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Effect of the interaction between elevated CO_2, temperature and drought during regrowth of nodulated alfalfa plants

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SUMMARY – The rising atmospheric CO_2 concentration resulting from industrial development may change photosynthesis and plant growth. However, there is a lack of information concerning the combined effect of factors such as CO_2, temperature and water availability on plant regrowth following cutting or grazing, the usual managing methods of forage legumes like alfalfa. In all Mediterranean regions of the world, summer drought has been recognized as the major stress that limits plant species distribution and growth. The aim of the work was to study the interactive effect of CO_2, temperature and water availability in nodulated alfalfa during regrowth. The higher drought tolerance observed during the regrowth period may be related to higher mass and reserves accumulated in the roots. Nitrogen pools in alfalfa taproot, especially vegetative storage proteins (VSP), affect the regrowth of new shoots. After one month of regrowth, drought increased the root VSP concentration.

Keywords: Alfalfa, drought, regrowth, temperature, vegetative storage proteins.

RESUME *– "Effet de l'interaction entre un CO_2 élevé, la température et la sécheresse pendant la repousse de plantes de luzerne nodulées ". L'augmentation de la dose de CO_2, provoquée par l´activité industrielle, pourrait bien affecter la photosynthèse et la croissance de la plante. Cependant, il y a un manque d'information concernant l'effet combiné du CO_2, de la température et du dosage d'eau sur la repousse de plantes de luzerne après la fauche. L'objectif de cette recherche est l'étude de l'interaction entre le CO_2, la température et le dosage de l'eau dans la repousse des plantes de luzerne nodulées. L'amélioration de la tolérance à la sécheresse observée pendant la repousse de la luzerne par rapport à la première pousse, pourrait être liée à l'accumulation de réserves dans les racines. Les réserves d´azote de la racine principale de la luzerne, spécialement les protéines végétatives de réserve (VSP), pourraient bien conditionner la repousse de la nouvelle partie aérienne. Après un mois de repousse, la sécheresse augmente le niveau de VSP.*

Mots-clés : *Luzerne, sécheresse, repousse, température élevée, protéines végétatives de réserve.*

Introduction

Economic development and progressive industrialization are responsible for increasing atmospheric CO_2 concentration from the end of the eighteenth century to the present day. CO_2 has greenhouse gas properties, contributing to a rise in temperature and the phenomenon known as global warming. The IPCC predicts that temperatures will rise about 1.4-5.8°C by the middle of this century (IPCC, 2007).

Many studies have reported the beneficial effect of increasing CO_2 concentration on growth in alfalfa and other species. However, it is well known that elevated CO_2 effect on growth depends on others factors such as temperature, relative humidity, drought or mineral nutrition. In all Mediterranean regions of the world, summer drought has been recognized as the major stress that limits plant species distribution and growth. Water availability is a factor that will especially affect plant life in these areas where the general circulation model (GCM) predicts drier conditions due to an increase of air temperatures and water deficit (Houghton *et al.*, 2001).

Generally, most of the studies concerning the response of plant growth to high CO_2 alone or in combination with other environmental factors such as water availability, temperature, and mineral nutrition have focused on vegetative normal growth. However, very little attention has been paid to the effects of combined factors during regrowth after mechanical cutting or pasturage, which is the usual method of managing forage legumes like alfalfa. Regrowth after defoliation requires the mobilization of C and N reserves accumulated previously in specific storage organs. In alfalfa, N reserves in the roots have been shown to be very important after partial or total removal of the photosynthetic area (Ourry *et al.*, 2001). Root carbon reserves are mainly used for respiration in remaining organs (Avice *et al.*, 1996), and contrasts with the view that shoot regrowth is carbohydrate-driven. The N reserves, however, are used to support shoot regrowth. Since defoliation causes great reduction of mineral N uptake and symbiotic N_2 fixation (Vance *et al.*, 1979), the mobilization of stored N is the key to providing N to the new regrowing shoots during the first days after cutting (Avice *et al.*, 1997).

The taproot is the main storage organ in alfalfa, and contains the most important soluble N pools: amino acids and soluble proteins. Among the soluble proteins, the four abundant polypeptides of 57, 32, 19 and 15 kDa have been identified in the taproots. These proteins, involved in the temporary storage of N, can represent up to 40% of the total soluble proteins and possess characteristics of vegetative storage protein (VSP) (Staswick *et al.*, 1994).

The objective of the present study was to determine the effect of CO_2 (ambient, around 350 μmol mol^{-1} *versus* 700 μmol mol^{-1}), temperature (ambient *versus* ambient + 4℃) and water availability (well-watered *versus* partially-watered) on biomass production (total dry matter), and their consequences on the accumulation of taproot N (especially VSP) and C (soluble sugars, starch) reserves in nodulated alfalfa after one month of regrowth.

Materials and methods

Alfalfa (*Medicago sativa* L. cv. Aragón) seedlings inoculated three times with *Sinorhizobium meliloti* strain 102F78, were planted in 13-l pots (20 plants per pot) containing a mixture of perlite-vermiculite (2:1, v/v). Plants were grown in a greenhouse at 25/15ºC, 50% RH, with a 14 hr photoperiod under natural daylight supplemented with fluorescent lamps and irrigated alternatively with Evans N-free solution and distilled water to avoid salt accumulation in the substrate. Thirty-day-old plants were placed into two temperature gradient greenhouses in an alfalfa field at the Muñovela farm of the Instituto de Recursos Naturales y Agrobiología (CSIC, Salamanca, Spain). The temperature gradient greenhouses were the same of Aranjuelo *et al*. (2005). Plants were divided into all combinations with respect to CO_2 concentration, temperature and water availability giving eight treatments. One greenhouse was maintained at an ambient CO_2 concentration level (around 400 μmol mol^{-1} CO_2), and the other at an elevated CO_2 level (around 700). Each greenhouse was divided into three modules, thereby providing different temperature values. The middle module was considered the transition module, and no experimental plants were included. In each greenhouse, the inlet module was maintained at an ambient temperature and the outlet module at an ambient temperature + 4℃.The maximum soil volumetric water content (θv), corresponding to the well-irrigated treatments, was around 0.4 cm^3 cm^{-3}. Drought treatments were kept on a 50% θv (around 0.2 cm^3 cm^{-3}).

Therefore eight treatments were considered: (i) well watered at ambient temperature and CO_2, (ii) well watered at ambient temperature and elevated CO_2, (iii) well watered at ambient temperature + 4ºC and ambient CO_2, (iv), well watered at ambient temperature + 4ºC and elevated CO_2, (v) partially watered at ambient temperature and CO_2, (vi) partially watered at ambient temperature and elevated CO_2, (vii) partially watered at ambient temperature + 4ºC and ambient CO_2, and (viii) partially watered at ambient temperature + 4ºC and elevated CO_2.

After one month of growth in these conditions (60-day-old-plants), the above parts of the plants were removed at a 5 cm stem height. The remaining organs (remaining shoots, taproots and nodules) were maintained in the greenhouses for a further month under the same treatments in order to develop new shoots (regrowth). Two plant harvests were performed: the first after one month of growth in the greenhouses (60-day-old plants) and corresponding to the vegetative normal growth harvest of alfalfa, and the second 30 days after cutting and corresponding to the regrowth harvest (90-day-old plants). In this paper only data from the second harvested were included (regrowth).

Plant total dry matter (Total DM), root/shoot ratio (Shoot/Root), taproot total nitrogen (N), taproot total soluble proteins (TSP) and soluble sugars (TSS), starch, vegetative storage proteins (VSP), including 32, 19 and 15 kDa molecular mass polypeptides, and the ratio VSP/ root TSP were measured. More details about the experimental design, methods, parameters and statistical analysis are given elsewhere (Erice *et al*., 2007).

Results and discussion

At the end of the regrowth period (90-day-old plants), total DM increased in response to elevated CO_2 in well-watered and partially-watered plants, with the exception of well-watered plants grown at an ambient temperature (Table 1). Partially-watered (drought) treatments reached an average of 78% of well-watered DM. The root/shoot ratio after the 30-day regrowth, regardless of water regimes, was unaffected by CO_2 or temperature. Reduced water availability in drought plants increased root/shoot values significantly. At the end of the regrowth period elevated CO_2 enhanced N taproot concentration under drought conditions (Table 1). After 1 month of regrowth, the higher taproot starch concentration was reached by well-watered plants grown under ambient temperature, especially those grown under conditions of elevated CO_2 (Table 1). At the end of the regrowth period, taproot TSS in well-watered treatments was enhanced by elevated CO_2 and lowered by elevated temperature (Table 1). Drought did not affect taproot TSS concentration during this period.

Table 1. Effect of CO_2, temperature and water availability on total dry matter (Total DM, g plant^{-1}), root/shoot ratio (Shoot/Root, g g^{-1}), root total nitrogen (N, mg plant^{-1}), taproot total soluble sugars (TSS, mg plant^{-1}), and starch concentrations in nodulated alfalfa after 1 month of regrowth. Meaning of treatment abbreviations: Well-watered, soil volumetric water content of 0.4 cm^3 cm-^{3}; partially-watered, soil volumetric water content of 0.2 cm^3 cm^{-3}; Amb CO_2, ambient CO_2 around 400 μmol mol^{-1}; 700 CO_2, elevated CO_2 around 700 μmol mol^{-1}; Amb, ambient temperature; Amb + 4ºC, ambient temperature + 4ºC. Mean followed by the same letter are not significantly different (P>0.05)

Treatments H_2O-CO_2-T	Parameters				
	Total DM	Root/Shoot	N	TSS	Starch
Well-watered-Amb-Amb	4.7 ± 0.2 a	2.2 ± 0.1 ab	83 ± 8 a	21 ± 2 a	255 ± 14 a
Well-watered-700-Amb	4.3 ± 0.3 a	1.8 ± 0.1 b	60 ± 8 b	27 ± 3 b	395 ± 20 b
Well-watered-Amb-Amb+4ºC	4.0 ± 0.3 a	1.9 ± 0.1 b	65 ± 8 b	12 ± 1 c	125 ± 13 c
Well-watered-700-Amb + 4ºC	5.8 ± 0.2 b	1.8 ± 0.0 b	80 ± 4 a	19 ± 1 a	157 ± 13 d
Partially-watered-Amb-Amb	2.8 ± 0.3 c	2.2 ± 0.1 a	45 ± 4 c	22 ± 1 a	146 ± 25 dc
Partially-watered-700- Amb	4.0 ± 0.2 a	2.2 ± 0.0 a	73 ± 10 a	23 ± 4 a	184 ± 30 d
Partially-watered-Amb-Amb + 4ºC	3.2 ± 0.2 c	2.0 ± 0.1 a	51 ± 4 c	21 ± 2 a	202 ± 34 d
Partially-watered-700-Amb + 4ºC	4.5 ± 0.3 a	2.1 ± 0.3 a	65 ± 8 b	25 ± 1 b	151 ± 14 d

At the end of the regrowth period, the taproot TSP content (Table 2) was reduced by CO_2 in well-watered plants but increased in drought treatments. Drought increased the TSP content significantly under elevated temperature conditions. Elevated CO_2 significantly enhanced VSP taproot content (Table 2) except in case of well-watered plants grown at ambient temperature. The VSP/TSP ratio was enhanced by elevated CO_2 under well-watered conditions.

Table 2. Effect of CO_2, temperature and water availability on taproot total soluble proteins content (TSP, mg plant^{-1}), taproot vegetative storage proteins (VSP, mg plant^{-1}), and TSP/VSP ratio (mg mg^{-1}) in nodulated alfalfa after 1 month of regrowth. The rest as the Table 1

Treatments H_20-CO_2-T	Parameters		
	TSP	VSP	VSP/TSP
Well-watered-Amb-Amb	109 ± 13 a	16 ± 2 abd	0.16 ± 0.00 a
Well-watered-700-Amb	62 ± 7 bc	14 ± 1 bc	0.23 ± 0.02 b
Well-watered-Amb-Amb+4ºC	76 ± 7 b	12 ± 1 c	0.17 ± 0.00 a
Well-watered-700-Amb + 4ºC	56 ± 7 c	20 ± 0 d	0.37 ± 0.04 c
Partially-watered-Amb-Amb	65 ± 7 bcd	12 ± 2 c	0.18 ± 0.00 a
Partially-watered-700- Amb	104 ± 7 a	18 ± 1 d	0.17 ± 0.01 a
Partially-watered-Amb-Amb + 4ºC	84 ± 7 d	14 ± 1 bc	0.17 ± 0.01 a
Partially-watered-700-Amb + 4ºC	113 ± 15 a	18 ± 1 d	0.16 ± 0.01 a

Drought limits growth and shoot regrowth after cutting, but significantly increase the root biomass (Table 1) and the TSP content in taproot during regrowth period (Table 2). As a consequence, in the absence of N limitation, drought stress may enhance the availability of N compounds to the taproots, accumulating high amounts of TSP and VSP which corresponded to the increase of their contents and the size of the taproot. These data suggest that the root N enrichment observed under drought conditions can redirect metabolic pathways to VSP synthesis possibly via an induction by ABA. Furthermore the VSP accumulation in vacuolar compartment reinforces the idea that VSP may significantly participate to maintain the cellular osmolarity equilibrium and/or N metabolism.

Conclusions

After one month of regrowth, drought under elevated CO_2 increased the VSP level and confirmed an expectation of greater forage production in drought treatments under equal regrowth conditions during the next cutting-regrowth cycle. A better knowledge and control of VSP taproot accumulation is essential for alfalfa yield improvement in order to obtain maximum production and maintain the perenniality of alfalfa yield.

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