols, indicate ± SE for n=3.

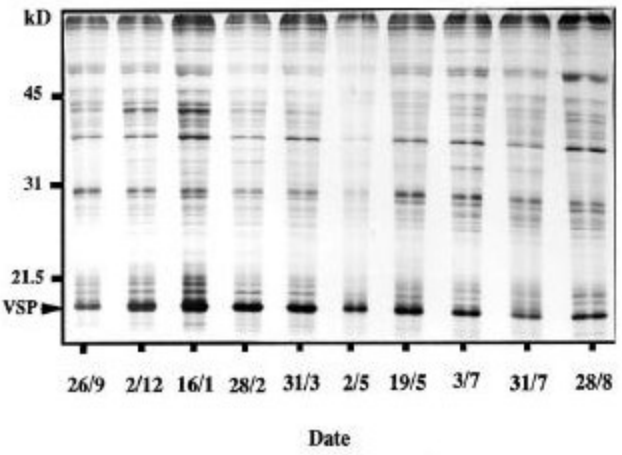

Figure 2
SDS-page pattern of soluble proteins of cv Aran stolons. Each well was loaded with proteins extracted from the same amount (400 µg) of dry weight. Vegetative storage proteins are identified by an arrow. The sampling date is given for each well. The positions of molecular weight markers are given in the left side.