

## Growth and mineral content of young chestnut trees under controlled conditions of nutrition

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**Summary** – The growth of young forest trees under conditions of controlled nutrition, limiting export and import of nutrients, is an efficient tool to obtain a rapid understanding of the direct effects of fertilization. This approach reveals the ability of chestnut trees to (i) grow in a poor soil with no additional supply of minerals for at least 2 years and (ii) draw elements from the mineral reserve of the soil. The growth of trees is enhanced by supplying nutrients, especially NPK. These nutrients directly modify the element availability in the soil and increase its pH. They also induce variations in cation content within different organs, eg, significant increases in calcium and magnesium but not in potassium content. Moreover, manganese seems to be important for the cationic balance in all organs as it is accumulated when trees are unfertilized but not when quick-lime is supplied.

**calcium / *Castanea sativa* / fertilization / growth / mineral nutrition**

**Résumé – Croissance et contenu minéral de jeunes châtaigniers cultivés en conditions nutritives contrôlées.** La culture contrôlée de jeunes arbres forestiers, en limitant les entrées et sorties d'éléments minéraux, permet d'évaluer rapidement les effets directs de la fertilisation. Ainsi, le châtaignier est capable de pousser pendant au moins deux ans sur un substrat pauvre et sans amendement, montrant ainsi son aptitude à puiser des éléments dans la réserve minérale du sol. Toutefois, une fertilisation, notamment par NPK, améliore sa croissance. Ces apports modifient la disponibilité des éléments dans le sol, y augmentent le pH et provoquent des variations des contenus cationiques dans les arbres : augmentation des teneurs en calcium et en magnésium mais pas en potassium. Cependant, le manganèse semble jouer un rôle important dans la balance cationique, puisqu'il s'accumule dans les arbres non fertilisés et qu'un apport de chaux vive provoque l'effet inverse.

**calcium / *Castanea sativa* / croissance / fertilisation / nutrition minérale**

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## INTRODUCTION

Growth of trees is generally related to mineral nutrition. Some deficiencies greatly affect growth, when cations are lacking (Shear and Faust, 1980; Spiers and Braswell, 1994) and particularly calcium deficiency (Davis, 1949; Ramalho et al, 1995). However, the chestnut tree is known for its ability to grow on poor ground (Bourgeois, 1992). In spite of interesting chemical and physical wood qualities, a wood failure known as ringshake frequently occurs with disastrous marketing consequences. Chanson et al (1989) have hypothesized that this cohesion breakdown is located in the middle lamella, a cell wall area rich in pectins. These acid polysaccharides are known to be stabilized by calcium (Demarty et al, 1984; Jarvis, 1984) and involved in modification of cell adhesion (Liners et al, 1994). These data suggest the potential role of calcium in ringshake. Thus, this wood failure could be related to calcium nutrition and its availability in soil.

Soils in the Limousin (France) are acidic and relatively poor in available nutrients (Verger et al, 1985, 1994). The aim of the present work was to determine whether fertilizer treatments can modify the growth of young chestnut trees grown in these soils and affect the cation content of different organs (roots, bark, de-barked stems, leaves), especially divalent cations. This study was carried out in a greenhouse in order to limit cation imports and exports and to control environmental factors.

## MATERIAL AND METHODS

### Material

One-year-old chestnut trees (*Castanea sativa* Miller) were planted in March 1994 in 8-L PVC pots. The culture substratum was composed of a C horizon of Limousin (middle west of France) chestnut forest soil (mesotrophic brown soil)

mixed with river sand (2:1 weight ratio), which represented a poor exchangeable mineral element substratum. This substratum was acidic (pH H<sub>2</sub>O = 5.1; pH KCl = 4.3) and was characterized by a very low cation exchange capacity of 1.32 cmolc.kg<sup>-1</sup> with exchangeable basic cations: Ca<sup>2+</sup>: 0.30 cmolc.kg<sup>-1</sup>; Mg<sup>2+</sup>: 0.12 cmolc.kg<sup>-1</sup>; K<sup>+</sup>: 0.18 cmolc.kg<sup>-1</sup> and a total acid cations: H<sup>+</sup>: 0.15 cmolc.kg<sup>-1</sup>; Al<sup>3+</sup>: 0.40 cmolc.kg<sup>-1</sup> (Freyssac et al, 1994).

Ten young trees were kept in order to quantify the element contents at the time of planting. The others were distributed between five different fertilizer treatment groups (20 trees each).

### Fertilizer supplies

The young plants were grown under five different sets of conditions, A, B, C, D and O.

A consisted of a single supply of quick-lime (2 cmolc of Ca<sup>2+</sup>/kg of soil, corresponding to 1 000 kg.ha<sup>-1</sup> (94.0% CaO) for forest fertilization);

B consisted of a single supply of Ca + Mg (2 cmolc of Ca<sup>2+</sup> and 0.5 cmolc of Mg<sup>2+</sup> per kg of soil, corresponding to 2 000 kg.ha<sup>-1</sup> (42.0% CaO + 10.0% MgO));

C consisted of B conditions + macroelements (ammonium nitrate, potassium oxide and phosphate at 500 kg.ha<sup>-1</sup>, for N (16.8% N-NO<sub>3</sub>, 16.8% N-NH<sub>4</sub>) and also for PK (18.5% P<sub>2</sub>O<sub>5</sub>, 24.0% K<sub>2</sub>O));

D consisted of C conditions + trace elements (Calmagol H, Holimco, 50 kg.ha<sup>-1</sup> with a composition of: Ca: 32.5%, Mg: 3.3%, Fe: 0.7%, Mn: 0.007%, Cu, Co and Ni traces);

O was a control with no additional elements.

The letters A, B, C, D and O will be taken to mean the trees and/or conditions under which they were grown.

The fertilizers were mixed into the substratum, pot by pot, before planting. Experiments were performed in semi-controlled conditions in the greenhouse with temperature measurement. The temperature varied from a minimum of 2 °C during the winter to a maximum of 45 °C in the summer. Being protected against rain fall, the trees were watered with deionized water exclusively between one and eight times per month depending on temperature. Moreover, leaves from each batch were collected when they fell,

then reduced to a powder for further analysis. An aliquot was used for mineral composition (data not shown) and the rest was added, in late January 1995, to the surface of the corresponding substratum of the trees that were not destructively harvested.

## Methods

Growth parameters, such as height, base diameter (at soil surface), number of branches and sum of annual shoot length, were measured for each tree in March 1994 (planting), September 1994 and June 1995.

The fourth leaf from each apex was gathered 15 days before harvesting in September 1994 and June 1995, and also the foliage of trees was harvested in order to estimate the leaf area by cutting it up and weighing paper copies.

Trees were grouped into three categories for each treatment: small, medium and large, according to the sum of annual shoot length. Three plants, the medium-sized trees of each category, were harvested to provide material for mineral content at each harvest time except at initial planting when six trees were randomly sampled. Roots (washed with deionized water), bark, leaves and de-barked stems were manually separated and oven dried at 80 °C for 2 h then at 60 °C for 48 h (to a constant mass). Plant materials were then weighed and powdered using a ball-bearing shaker. Except for current year de-barked stems of which there was insufficient quantity, 0.2 g of each sample was weighed out and digested for 10 min at 600 °C in 14 mL of a 2:6:6 (v/v/v) mixture of H<sub>2</sub>SO<sub>4</sub>, HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> according to Hoenig and Vanderstappen (1978). Concentrations of Ca, Mg and Mn were determined by atomic absorption and K contents by atomic

emission spectrophotometry (Atomspek H1170-Hilger & Watts).

The substrata of the three harvested plants for each treatment were mixed together at harvest time (September 1994, January 1995 and June 1995). As powdered leaves were applied in January 1995, the upper 5 cm, where there were no roots, were not taken into account. The pH of the air-dried and sieved (2 mm) samples of substratum was measured in deionized water (w/w, 2:5) after standing overnight. The pH was also determined for samples collected in March 1994 before planting.

## Statistical analysis

Growth parameter analyses were expressed as the mean of 20 individual values at the beginning of the study, to 10 individual values at the end, and the Mann and Whitney U test (1947) was applied with a threshold of 5%.

Mineral analyses were performed individually, on three different trees for each treatment at the sampling date. Results were expressed as the mean of the three values and the Mann and Whitney U test (1947) was again applied, with a threshold of 5%.

## RESULTS

### Growth parameters

At the time of planting (March 1994), the heights of the 1-year-old chestnut trees ranged from 22 to 52 cm. The mean heights (table I) and base diameters (table II) of trees

**Table I.** Average height of chestnut trees (cm) in relation to fertilizer supplies. Each value represents a mean  $\pm$  SE from 10 to 20 trees. Values in rows followed by the same letter are not significantly different (threshold of 5%) according to Mann and Whitney U test (1947). Fertilizer treatments correspond to (O) control unfertilized, (A) a quick-lime, (B) a calcomagnesian, (C) a macroelement and (D) macro + microelement supplies.

Date	O	A	B	C	D
March 94	35.5 $\pm$ 1.3a	32.4 $\pm$ 1.6a	35.2 $\pm$ 1.8a	35.3 $\pm$ 1.3a	34.4 $\pm$ 1.6a
Sept 94	45.5 $\pm$ 2.4a	42.5 $\pm$ 2.1a	45.5 $\pm$ 2.6a	47.3 $\pm$ 2.3a	48.4 $\pm$ 2.3a
June 95	51.5 $\pm$ 3.1a	46.8 $\pm$ 2.8ab	46.9 $\pm$ 3.3ab	54.5 $\pm$ 2.2bc	62.6 $\pm$ 3.9c

**Table II.** Average base diameter of chestnut trees (mm) in relation to fertilizer supplies. Each value represents a mean  $\pm$  SE from 10 to 20 trees. Values in rows followed by the same letters are not significantly different (threshold of 5%) according to Mann and Whitney U test (1947). Abbreviations for treatments are the same as those in table I.

Date	O	A	B	C	D
March 94	8.2 $\pm$ 0.2a	6.9 $\pm$ 0.3b	8.6 $\pm$ 0.4a	8.5 $\pm$ 0.3a	8.0 $\pm$ 0.3a
Sept 94	9.6 $\pm$ 0.3ab	9.0 $\pm$ 0.3a	10.1 $\pm$ 0.4bc	10.8 $\pm$ 0.2c	10.1 $\pm$ 0.2b
June 95	10.1 $\pm$ 0.4a	9.6 $\pm$ 0.4ab	11.3 $\pm$ 0.6bc	11.7 $\pm$ 0.4c	11.4 $\pm$ 0.3c

**Table III.** Sum of annual shoot length of chestnut trees (cm) in relation to fertilizer supplies and number of ramifications, in parentheses. Each value represents a mean  $\pm$  SE from 10 to 20 trees. Values in rows followed by the same letters are not significantly different (threshold of 5%) according to Mann and Whitney U test (1947). Abbreviations for treatments are the same as those in table I.

Date	O	A	B	C	D
Sept 94	36.0 $\pm$ 3.9ab (4.5 $\pm$ 0.4a)	32.2 $\pm$ 3.0a (4.2 $\pm$ 0.3a)	39.5 $\pm$ 5.0abc (5.5 $\pm$ 0.6a)	51.0 $\pm$ 3.4c (5.2 $\pm$ 0.6a)	48.2 $\pm$ 4.5bc (4.3 $\pm$ 0.4a)
June 95	10.1 $\pm$ 1.6ab (6.6 $\pm$ 0.7a)	9.8 $\pm$ 1.9a (7.0 $\pm$ 0.8a)	15.0 $\pm$ 2.1b (8.9 $\pm$ 1.3ab)	44.2 $\pm$ 3.9c (13.4 $\pm$ 1.4c)	44.5 $\pm$ 2.9c (11.8 $\pm$ 1.5bc)

were similar for all treatments except A, where the trees were 10% smaller than those in the other treatments, by chance.

Six months later (September 1994), the heights of the trees varied individually from 28 to 66 cm but the treatment mean values were not significantly different (table I). The same pattern was observed in the diameter measurements (table II). In September 1994, significant increases in the sums of the shoot length were observed in C and D compared to O and A (table III). The B trees produced an intermediate effect. However, no significant effect was seen in terms of the number of ramifications.

During the second growing season (June 1995), the C and D trees were significantly greater in terms of all growth parameters measured, especially for the sums of shoot length, which were four times greater than in O (tables I, II and III). As far as all measurements were concerned, no significant effects were observed for A. A slight effect

was observed for treatment B, in comparison with O, but only the diameters were significantly higher (table II).

From 1994, the area of the fourth leaf of the D trees was significantly higher than that of O trees (table IV). This difference

**Table IV.** Average leaf area of the fourth leaf of chestnut trees (cm<sup>2</sup>) in relation to fertilizer supplies. Each value represents a mean  $\pm$  SE from 10 to 20 trees. Values in columns followed by the same letters are not significantly different (threshold of 5%) according to Mann and Whitney U test (1947). Abbreviations for treatments are the same as those in table I.

Treatments	September 94	June 95
O	37.2 $\pm$ 14.6a	33.1 $\pm$ 6.9ab
A	39.5 $\pm$ 16.3ab	30.4 $\pm$ 15.1a
B	38.9 $\pm$ 19.1ab	35.4 $\pm$ 12.2ab
C	45.2 $\pm$ 22.5ab	50.0 $\pm$ 14.3c
D	50.6 $\pm$ 18.2b	55.0 $\pm$ 25.1c

**Table V.** Average foliage surface of chestnut trees ( $\text{cm}^2$ ) in relation to fertilizer supplies. Each value represents a mean  $\pm$  SE from three trees. Values in columns followed by the same letters are not significantly different (threshold of 5%) according to Mann and Whitney U test (1947). Abbreviations for treatments are the same as those in table I.

Treatments	September 94	June 95
O	698 $\pm$ 282abc	420 $\pm$ 213a
A	653 $\pm$ 253ab	445 $\pm$ 337ab
B	615 $\pm$ 114a	775 $\pm$ 175b
C	1068 $\pm$ 356abc	1605 $\pm$ 180c
D	938 $\pm$ 88c	1485 $\pm$ 230c

was even greater in June 1995, depending on the fertilizer supplies. Thus, C and D induced a significant increase in the area of the 4th leaf compared to the other treatments. Moreover, the total foliage area showed the same trend (table V). In 1995, this value was four times greater in C and D, and intermediate in B trees compared to O and A trees.

### pH substratum evolution

Before both fertilization treatments and planting, the pH of the substratum was 5.1 but increased to pH 6.4 where quick-lime (calcium supply) was added. Furthermore,

**Table VI.** Substratum pH values in relation to fertilizer supplies. Each value represents a pH value of a mixture of three total substrata. Abbreviations for treatments are the same as those in table I.

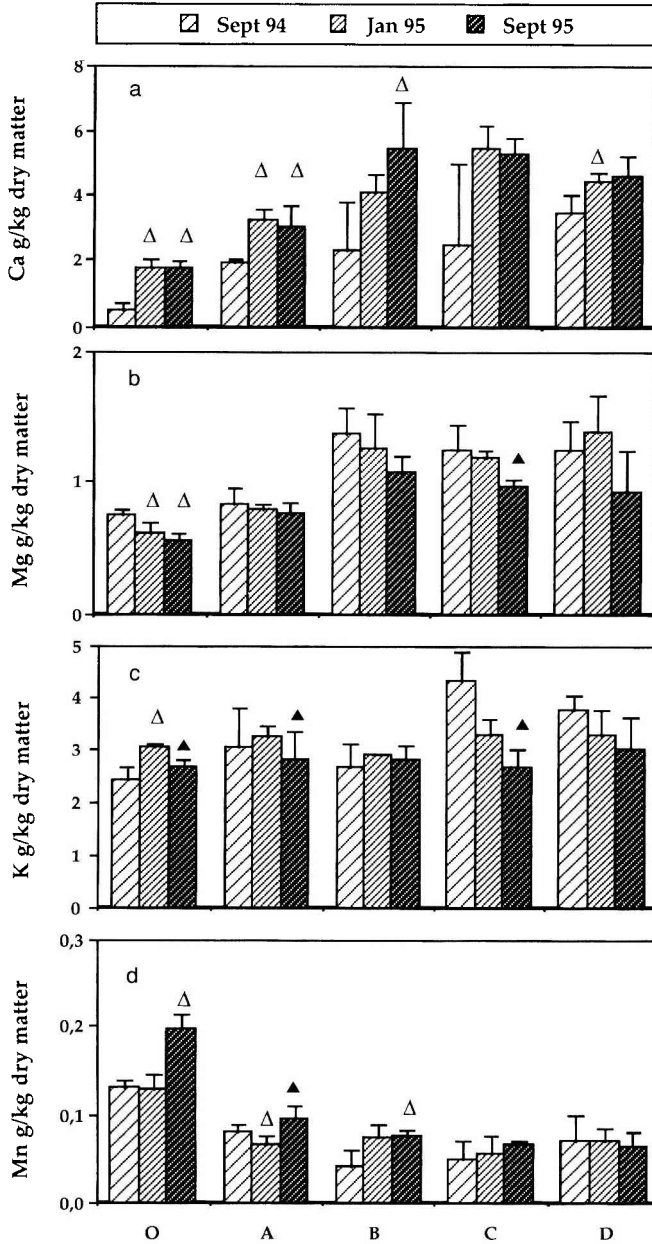
Date	O	A	B	C	D
March 94	5.1	6.4	6.9	6.8	6.8
Sept 94	5.1	6.0	7.1	7.5	7.4
Jan 95	5.1	6.2	7.3	7.2	7.3
June 95	5.2	6.1	7.5	7.7	7.1

the pH was greater (6.8–6.9) where the substrata were supplemented with the other fertilizers (table VI). The substratum pH remained acidic and stable under O and slight acidification was observed under A. In contrast, pH values increased slightly but irregularly under other conditions, during the experimental period.

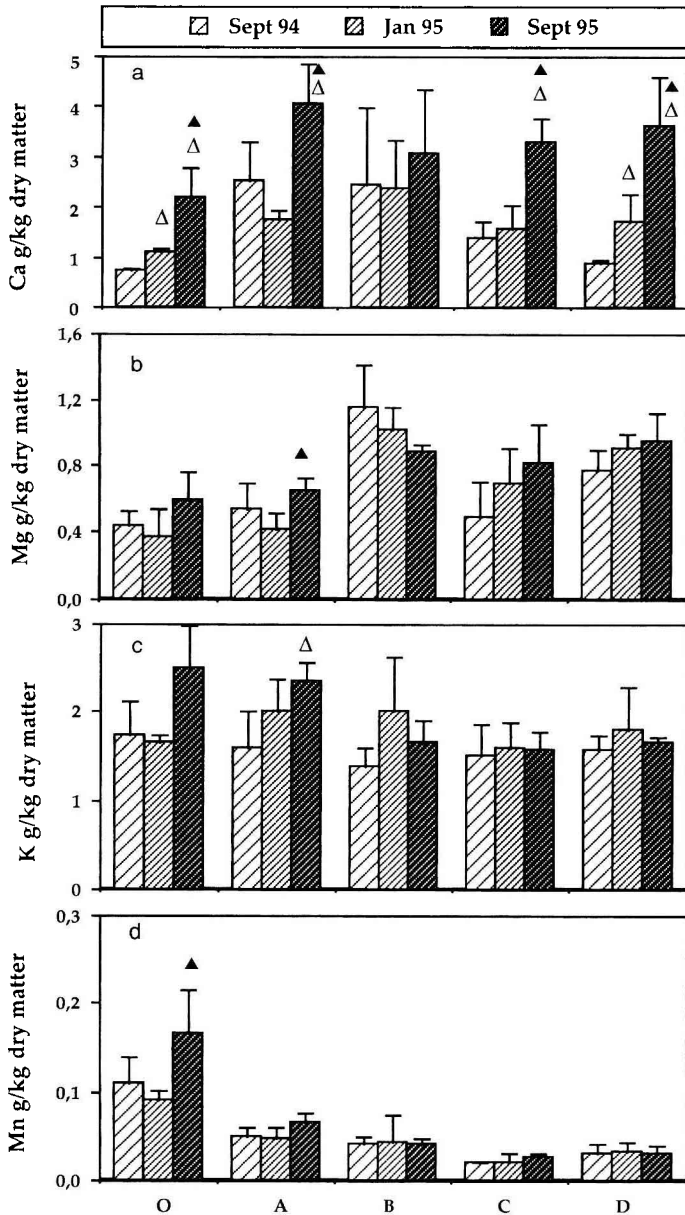
### Mineral contents

At each sampling date, the root Ca levels (fig 1a) were at least twice as high where fertilizer was supplied (A, B, C and D) than without (O). Under O, a very low Ca concentration was observed in September 1994, which increased significantly as early as January 1995 and was found to be identical in September 1995 (four times less in 1994 than in January and September 1995). As early as September 1994, the same trend, ie, an increase in Ca between September 1994 and January 1995 and a slight variation until September 1995, was observed but less markedly for the same set of conditions. The root Mg levels in September 1994 (fig 1b) were greater by up to 75% for trees supplied with Mg (B, C and D), compared to those that did not receive Mg (O, A). In September 1995, Mg levels were 25% lower than in 1994 in all cases. The K levels (fig 1c) showed a slight treatment effect where K was supplied (C and D) in September 1994. And a decreasing trend of K concentrations occurred under O and A between January and September 1995 and under C and D between September 1994 and September 1995. The Mn levels (fig 1d) were twice as high without fertilizer (O) than under other conditions and increased in the second year, especially in O but not for D.

The Ca levels in de-barked stem (fig 2a) showed a significant increase (double) from September 1994 to September 1995 for A, C, D and O but not for B. The increase in Ca concentration when calcium was applied



**Fig 1.** Effects of fertilization on calcium (a), magnesium (b), potassium (c) and manganese (d) concentrations ( $\text{g}\cdot\text{kg}^{-1}$  dry matter) in chestnut roots. ( $\Delta$ ) and ( $\blacktriangle$ ) cation concentrations varying significantly in comparison with September 1994 and January 1995, respectively, by Mann-Whitney U test (threshold 5%). Fertilizer treatments correspond to (O) control unfertilized, (A) a quick-lime, (B) a calcomagnesian, (C) a macroelement and (D) macro + microelement supplies.



**Fig 2.** Effects of fertilization on calcium (a), magnesium (b), potassium (c) and manganese (d) concentrations ( $\text{g}\cdot\text{kg}^{-1}$  dry matter) in chestnut de-barked stems. ( $\Delta$ ) and ( $\blacktriangle$ ) cation concentrations varying significantly in comparison with September 1994 and January 1995, respectively, by Mann-Whitney U test (threshold 5%). Abbreviations for treatments are the same as those in figure 1.

(quick-lime as well as CaMg) also revealed a fertilizer effect. The Mg levels followed the same pattern: they increased when magnesium was applied (fig 2b), and a tendency to increase over time was observed for all treatments, except B. The concentration of K decreased with the addition of fertilizer (fig 2c). The K levels increased in 1995 in O and A and remained constant for the others. The highest Mn levels (fig 2d) were obtained in O and gradually decreased from A to C and D. In addition, the Mn concentrations of O trees were higher in September 1995 than in 1994.

The Ca levels in bark (fig 3a) were double those of the control in treatments where Ca was supplied. They revealed an increase from September 1994 to September 1995 (except in the control), which was particularly marked in A, C and D. In figure 3b, an increase (50%) in the Mg level was observed in Mg supplied trees as well as a significant Mg decrease from September 1994 to September 1995. The K levels showed no significant treatment effects. There was a trend in K concentration, an increase in O and A but a decrease in C and D, over time (fig 3c). The Mn concentrations (fig 3d) were twice as high in O and tended to gradually decrease from A to C and D.

The results presented in figure 4a show a strong fertilizer effect on Ca levels in leaves, which tripled in treatments where calcium was supplied. These levels remained similar, between harvest dates. In September 1994, an increase in Mg concentrations (fig 4b) was observed for the three treatments that contained a magnesium supply, whereas these levels decreased steeply and were statistically significant (30–40%) in September 1995 (B, C and D). The pattern of K revealed highest levels in O and A. This situation was more marked during the second year (fig 4c). The Mn levels (fig 4d) were higher in O and A than in the three other treatments in September 1994. In September

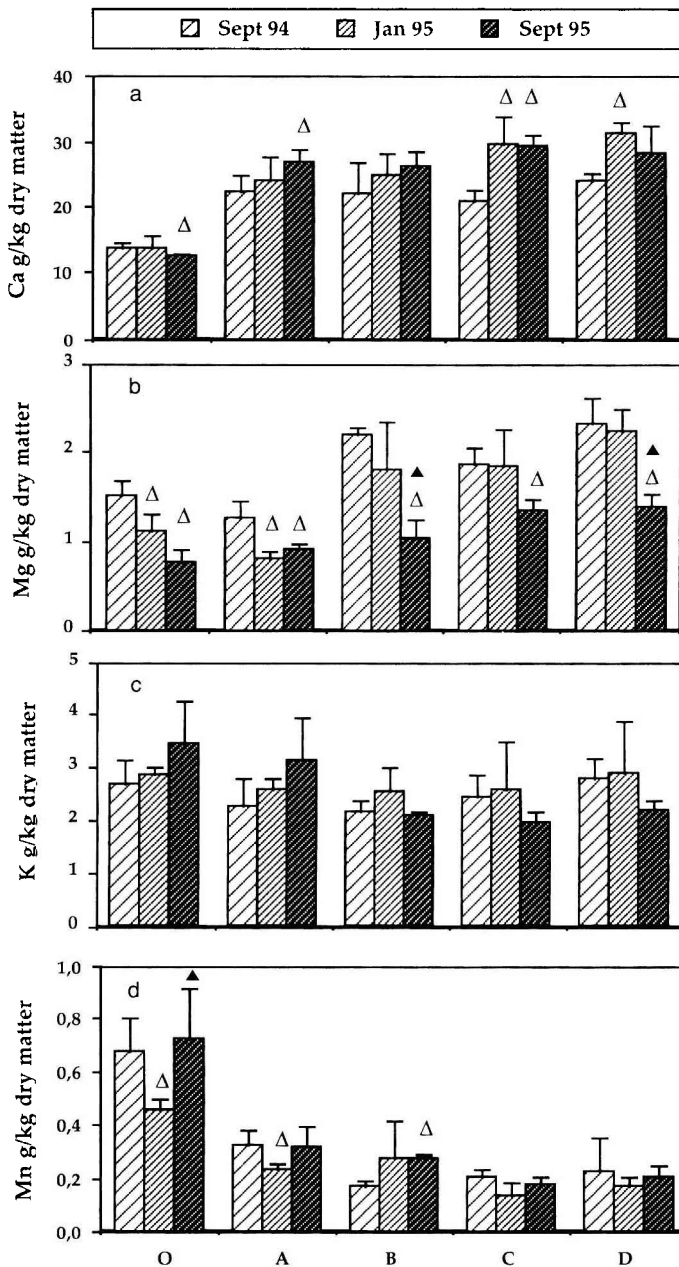
1995, the Mn level was also higher in O trees than in the others.

## DISCUSSION

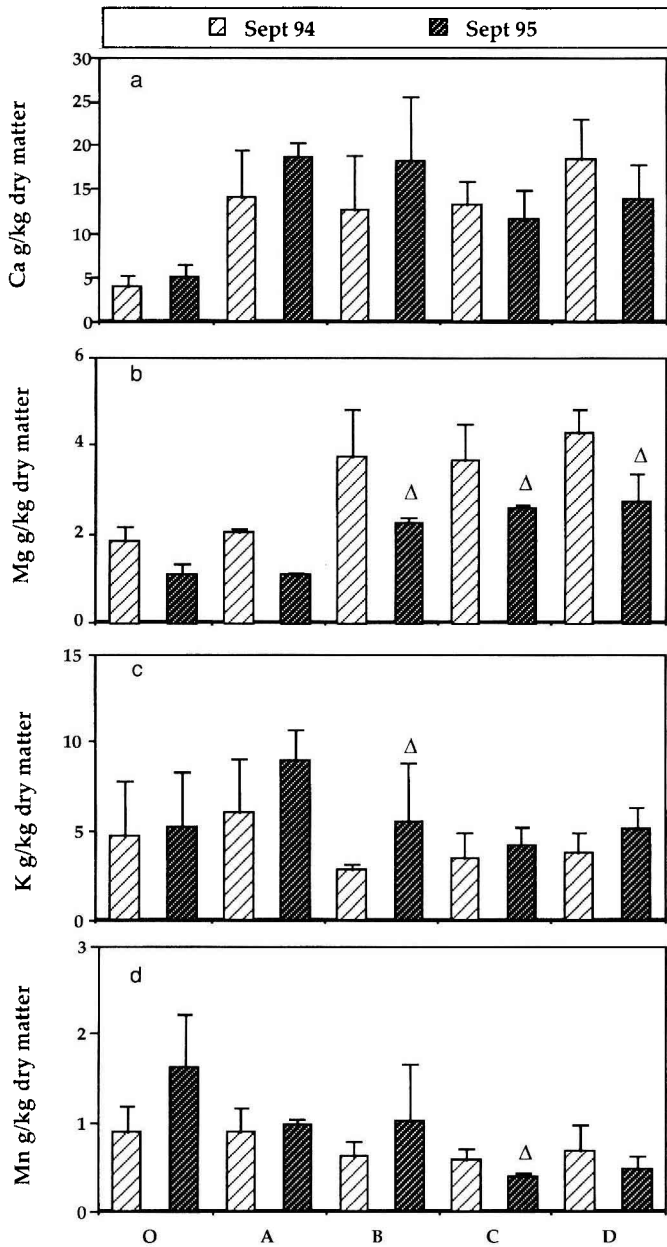
In forest nutrition studies (Hytonen, 1995), experiments are generally carried out over several years in order to obtain significant treatment effects and to limit the variability due to both climatic and biotic factors. It is evident that to work under natural conditions, many parameters and interactions must be managed and it is thus necessary to simplify the experiments. In this study, the experiments were conducted under closed conditions in a greenhouse. The plants were protected from the weather so that a number of physical and biological factors that can represent export (loss by percolation, flora, fauna, etc) or import (rain-fall element, decomposition of pre-existing litter, etc) parameters were controlled in this study. Apart from the fact that the mineral composition of the substratum was already known (Freyssac et al, 1994), supplies were tightly controlled, deionized water was used for watering and finally the leaf mineral composition was quantified at leaf fall in order to take it into account (data not shown). This experimental approach results in a better understanding of fertilization effects by reducing annual variations in atmospheric and biotic factors (Ranger, 1981).

Following Bourgeois (1992), we confirmed that chestnut trees are able to grow for at least 2 years on an exchangeable element-poor substratum such as the C Horizon of mesotrophic brown soil (Verger et al, 1985, 1994) watered only with totally deionized water. This growth capacity on a substratum poor in exchangeable elements could reveal an ability to draw mineral elements from the soil reserve, as hypothesized by Brethes and Nys (1975) with resinous trees.





**Fig 3.** Effects of fertilization on calcium (a), magnesium (b), potassium (c) and manganese (d) concentrations ( $\text{g}\cdot\text{kg}^{-1}$  dry matter) in chestnut bark. ( $\Delta$ ) and ( $\blacktriangle$ ) cation concentrations varying significantly in comparison with September 1994 and January 1995, respectively, by Mann-Whitney U test (threshold 5%). Abbreviations for treatments are the same as those in figure 1.



**Fig 4.** Effects of fertilization on calcium (a), magnesium (b), potassium (c) and manganese (d) concentrations ( $\text{g}\cdot\text{kg}^{-1}$  dry matter) in chestnut leaf. ( $\Delta$ ) cation concentrations varying significantly in comparison with September 1994, by Mann–Whitney U test (threshold 5%). Abbreviations for treatments are the same as those in figure 1.

Growth parameter analysis shows some differences depending on both culture duration and fertilizer effects. A slight growth increase was observed from September 1994, probably owing to NPK supply (C and D). The leaf areas followed a similar pattern. During the second year, these observations were confirmed. All growth parameters increased significantly following an NPK (including calcium and magnesium) supply, but to a lesser extent with CaMg. Some differences concerning leaf morphology and colour were also observed in 1995. Leaves were either rounded, large and deep-green or small, elongated and yellow-green with or without an NPK supply, respectively (data not shown). Taken together these results give rise to two hypotheses: i) Ca and/or Mg are not the sole deficient element(s) in the substratum used; ii) the fertilizer supplies modify the cation balance in soil. The nutrient ratios in soil affect absorption and translocation, and consequently growth (Shear and Faust, 1980; Jadczyk and Lenz, 1994).

The addition of Ca and CaMg increases the growth, which shows the deficiency of both elements in the substratum. A greater effect on growth and foliage development is observed when NPK fertilizer is added, indicating that at least one of them (N, P or K) is initially insufficient. In addition, nutrient supplies can modify the cation balance either by a direct effect or via a pH variation and consequently modify the cation absorption (Maas et al, 1969; Iyengar and Reddy, 1993; Jadczyk and Lenz, 1994; Ramalho et al, 1995). Moreover, some studies show correlations between soil acidification and cation availability (Ross et al, 1985; Whigham and Richardson, 1988; Neilsen et al, 1994; Ljungström and Nihlgard, 1995). In our study, all the fertilizer supplies induce an increase in soil pH. Used alone, calcium enhances the soil pH but less than when it is added with other elements (Mg and NPKMg). Chestnut trees, which are known for their ability to grow in acid soils, have

shown a marked tendency to grow under slightly basic pH conditions. These results are not contradictory, indeed the basic pH is not related to limestone, which inhibits the growth of chestnut trees (Bourgeois, 1992).

The differences in both growth and foliage development that were observed between the first and second year could be attributed to nutrient storage within the plant, resulting from previous non-deficiency culture conditions. This is probably a sufficient reason to explain the marked growth responses during the second year.

In addition to the fertilizer effects on chestnut tree morphology, some fertilizer effects have been observed concerning the mineral contents. Results show different mineral levels according to the components tested. Thus, all minerals studied have the lowest concentrations in wood (de-barked stem) and root samples, whereas they are higher in bark and then in leaves for Ca and inversely for Mg, Mn and K. These results are in agreement with those reported in other species such as Scots pine (Ranger, 1981) and larch (Myre and Camire, 1994). In our experiments, the same trend is observed in mineral concentrations in all components, whatever the fertilizer effect. Moreover, we note that the leaf/any other component ratio (but only to a lesser extent with bark) of Mn concentrations are the highest (ten for Mn and three for Ca, Mg and K). Furthermore, the foliar area of the fourth leaf reveals the same pattern as the total foliage area and also the mineral content (data not shown). Thus, in agreement with previous works (Ljungström and Nihlgard, 1995; Ramalho et al, 1995), leaf sampling can be used to estimate the cation status in trees and is consequently a rapid method of revealing ion deficiencies. According to Ljungström and Nihlgard (1995), Ca and Mg concentrations increase markedly during the first year, with calcium and magnesium supplies, respectively. By contrast, the potassium supply

has no effect on any of the mineral contents studied, including itself. This element may be present in sufficient proportions in the plant, taking into consideration the K level in leaves (0.87%) under optimal nutritional conditions (Colin-Belgrand et al, 1993).

In addition, the concentration of each element varied differently within each component over time. For instance, the Ca level increased from September 1994 to September 1995, even under control conditions and more markedly with all other fertilizer supplies. This accumulation of Ca, with no additional supply, is observed in all components. Thus, this cannot be explained by a redistribution of stored calcium from one component to others. According to our experimental conditions, the only calcium source for the young trees cultured with no supply was the substratum, poor in exchangeable mineral elements (Freyssac et al, 1994). Consequently, the enhancement of calcium concentrations observed in all plant components could reveal the ability of chestnut to draw up mineral elements from the soil reserve, as observed in resinous trees (Brethes and Nys, 1975), via a disorganization of clay colloids, which increases ion availability. The evolution of Mg concentrations was the reverse: a decrease was observed during the experimentation time under all conditions, especially in leaves and bark, because it could have been absorbed mainly during the first months of culture and to a lesser extent later. The difference between the accumulation levels of these cations can be explained by the lower mobility of Ca (Ferguson and Bollard, 1976; Marschner, 1986; Hytönen, 1995) or differences in absorption rate between calcium and magnesium, via interactions between cations (Maas et al, 1969; Jadczyk and Lenz, 1994). In order to fully understand these phenomena, it will be interesting to take the substratum cation content into account.

Finally, we would like to point out the case of Mn whose levels are higher under

control conditions than all others. Other works show a decrease in leaf manganese concentration in relation to an increase in soil pH and a negative correlation between Mn leaf concentration and growth (Spiers and Braswell, 1994). Neilsen et al (1994) reported a maximum Mn content in leaves at pH 4.8 and a relationship between soil acidification and increasing solubility of toxic elements such as manganese and aluminium. In addition, variations in soil pH modify the cation availability. For example, a decrease in pH from 6.1 to 3.5 induces a 16-fold increase in extractable  $Mn^{2+}$  (Ross et al, 1985). Thus, these manganese and aluminium availabilities can cause phytotoxicity and imbalanced nutrition, aggravating the cation deficiencies. Since a calcium supply induces a strong decrease in Mn level, this study, in agreement with Ljungström and Nihlgård (1995), suggests that the manganese content of a plant component can be used as a marker of calcium and perhaps other mineral deficiencies.

In conclusion, our study shows the impact of some fertilizations on both growth and some mineral contents of young chestnut trees. It would be interesting to study their effects over several years. Our results, concerning the low cation concentrations in debarked stem, calcium in particular, without fertilization, give support to the hypothesis concerning the potential role of calcium in ringshake. It will be necessary to study mechanical resistance of wood in correlation with calcium content in order to find out if calcium deficiency *in situ* influences the mechanical qualities of wood. In the near future, we aim to specify the localization of cations *in situ*, using SIMS (secondary ion mass spectrometry), which appeared to be a useful means of studying the calcium distribution in plant tissues (Jauneau et al, 1992; Roy et al, 1995). It will also be important to link calcium localization to the content and metabolism of pectins.

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