

Effect of boron on nitrogen fixation and carbohydrate content in faba bean (*Vicia faba* L.)

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Abstract

Al Mohammad, H. and D. Poulain. 1996. Effect of boron on nitrogen fixation and carbohydrate content in faba bean (*Vicia faba* L.). Arab J. Pl. Prot. 14(2): 105-110.

One variety (Blandine) and two pure lines of faba bean were cultivated in a greenhouse with a nutrient solution containing three boron concentrations : 0, 2 and 32 mg B l⁻¹. The growth of the root system and nodulation, symbiotic fixation activity and modifications of leaves soluble sugars content were determined. Deficiency and excess of boron resulted in the restriction of root growth, diminution of the fresh weight of nodules per plant and acetylene reduction activity (ARA) level. Absence of boron in the nutrient solution resulted in an accumulation of soluble sugars in leaves, particularly sucrose. The negative effect of boron deficiency could be explained by a limitation of carbon supply to the nodules as well as by its direct effect on the ARA activity of nitrogenase. Boron toxicity may affect the activity of this enzyme by its antagonistic effect on molybdenum and/or by its effect on the availability of magnesium.

Key-words. Boron, carbohydrate, deficiency, excess, *Faba bean* L., nitrogen fixation.

Introduction

A number of studies have been carried out on boron requirement by legumes (9, 14, 17, 23). Generally, the need for this element by legumes is high compared to other families. Within legumes, it varies according to species; faba bean in particular is very sensitive to boron deficiency, but it is tolerant to relatively high concentrations of this element (19). Some of the symptoms observed in both situations are similar (reduction of growth and yellowing coloration of leaves) which might be directly or indirectly associated with poor nitrogen fixation and further assimilation. Indeed, in tomatoes both excess and deficiency of boron have been shown to induce an accumulation of nitrate, which is associated with a reduction in the activity of nitrate reductase (3).

The present work is aimed at analyzing the effect of different levels of boron on the development of the root system, nodulation and nitrogenase activity in faba bean.

Materials and methods

Production of plant material: The experiment was carried out in the greenhouse between February and June 1994, at the Department of Agronomy, ENSA, Rennes.

Three faba bean genotypes were used: a cultivated variety (Blandine) and two pure lines (C.3892 and C.469). They have been chosen because of their typical responses to different levels of boron (19). The plants were grown in pots (on perlite) in a nutrient solution optimized for faba bean, devoid of nitrogen and adjusted to pH 7 (Table 1). Three boron (B) concentrations were included in the nutrient solution : 0 mg B l⁻¹ corresponding to deficiency level ; 2 mg B l⁻¹ corresponding to optimal level and 32 mg B l⁻¹ corresponding to a toxicity level. A suspension of *Rhizobium leguminosarum* (produced *in vitro* in Petri dishes), was added to each pot 8 days after emergence. Four grains of the same cultivar were sown per pot, and the number of plants in each pot was thinned to three after emergence. The experiment

was carried out under natural sunlight at natural temperature (between 10 and 33°C).

Table 1. Chemical composition of the nutritive solution used for growing faba bean.

Macronutrients	mg.l ⁻¹	Micronutrients	mg.l ⁻¹
KH ₂ PO ₄	176.9	ZnSO ₄ .7H ₂ O	1
K ₂ SO ₄	166.4	CuSO ₄ .5H ₂ O	0.25
MgSO ₄ .7H ₂ O	105.9	(NH ₄) ₆ Mo ₇ O ₂₄ .4H ₂ O	0.05
CaCl ₂ .2H ₂ O	182.3	MnSO ₄ .H ₂ O	2
		HCl (N)	0.76 ml
		Iron masquolate (30%)	0.03 ml
		H ₃ BO ₃ deficiency: 0B	0
		H ₃ BO ₃ normal: 2B	11.43
		H ₃ BO ₃ toxicity: 32B	182.97

Assay of nitrogenase acetylene reduction activity (ARA):

Nitrogenase can reduce acetylene to ethylene. This latter property has been proposed as an indirect method of measuring nitrogen fixing activity (6). The rate of acetylene reduction activity has been used to estimate the capacity of symbiotic N fixation at different stages :

- at the beginning of vegetative growth, i.e. 36 days after emergence (28 days after inoculation) samples were taken at the rate of 3 pots of C.469 for each treatment.
- in the period of intensive shoot elongation, i.e. 47 days after emergence (39 days after inoculation) samples were taken at the rate of 3 pots of C.469, 2 pots of Blandine and 1 pot of C.3892 per treatment.
- at the beginning of flowering, i.e. 68 days after emergence (60 days after inoculation) samples were taken at the rate of 2 pots per treatment for each genotype.

On each sampling day, measurements were carried out on three plants of each pot : the plants were uprooted (stems and roots) and carefully removed from the perlite so as to obtain the complete root system and prevent the loss of

nodules. The traces of perlite remaining on the root were washed off. Measurements were carried out *in situ* in closed Erlenmeyer flasks, on non-excised nodulated roots following the method of Obaton *et al.* (16). 10% (v/v) of acetylene was injected into the incubation flask with the aid of a syringe. This concentration is adequate to saturate the nitrogenase and block the fixation of atmospheric nitrogen. Samples of internal atmosphere were taken after 90 minutes incubation. The ethylene formed was assayed by gas chromatography with an ionization detector. The results were expressed in two ways :

- the nitrogenase global activity (NGA) expressed in μmoles of ethylene formed $\cdot \text{h}^{-1} \cdot \text{plant}^{-1}$, which corresponds to the average activity measured on the three plants.
- the nitrogenase specific activity (NSA) of fresh nodules, expressed in μmoles of ethylene formed $\cdot \text{h}^{-1} \cdot \text{g}^{-1}$, corresponds to the ratio of the entire activity of the 3 plants to the weight of fresh nodules of the same plants.

Observation of root system: The root system of each plant was cut at the neck and the nodules were carefully removed and weighed. Their activity was rapidly assessed by observing the colour of the nodular tissue (red or pink colour indicates the presence of leghaemoglobin; in contrast, a greenish-white tint indicates inactivity of the nodules). However, the presence or absence of leghaemoglobin is not always a reliable indicator of the fixation capacity of bacteria, thus rendering this method of observation inaccurate. In fact, Davidson (4) has shown that green nodules harvested from field grown soybean had reduced levels of nitrogen fixation. The dry matter of the root system was determined by oven-drying at 105°C for 48 hours.

Determination of soluble carbohydrates: The leaves of three plants of the same pot were randomly selected and sampled at 40 and 54 days after emergence and lyophilized. Soluble carbohydrates were extracted by stirring for 1 hour in ambient deionized water as described by Timpa *et al.* (27). The aqueous extract was then filtered through a cellulose acetate filter (0.45 μm) and then immediately analyzed by HPLC ion exchange chromatography on Carbowac AP1 column (Dionex).

The detection, which was limited to glucose, fructose and sucrose was carried out by a Dionex A1 450 automatic detector. Two replicate runs were made by HPLC for each extraction. The results, calculated in relation to reference standards, were expressed in $\text{mg} \cdot \text{g}^{-1}$ DM. Statistical analysis was carried using the StatView analysis of variance.

Results

Effect of boron on the root system: Both deficiency and excess (toxicity) of boron led to statistically significant reduction of the root system irrespective of the stage of development and genotypes (Figure 1 A ; 2 B and D). The growth of this organ was greatly reduced by deficiency and rapidly stopped in the case of toxicity. In the latter case, only C.3892 showed signs of development.

Effect of boron on nodules: Irrespective of the genotype, boron deficiency resulted in reduced size of nodules associated with a greenish-brown coloration.

Deficiency and excess of boron induced a statistically significant reduction in the fresh weight of nodules in all genotypes (Figure 1 B ; 2 A and C). However, in contrast to what has been observed for the root system, the development of nodules was less affected by toxicity compared to deficiency, especially at 47 days after emergence. At this time the fresh weight of nodules differed with level of boron (2 and 32 $\text{mg} \cdot \text{l}^{-1}$) in all varieties; however, subsequent weight gain between the 47th and the 68th day was the same : 42 $\text{mg} \cdot \text{plant}^{-1} \cdot \text{d}^{-1}$ instead of 173 $\text{mg} \cdot \text{plant}^{-1} \cdot \text{d}^{-1}$ observed in control plants.

Effect of boron on ARA of nitrogenase: In general, the specific activity, varied according to genotype, and decreased with time. However, as observed for nodular development, specific activity was generally more affected by boron deficiency compared to toxicity. C.469 appeared very sensible to absence of boron (the NSA was 10% of the value observed with the control 68 days after emergence) compared to C.3892 (at the same date, NSA was 78% that observed with the control). A similar trend was observed in the case of toxicity: NSA was 23% and 62% that of control for C.469 and C.3892 respectively (Figure 3 A, B and C).

The global activity of nitrogenase (NGA) integrates the specific activity of the nodules and their weights. The results depend therefore on the relative evolution of these two parameters. In many cases, excess of boron resulted in significantly lower reduction of NGA than boron deficiency. At the last sampling, genotype C.469 was the most affected by excess of boron. Absence of this element resulted in reduction levels of NGA. In this case the genotype C.3892 was less affected than the control (Figure 4 A, B and C).

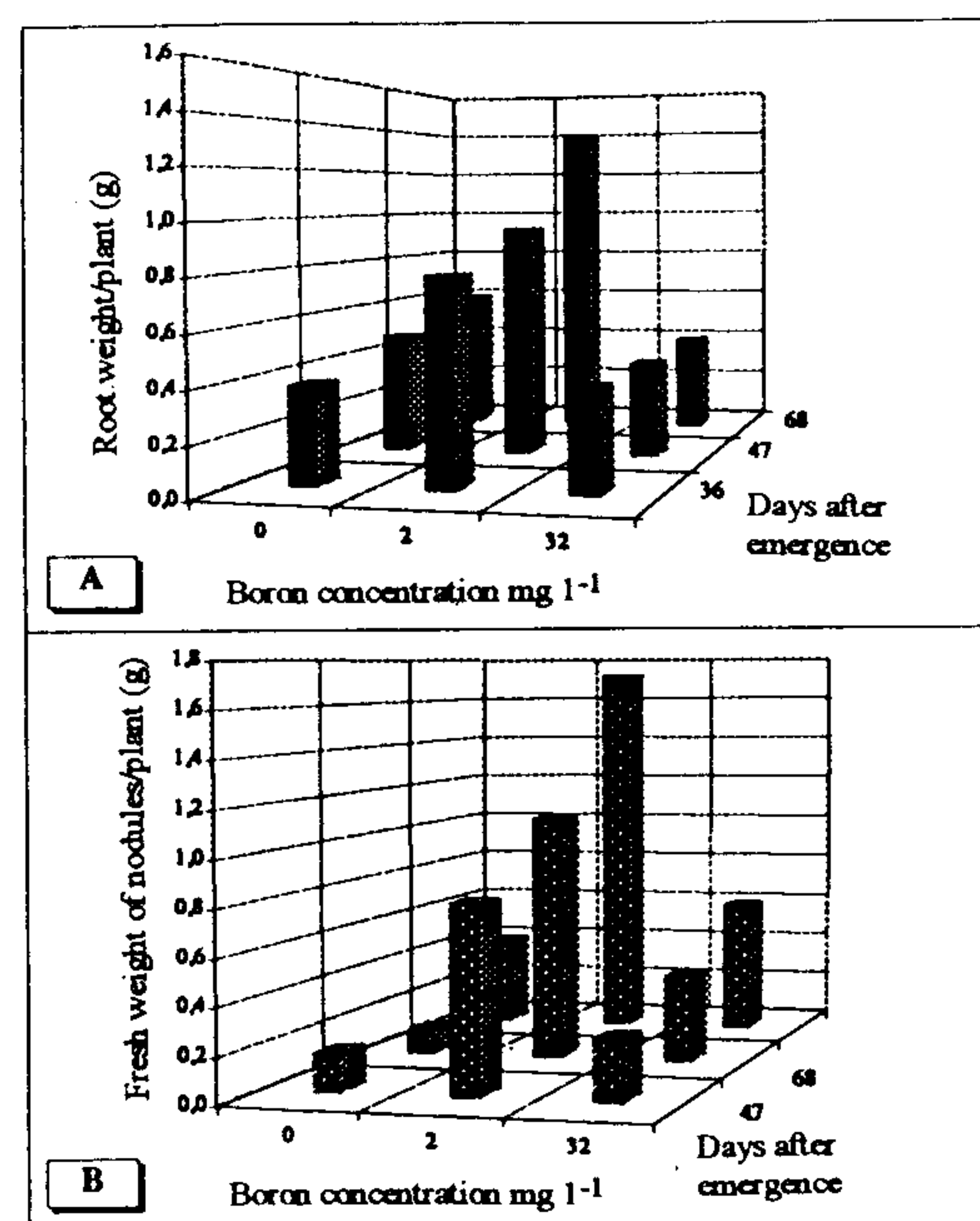


Figure 1. Effect of boron concentrations in media on the dry weight of root (A) and nodules fresh weight (B) for line C.469

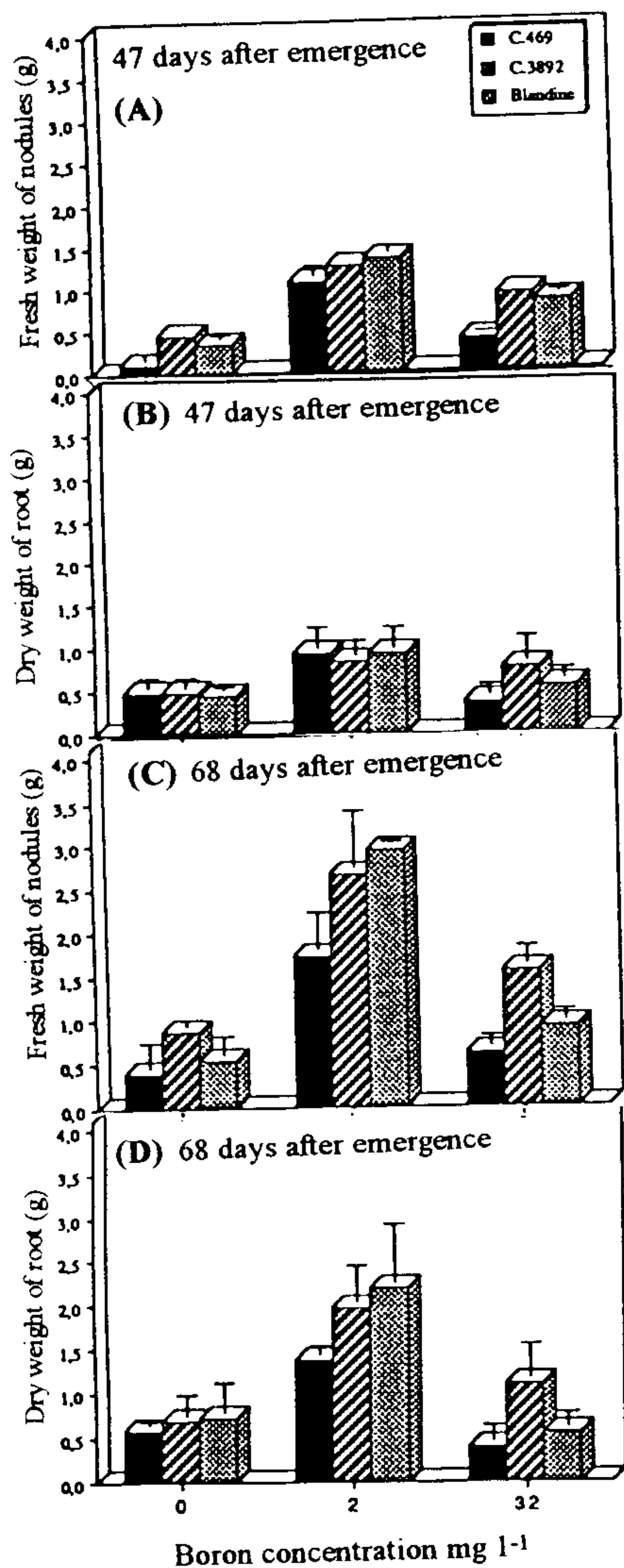


Figure 2. Effect of boron concentrations in media on fresh weight of nodules (A and C) and dry weight of root (B and D).

Effect of boron on carbohydrate contents: Variation in boron levels did not induce important modifications in total levels of carbohydrates in leaves at first sampling (Figure 5 A). A similar trend was observed for glucose, fructose and sucrose levels, even though boron deficiency led to a lower proportion of sucrose (78%) compared to the value observed in physiological and deficiency conditions (84%). Genotype influence on levels of soluble sugars were also observed at control levels of boron (Figure 6 A). Deficiency and excess of boron reduced the level of soluble sugars by 75% of that observed with the control in C.3892. In the case of Blandine, a similar trend was observed only with excess boron levels. In contrast, boron deficiency led to accumulation of soluble sugars in C.469, particularly glucose and fructose.

Deficiency symptoms appeared a few days before the second sampling. The symptoms, which were more severe on C. 469, appeared on the young leaves and growth regions of the three genotypes. In all genotypes (Figure 5 B) the quantity of soluble sugars (particularly sucrose) in the leaves of deficient plants was four times higher than that observed in control plants or those on excess boron. Absence of boron

resulted in the disturbance of the equilibrium between the different sugars, since the level of glucose + fructose represented almost 25% of the total as against 5% and 10% observed for the control and toxic levels. These effects were observed (with variable intensities) in the three genotypes (Figure 6 B).

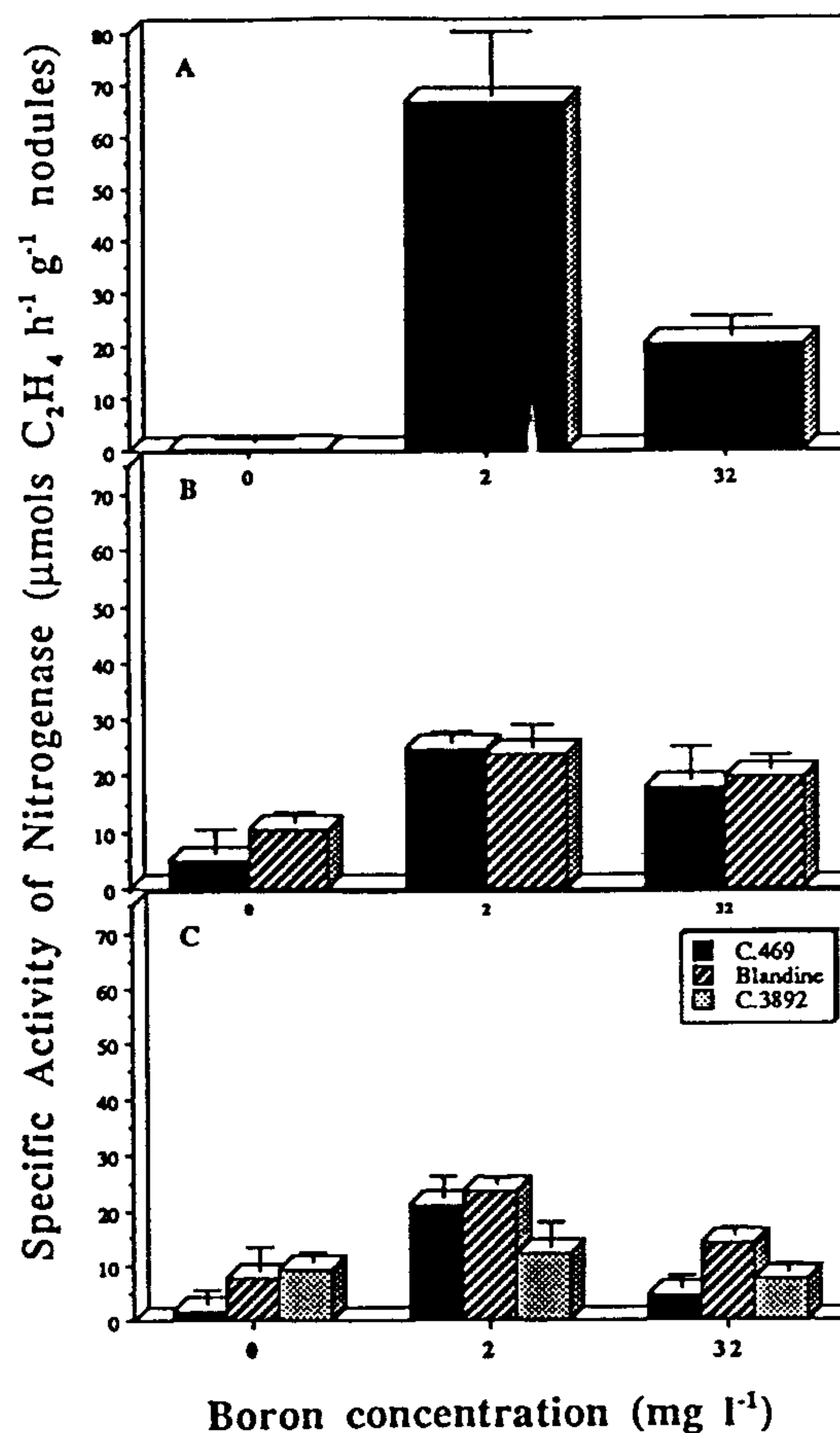


Figure 3. Effect of boron on specific activity of Nitrogenase (SAN), 36 (A) 47 (B) 68 (C) days after emergence and 27, 39 and 60 days respectively after inoculation.

Discussion

The absence of boron in the nutritive solution resulted in the simultaneous appearance of deficiency symptoms in the plants and high accumulation of soluble sugars in the leaves. Indeed, Sisler *et al.* (26) have already observed that the appearance of deficiency symptoms was associated with a reduction in the mobility of different sugars, especially sucrose. The increase in the quantity of sugars in the leaves could result either from increased biosynthesis or reduction in transport or both. The implication of boron in the different stages of metabolism of sugars has been reported by many authors (1, 2, 8, 11, 24).

The hypothesis that boron might stimulate the synthesis of sucrose has not been confirmed, and the present results do not provide such evidence. On the one hand, the simultaneous accumulation of other sugars such as glucose and fructose observed in this study shows that the effect of boron on accumulation of sugars is not restricted to sucrose alone. On the other hand glucose and fructose accumulation

could result from breakdown of sucrose by invertase in the absence of boron. It has been shown that the activity of invertase increases in the absence of boron (25). In contrast, in spite of contradictory explanations (8, 12, 13, 24), the effect of this element on the transport of sucrose out of the leaves has been confirmed by the low levels of sugars in the phloem of deficient plants. Consequently, the plant is incapable of fully exploiting the available photosynthates, and this leads to the reduction of the quantity of carbon available in the root system necessary for nitrogen fixation and assimilation of ammonium. Thus, the development of new organs is greatly reduced or even stopped. Indeed, we have shown that boron deficiency resulted in diminution of the growth of shoots as well as the yield of plants (19). A similar effect was found in the root system of deficient plants. It is highly probable that reduction in the root biomass limits water and mineral uptake which in turn contributes to a poor availability of nutrients in the shoot parts. Additionally, reduced availability of energy can lead to poor development and malfunction of the nodules as observed by Herdina and Silsbury (7). Restriction of nitrogen supply could be partially responsible for the symptoms observed on the leaves, which were similar to those observed in the case of nitrogen deficiency.

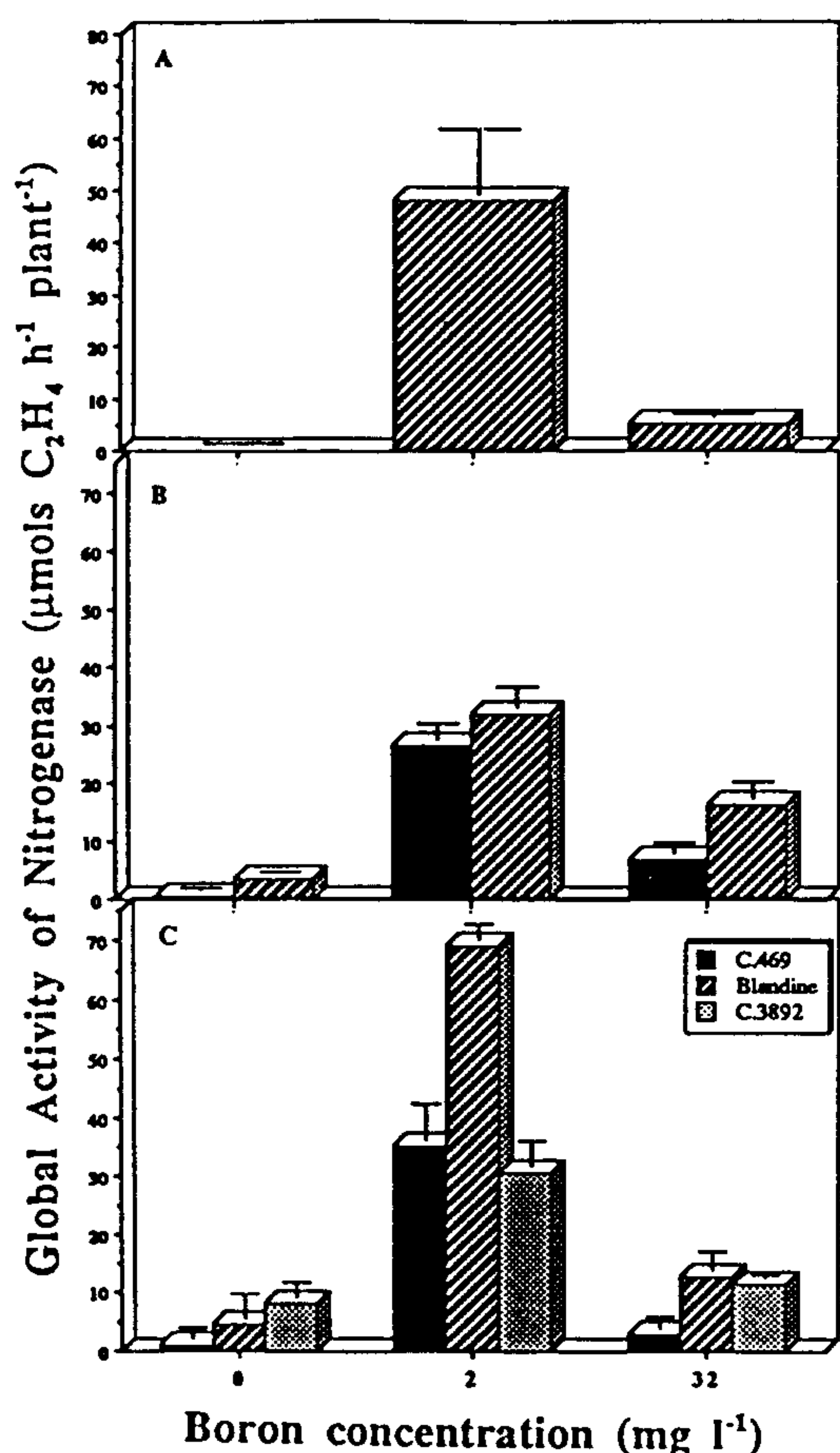


Figure 4. Effect of boron on Global activity of nitrogenase (GAN), 36 (A) 47 (B) 68 (C) days after emergence and 27, 39 and 60 days, respectively after inoculation.

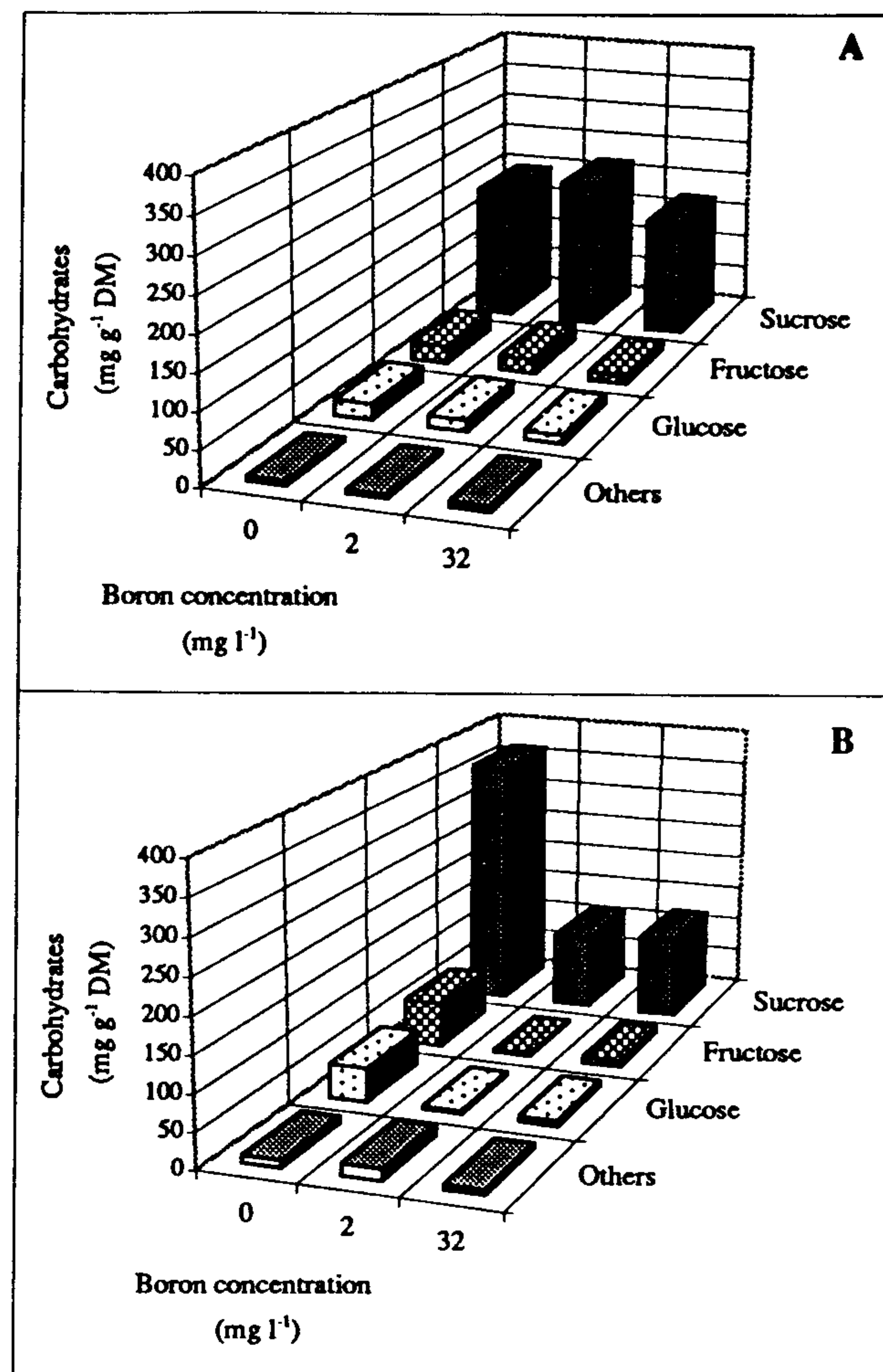


Figure 5. Effect of boron concentrations in culture media on total carbohydrates 40 (A) and 54 (B) days after emergence.

In other respects, data on the toxic effects of boron are scarce in the literature. The accumulation of sugars as a result of boron toxicity observed in our experiment agrees with the results of Scott (22) and Picchioni *et al.* (18). However, this phenomenon cannot be the result of restricted transport because in our experiment the plants exhibited relatively high growth rates despite the appearance of toxicity symptoms. Indeed, Scott (22) has reported high quantity of sugars in the root system of plants subjected to boron toxicity. The diminution of the weight of roots cannot be associated with the effect of boron on sugars. Shelp (24) reported that boron toxicity, but not deficiency, consistently affected the concentration and relative composition of amino acids. Kastori and Petrovic (10) have however reported that boron toxicity is associated with nitrate accumulation which affects the metabolism of nucleic acids and proteins, as well as energy turnover. Similar observations could explain the reduction in root growth and activity of nitrogenase observed in our experiments. In addition, the possible antagonistic effects of boron and/or formation of complexes with molybdenum (2), or a direct or indirect effect on the availability of magnesium could be other factors of interest. It has been shown that in cowpea (*Vigna unguiculata*) excess of boron led to a reduction in the quantity of magnesium (5) whereas magnesium supply reduced the toxic effects of boron on the yield of *Pisum sativum* (21).

These important differences observed between cultivars towards boron supply suggest differences in adaptive mechanisms to the excess or absence of this element. C.469 appeared to be very sensitive both in terms of the rapidity of appearance and severity of symptoms. In contrast, C.3892 showed certain adaptability to variation in the culture medium. These differences could be explained by variations in the quantities of phenolic compounds such as caffeic acid (unpublished results). Similarly, the affinity of boron to cis-hydroxyl radicals (13), leading to the formation of borate-sugar complexes, especially borate-fructose (29), could be an

important mechanism of tolerance to excess boron, partially explaining the diminution in the quantity of sucrose observed in plants under toxic conditions.

Our results indicated the importance of optimum boron supply for growth of faba bean. Indeed, the formation of nodules was very sensitive to boron deficiency (15) and the supply of this element has been shown to significantly increase nodulation and nitrogenase activity in cowpea (20) and soybean (28). The optimal dose of this element, which in our experiment corresponded to $5 \text{ kg} \cdot \text{ha}^{-1}$, also allowed high rates for nitrogen fixation.

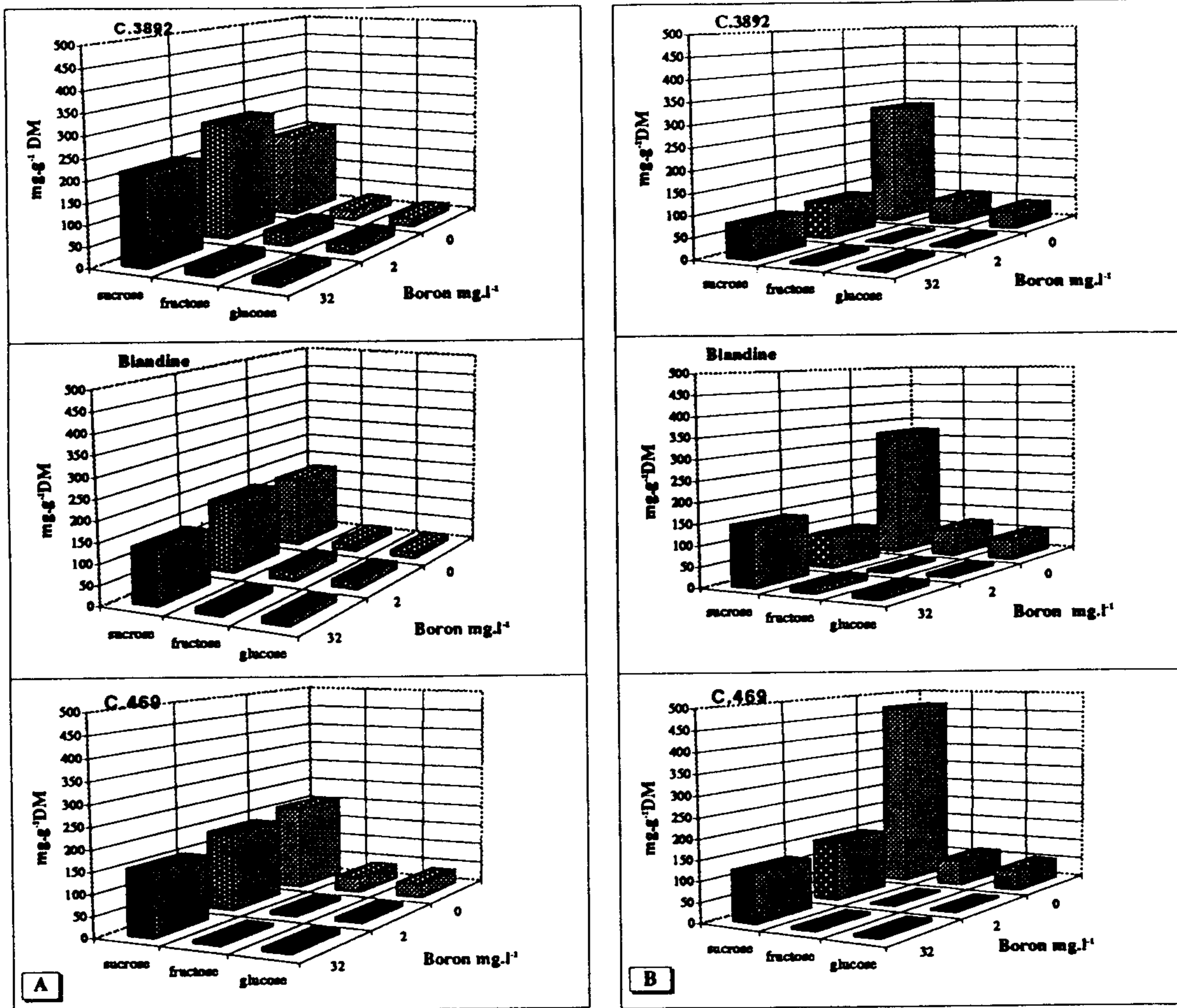


Figure 6. Effect of boron concentrations in culture media on total carbohydrates (glucose, fructose and sucrose) 40 (A) and 54 (B) days after emergence.

المخلص

المحمد، حسين ودومينيك بولان. 1996. تأثير البورون في التثبيت الحيوي للأزوت وفي المحتوى الكربوهيدراتي عند نبات الفول (*Vicia faba* L.). مجلة وقاية النبات العربية: 14(2): 105-110.

تم زراعة صنف (Blandine) وسلالتين نقيتين من الفول ذي الحبوب الصغيرة تحت ظروف البيت البلاستيكي وذلك باستخدام محلول غذائي بثلاثة تراكيز مختلفة من البورون (0، 2، 32 مل/لتر). تم خلال هذه التجربة دراسة تأثير البورون في المجموع الجذري والعقد الجذرية، كما تم قياس نشاط البكتيريا المثبتة للأزوت وتحديد محتوى الأوراق من السكريات النوية. بينت النتائج أن نقص وزيادة عنصر البورون يحد من نمو المجموع الجذري ويقلل بشكل معنوي من الوزن الرطب للعقد الجذرية للنبات الواحد، كما أنه يخفض بشكل معنوي النشاط التثبيتي لإنزيم النتروجيناز (ARA). بالإضافة إلى أن نقص البورون يؤدي إلى تراكم كبير للسكريات النوية في الأوراق وبشكل خاص سكر القصب. إن انعكاس نقص البورون سلباً على التثبيت الحيوي للأزوت يمكن تعليقه من خلال انخفاض التغذية الكربوهيدراتية وكذلك لوجود علاقة مباشرة ما بين البورون وإنزيم النتروجيناز. أما فيما يتعلق بفرط البورون فهو يؤثر في نشاط إنزيم النتروجيناز من خلال علاقة التضاد الموجودة بين البورون الزائد والموليبدينوم وأيضاً من خلال تأثيره في توافر المغنيزيوم.

كلمات مفتاحية: البورون، كربوهيدرات، نقص، سمية، الفول، تثبيت الأزوت.

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