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Growth, mineral nutrient content and nitrogen metabolism in *Laccaria laccata*-inoculated and non-inoculated Douglas fir seedlings during their growth period

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Introduction

Enhanced plant growth caused by ectomycorrhizal inoculation in the nursery is well documented: thus *Laccaria* strains have proven to be very efficient for improving growth of various conifers (Le Tacon and Bouchard, 1986). The investigations described herein were carried out to obtain information on physiological and biochemical modifications that can explain such stimulation. The present study compared the seasonal changes in protein and amino acid levels, aspartate aminotransferase activity and nutrient content in both *L. laccata*-inoculated and non-inoculated Douglas fir grown in a forest nursery.

Materials and Methods

After 1 yr of growth in the nursery, 12 seedlings from both untreated and treated plots were collected at random, bimonthly from May to September. Root and top fresh weights and heights were measured for each seedling. Parts of the

needles and roots were removed immediately after collection for amino acid, protein and enzyme determinations. Aspartate aminotransferase (AAT) activity was determined spectrophotometrically and proteins were measured using a colorimetric method. Amino acids were analyzed using an HPLC procedure. Total N was measured by a microKjeldahl method, total P by a colorimetric method and K and Mg by atomic absorption spectrophotometry.

Results

By the end of the growing period, *L. laccata*-inoculated seedlings were significantly higher (not shown) and had accumulated more than 2-fold the fresh matter of the controls (Table I). In expanding shoots, N, P, K and Mg concentrations decreased rapidly until mid-August (Fig. 1 A, B, C, D) and tended to increase after growth ceased. No significant difference was found in nutrient analysis of stems and roots between treated and untreated plants (not shown). By contrast, statistically significant (ANOVA, $P = 0.05$) changes were observed for leaf nutrient analysis

Table I. Seasonal variations of fresh matter (g/plant \pm SD) in *L. laccata*-inoculated and non-inoculated (control) Douglas fir, 1 yr old at the beginning of the experiment.

	Date of sampling			
	May 11	June 15	July 20	Sept. 8
Non-inoculated controls	0.6 \pm 0.3	4.2 \pm 1.3	7.1 \pm 1.6	7.9 \pm 1.3
<i>L. laccata</i> -inoculated fir	4.6 \pm 1.2	11.2 \pm 2.1	16.3 \pm 2.3	16.8 \pm 2.7

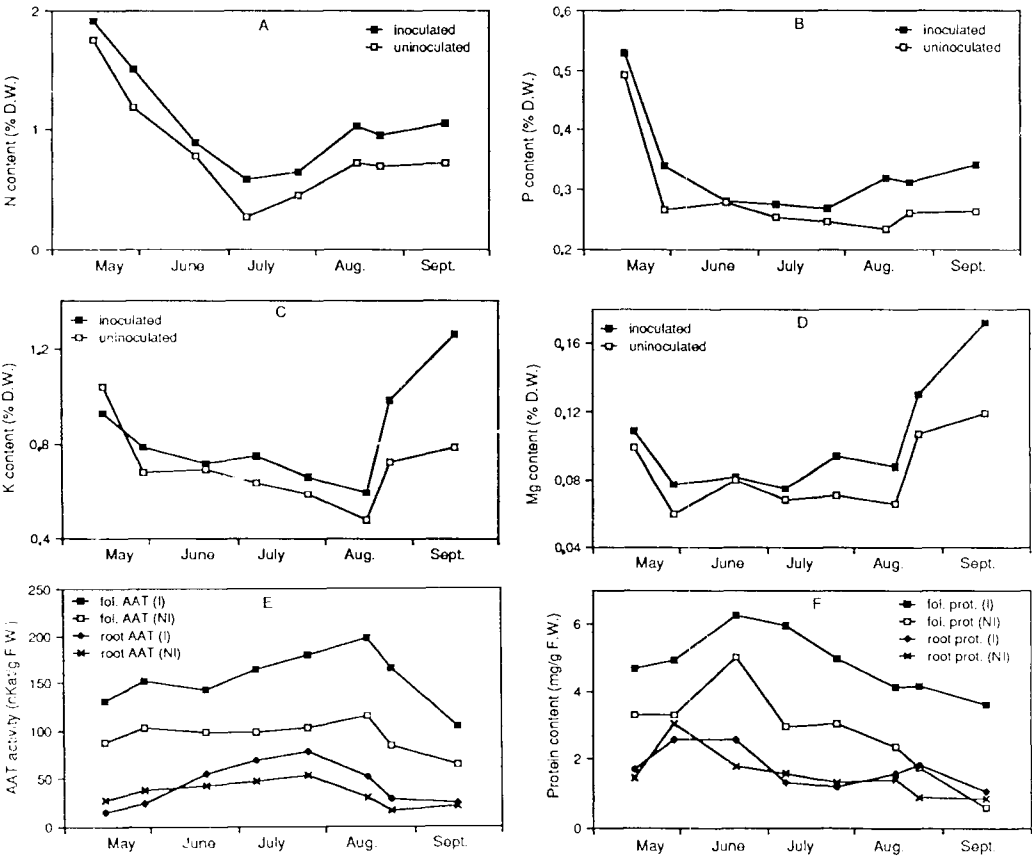


Fig. 1. Seasonal changes in nutrient and protein contents, AAT activity of *L. laccata*-inoculated or non-inoculated Douglas fir seedlings grown in a forest nursery. (fol: foliar; I: inoculated; NI: non-inoculated). **A:** leaf nitrogen; **B:** leaf phosphorus; **C:** leaf potassium; **D:** leaf magnesium; **E:** leaf and root AAT activities; **F:** leaf and root proteins.

between treatments and, more particularly, by the end of the growing season (Fig. 1A, B, C, D).

Concerning protein amounts and AAT activity levels, high and significant differences (ANOVA, $P = 0.05$) were found in

Table II. Root and leaf concentrations ($\mu\text{mol/g DW}$) of free amino acids in *L. laccata*-inoculated and non-inoculated Douglas fir seedlings.

Free amino acid	May 11				June 15				July 20				August 26			
	foliar analysis		root analysis		foliar analysis		root analysis		foliar analysis		root analysis		foliar analysis		root analysis	
	inocul.	uninoc.	inocul.	uninoc.	inocul.	uninoc.	inocul.	uninoc.	inocul.	uninoc.	inocul.	uninoc.	inocul.	uninoc.	inocul.	uninoc.
Ala	24.3	18.7	32.0	27.9	30.0	21.8	36.0	23.5	<u>45.2</u>	<u>27.6</u>	46.0	37.5	28.2	26.2	<u>119.5</u>	<u>83.3</u>
γ Aba	47.9	45.1	33.1	41.5	34.5	26.6	37.2	38.5	14.5	12.5	29.0	18.0	23.8	24.8	68.5	56.2
Arg	19.3	15.3	34.1	39.1	10.5	12.3	11.4	12.1	5.9	6.8	22.7	25.6	<u>37.5</u>	<u>30.2</u>	<u>112.4</u>	<u>102.5</u>
Asp	46.1	42.6	26.8	28.0	24.5	21.3	24.5	23	19.3	20.6	28.5	24.5	18.4	15.6	27.5	20.5
Gln	41.5	40.8	23.8	16.0	<u>24.1</u>	<u>9.34</u>	13.4	12.8	<u>13.9</u>	<u>6.8</u>	<u>18.0</u>	<u>9.2</u>	<u>15.1</u>	<u>6.5</u>	<u>51.5</u>	<u>24.1</u>
Glu	10.3	7.7	7.9	6.9	8.1	8.5	6.1	7.5	6.3	7.1	4.2	3.8	9.3	6.5	5.1	6.2
Pro	9.8	12.5	18.8	21.8	<u>6.6</u>	<u>23.9</u>	<u>8.0</u>	<u>17.3</u>	11.4	7.5	7.1	9.7	<u>4.1</u>	<u>12.4</u>	<u>10.4</u>	<u>19.3</u>

Underlined figures denote significant differences.

foliar analysis between inoculated seedlings and controls: leaves of inoculated Douglas fir accumulated higher protein amounts (Fig. 1F) and showed greater AAT capacities (Fig. 1E). By contrast, there was no difference in root analysis between treatments (Fig. 1E, F).

Alanine, aminobutyric acid, glutamine, aspartic acid, arginine, proline and glutamic acid were the major components of the free amino acid pools in root and leaf tissues. Alanine and glutamine in both root and leaf tissues appeared to be in higher amounts in *L. laccata*-inoculated Douglas fir, whereas proline preferentially accumulated in non-inoculated seedlings (Table II).

Discussion and Conclusion

Growth measurements reaffirmed the well-established importance of *L. laccata* strain 238 for improving seedling growth of Douglas fir (Le Tacon and Bouchard, 1986). Leaf nutrient content following inoculation also confirmed the importance of ectomycorrhizae for nutrient uptake, as reviewed by Harley (1969).

Our results also suggested that inoculations caused changes in the biochemical processes of the host tissues as demonstrated earlier (Nemec and Meredith, 1981; Krishna and Bagyar, 1983). Thus, alanine, proline and glutamine contents, protein amounts and AAT activity levels differed between controls and treated plants.

As a hypothesis, we might propose that lower AAT activity combined with a higher proline amount in non-inoculated Douglas fir characterized seedlings in stress-inducing situations. AAT is often considered as an indicator of physiological activity in plants affected by various internal or external factors (Cooper and Hill-Cottingham, 1974) and proline accumulation described as a response to environmental stress factors (Bode *et al.*, 1985). Inoculation with efficient mycorrhizal strains might provide a way to suppress or at least to attenuate stress-inducing situations.

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