Symbiosis, 9 (1990) 83–86
Balaban Publishers, Philadelphia/Rehovot

Aluminium Tolerance of the Ectomycorrhizal Fungus *Suillus Variegatus**

H. VÄRE and A.M. MARKKOLA

Department of Botany, University of Oulu, Linnanmaa, SF-90570 Oulu, Finland.

Abstract

The aluminium and phosphorus of an ectomycorrhizal fungus, *Suillus variegatus*, grown on a petri plate was studied using a spectrometer and scanning transmission electromicroscope with electron dispersion photometer. Higher concentrations of soluble Al in the growth medium resulted in the abundant formation of aluminium polyphosphate granules in the cell walls. Although Al was detoxified by P, it led to decreased growth, because P was bound in inactive form.

Introduction

Aluminium tolerance has been investigated intensively in higher plants but experiments on fungi have mainly been restricted to pathogen fungi, with very little work done on ectomycorrhizal fungi. When Al penetrates the cell wall, it may interfere with growth by binding to soluble P. Since some strains of *Suillus variegatus* withstand 15 000 ppm soluble Al (Hintikka 1987), about 50 times more than its mycorrhizal symbiont, *Pinus sylvestris*, the present experiment was undertaken to study the possibility of an Al detoxification mechanism in *S. variegatus*.

Material and Methods

Pure cultures of *Suillus variegatus* (Fr.) O. Kunze isolated from a fruitbody were grown on petri plates (pH 4.5) containing modified Melin-Norkrans (MMN) media and various concentrations of aluminium (0, 2, 3, 7, 14, 28, 138, 500, 1000 and 5000 ppm) in the form of $AlCl_3 \cdot 6H_2O$. The fungal mycelium grown on all Al concentrations was analyzed by spectrometry (Chen et al., 1956) to measure P and by atomic

*Reviewed

absorption spectrometry (AAS) to measure Al and their exact locations in the fungal cells was revealed by scanning transmission electron microscopy with dispersion spectrometry (STEM-EDS).

All results were analyzed by one-way ANOVA and a correlation analysis was utilized to interpret the relationship between P/Al ratio (500, 1000 and 5000 ppm at the growth media) and the growth of *Suillus variegatus* hyphae.

Results and Discussion

S. variegatus showed optimum growth with 3 ppm Al in the growth medium (Fig. 1),

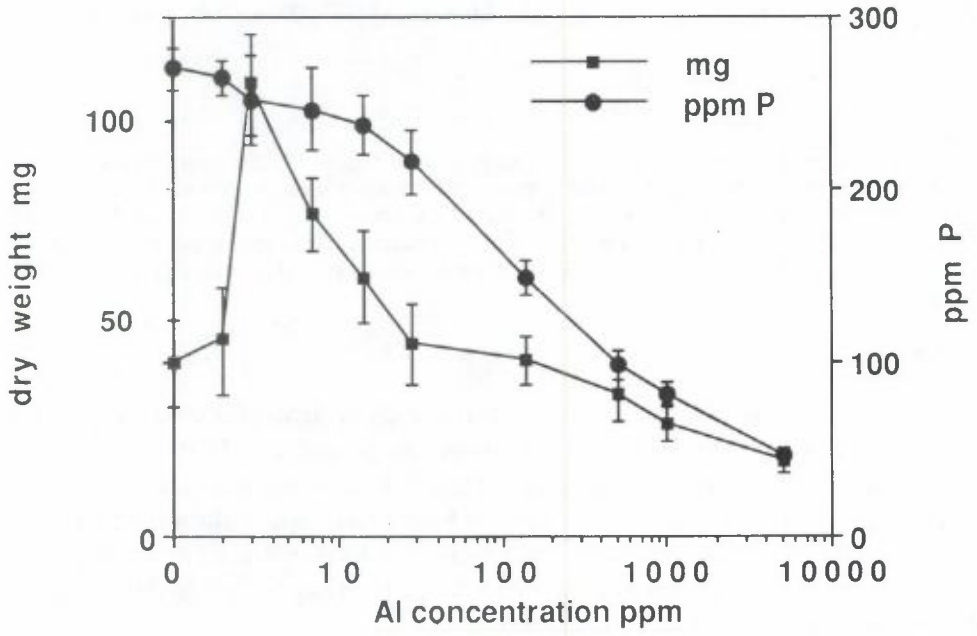

Figure 1. Dry weight (mg) of the mycelium of Suillus variegatus grown in MMN media with varying Al concentrations (0, 2, 3, 7, 14, 28, 138, 500, 1000, 5000 ppm) and mycelium P concentration measured by spectrometer, n = 10.

the difference with other concentrations being significant ($F = 83.5$, $p < 0.001$). At 5000 ppm the final dry weight of the mycelium was only one fifth of the optimum, but the mycelium was still vital. The growth of fungal mycelium decreased as the cell P concentrations decreased on increasing Al concentartions in the growth medium. The total P was very low in the mycelium grown on higher Al concentrations, corresponding to mycelia grown in impoverished medium P (Mousain & Salsac 1984).

The P/Al ratio measured by AAS showed a significant correlation with mycelium dry weight ($r = 0.792$, $p > 0.001$), and STEM-EDS revealed an abundant formation of aluminium polyphospate (APP) granules (Fig. 2) from 500 ppm Al upwards in the

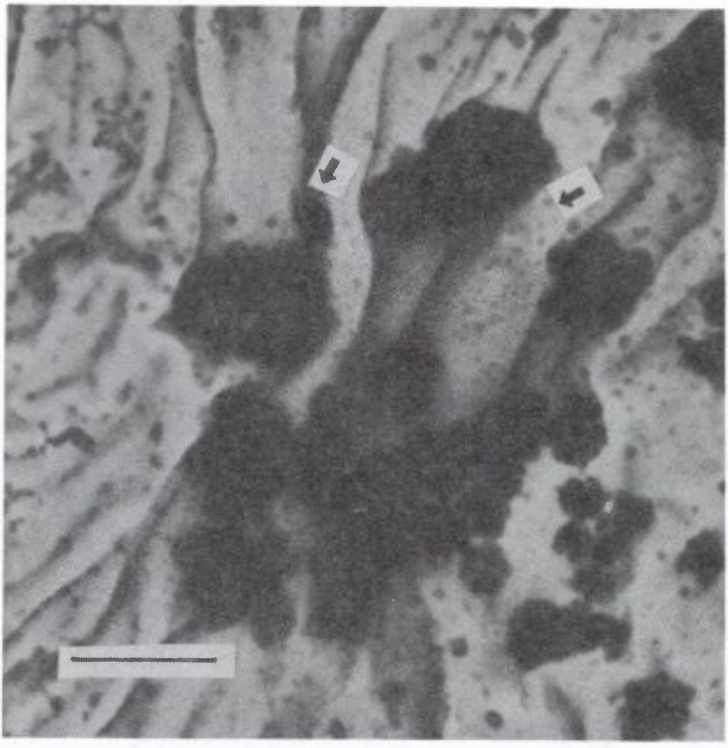

Figure 2. Scanning transmission electron micrograph of *Suillus variegatus* hyphae grown in 5000 ppm soluble Al. The arrows indicates the places (granule and cytoplasm between granules) of measurements made by STEM-EDS. The bar represents 0.1 μm.

growth medium, the P/Al ratio of these granules also showing a significant correlation with mycelium dry weight ($r = 0.918$, $p < 0.001$). This shows that more Al is bound to P in higher Al concentrations in the growth media, which simultaneously leads to decrease in growth. Analysis of Al and P by STEM-EDS showed a highly significant difference between the cytoplasm and granules ($p < 0.001$), Al concentrations being higher in granules. The results indicate that Al is at least partly detoxified by the formation of APP granules, a mechanism which is dependent on the energy provided. Reduction of the sugar concentration by 50% inhibits the growth of the fungal mycelium and causes the formation of APP granules to occur at lower Al concentrations

in the growth medium. Al may be detoxified by organic acids (Haug 1980), the production of which has been investigated by some ectomycorrhizal fungi (Lapeyrie et al., 1987). The formation of APP granules has been reported in algae (Petterson et al., 1985) and that of polyphosphate granules is well documented in ectomycorrhizas (e.g. Ashford et al., 1986).

The Al tolerance of ectomycorrhizal fungi may be a valuable tool for consideration when replanting forests on acidified soils, which may have increased soluble Al concentrations.

REFERENCES

Ashford, A.E., Peterson, R.L., Dwarte, D., and Chilvers, G.A. 1986. Polyphosphate granules in eucalypt mycorrhizas: determination by energy dispersive x-ray microanalysis. *Canadian Journal of Botany* **64:** 677–687.

Chen, P.S., Toribara, T.Y., and Warner, H. 1956. Micro-determination of phosphorus. *Analytical Chemistry* **28:** 1756–1758.

Haug, A. 1984. Molecular aspects of aluminum toxicity. *CRC Critical Reviews in Plant Science* **1:** 345–373.

Hintikka, V. 1987. Aluminium and manganese tolerance in ectomycorrhizal and saprophytic basiodiomycetes. *Aquilo Serie Botanica* **25:** 68–70.

Lapeyrie, F., Chilvers, G.A., and Bhem, C.A. 1987. Oxalic acid synthesis by the mycorrhizal fungus *Paxillus involutus* (Batsch. ex Fr.) Fr. *New Phytologist* **106:** 139–146.

Matsumoto, H., Hirasawa, E., Torikai, H., and Takahashi, E. 1976. Localization of absorbed aluminium in pea root and its binding to nucleic acids. *Plant & Cell Physiology* **17:** 127–137.

Mousain, D. and Salsac, L. 1984. Effet du phosphate et du calcium sur la croissance et l'accumulation ionique chez les champignons ectomycorhiziens en culture in vitro. *Canadian Journal of Botany* **62:** 2600–2609.

Petterson, A., Hällbom, L., and Bergman, B. 1986. Aluminium uptake by *Anabaena cylindrica. f General Microbiology* **132:** 1771–1774.