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Seasonal fluctuations of photosynthesis and its pigments in 1-year mycorrhized spruce seedlings

D. Vodnik, N. Gogala

Department of Biology, Biotechnical Faculty, Večna pot 111, SLO-61001 Ljubljana, Slovenia

Abstract. Specimens of spruce *Picea abies* (L.) Karsten were inoculated with the fungi *Laccaria laccata, Pisolithus tinctorius* and *Lactarius piperatus* in a nursery at the time of sowing. The 1-year seedlings were then tested in two growth periods for their photosynthesis, chlorophyll and carotenoid levels, and water potential; their roots were examined with a scanning electron microscope. Increased photosynthetic activity was determined at the start of the growth season in only those seedlings inoculated with the fungus *Laccaria laccata.* The levels of chlorophyll and carotenoids measured in September in the needles of all three mycorrhized groups of plants were higher than in the controls.

Key words: Ectomycorrhiza – Inoculation – Photosynthesis – Photosynthetic pigments

Introduction

The application of mycorrhizae to forestry planting in Slovenia began in the Department of Biology of the Biotechnical Faculty in Ljubljana and the nursery Omorika at Pohorje in the 1980s. The fungus *Laccaria laccata* proved to be an appropriate mycorrhizal symbiont of the spruce *Picea abies* after vegetative inoculation in bare-root seedling cultivation. Good results were also obtained with another often-used spruce symbiont, the fungus *Pisolithus tinctorius.* In less than a year after inoculation, the seedlings were stronger and larger, and fewer plants showed chlorosis or other signs of illness compared to the controls. The greatest mycorrhizal colonization and the most favorable effects were seen after inoculation by *Laccaria laccata.* Less favorable results were obtained after inoculation with *Lactarius piperatus* (Gabrovšek and Gogala 1990).

Laccaria laccata is a known successful symbiont of the spruce. The high degree of infectivity (Molina 1982), its antipathogenic activity (Sampagni et al. 1985; Unestam et al. 1987), and its extremely positive influence on the growth of different tree species were the reason for its introduction into nursery programs, particularly in Europe. Increased early development is of economical interest as 2-year inoculated seedlings are suitable for transplantation (Marx et al. 1991). Some research also demonstrated that the advantages of seedlings inoculated with *Laccaria laccata* continue after transplantation onto permanent sites (Le Tacon et al. 1992). Our tests used a strain of *Laccaria laccata* isolated from the region of Pohorje. This area is characterized by acid soil; here mycorrhized plants are more successful than unmycorrhized ones.

Similar stimulative effects on the growth of the spruce are also shown by *P. tinctorius.* We chose this fungus because of its previous use in well-tested reforestation attempts in ecologically damaged areas (Marx et al. 1991). The regions of Pohorje and the upper Mežica valley suffer from the effects of high SO_2 emission and some forest areas are completely denuded and eroded. Our seedlings are destined for these sites.

The improved mineral metabolism of mycorrhized plants may indirectly affect photosynthesis. It is also known that the use of photosynthates by mycorrhizal fungi directly affects carbon assimilation of the plant. Photosynthesis is mainly increased by the colonization of the root system (Nylund and Wallander 1989). Mycorrhizal infection is also said to positively influence resistance to water stress (Reid 1979; Harley and Smith 1983).

We followed several physiological parameters in 1-year seedlings inoculated with *Laccaria laccata, P. tinctorius* and *Lactarius piperatus*: the photosynthetic activity, the levels of some pigments in the needles, and the water potential. We also examined the roots with a scanning electron microscope (SEM) to determine the presence of the mycorrhizal colonization and its intensity.

Correspondence to: D. Vodnik

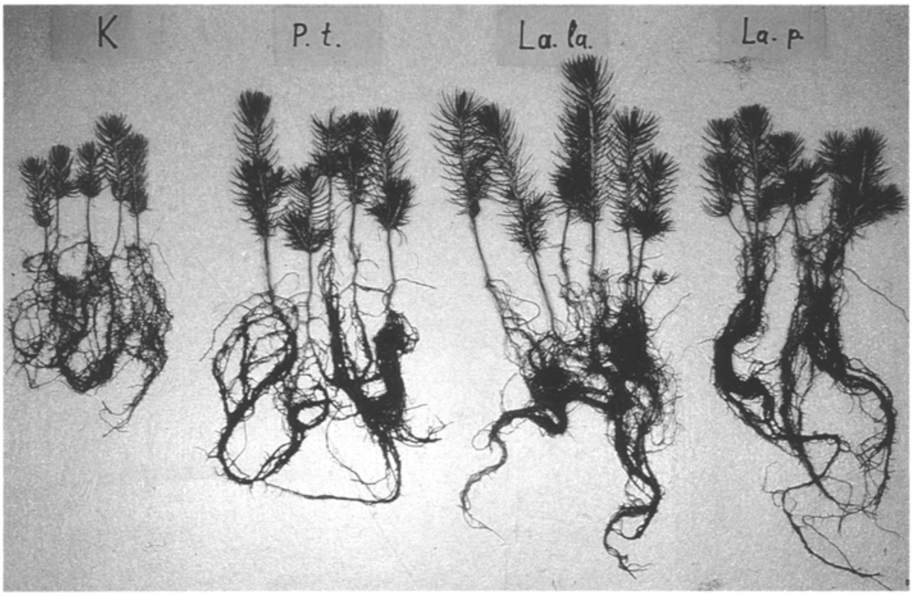

Fig. 1. One-year spruce seedlings after inoculation with the fungi *Laccaria laccata* (La. la.), *Pisolithus tinctorius* (P. t.) and *Lactarius piperatus* (L. p.). K, Control

Materials and methods

Inoculum preparation and inoculation

Laccaria laccata (Scop. ex Fr.) Bk. et Br., *P. tinctorius* (Pers.) Coker et Couch and *Lactarius piperatus* (L. ex Fr.) Gray, from the fungal collection of the Department of Biology, Biotechnical Faculty, University of Ljubljana, were grown on M-40 medium (Stevens 1974). Three-week-old cultures were transferred onto sterilized vermiculite-peat substrate admixed with liquid M-40 medium in PVC bags. The mycelium was grown in the dark at 25° C. After 3 months, it had completely overgrown the substrate.

The nursery Omorika at Pohorje is characterized by dystric brown soil, cambisol. The greenhouse soil was covered with the basic substrate consisting of a mixture of peat and vermiculite in a ratio of 4:1 (modified after Marx et al. 1982), treated with Basamide. In May 1991, we buried approximately 2 l/m^2 of the washed inoculum into the upper layer of the basic substrate (Marx et al. 1991). The spruce seeds, provenience Brezovec, Pohorje (elevation 700 m), were sown manually. No chemicals were used during the experiment; the seedlings were hand weeded and watered during the dry season. The control series consisted of plants grown on a similarly prepared substrate without the addition of fungi.

Measurements and analyses

Sampling and measurements were performed on 1-year seedlings in May and September 1992. Each sample consisted of 10 plants.

We followed the photosynthesis in the seedlings with a portable CO_2 analyzer LCA-2 (ADC Instruments). The preliminary measurements were taken at a time of varying light conditions; later measurements were performed under light conditions that did not limit photosynthetic activity. In the tested plants, we also determined the levels of chlorophylls a and b and carotenoids in the needles according to Lichtenthaler's spectrophotometric method (1987). The water potential was measured with a pressure chamber. Additionally, we checked the rootlets with an SEM (Jeol JSM-840A). The samples were prepared by using a modified Crowley and Reid's method (1985) in which 1% glutaraldehyde and sodium cacodylate were used for fixation. Student's *t*-test was used for statistical evaluation (* $P<0.05$, ** $P<0.01$).

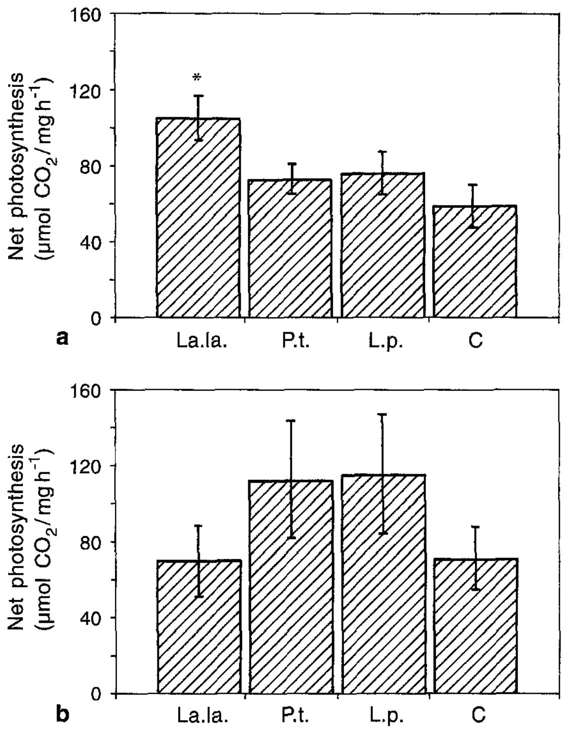

Fig. 2a, b. Photosynthesis of 1-year spruce seedlings (per mg chlorophyll a+b) inoculated with various fungi (abbreviations as in Fig. 1). C, controls; **a** May 1992; **b** September 1992. The bars indicate standard errors

Results

All 1-year seedlings inoculated at the time of sowing with an inoculum of one of three mycorrhizal fungi showed a markedly increased growth rate in comparison to the uninoculated plants (Fig. 1).

Photosynthetic measurements of the inoculated and control seedlings in varying light conditions showed no significant differences. We determined the saturation light conditions and in later field work only used results obtained in optimal lighting. In May, we measured the highest net photosynthesis in the seedlings infected with *Laccaria laccata,* but in September these same seedlings showed relatively low values, approximating those of the controls. High but extremely variable values were found in the other two groups mycorrhized with *P. tinctorius* and *Lactarius piperatus* (Fig. 2a,b).

May measurements showed the greatest concentrations of chlorophyll a to be in the needles of the control seedlings. Seedlings colonized by *Laccaria laccata* had the lowest values in May but by September they had increased fourfold. The other two inoculated groups also showed markedly increased values in the autumn as compared to the spring. Chlorophyll a values for control plants lagged behind the mycorrhized plants by the autumn. The concentrations of chlorophyll b were also lowest in the mycorrhized seedlings in spring but in the control plants in the autumn. Again, the greatest differences were seen in those inoculated with *Laccaria laccata.* The ratio between chlorophylls a and b was consistently relatively low, i.e. about 2:1. It only increased slightly in the inoculated seedlings in the autumn (Fig. 3a,b). A similar seasonal trend was also seen in the concentrations of carotenoids in the seedling needles (Fig. 4).

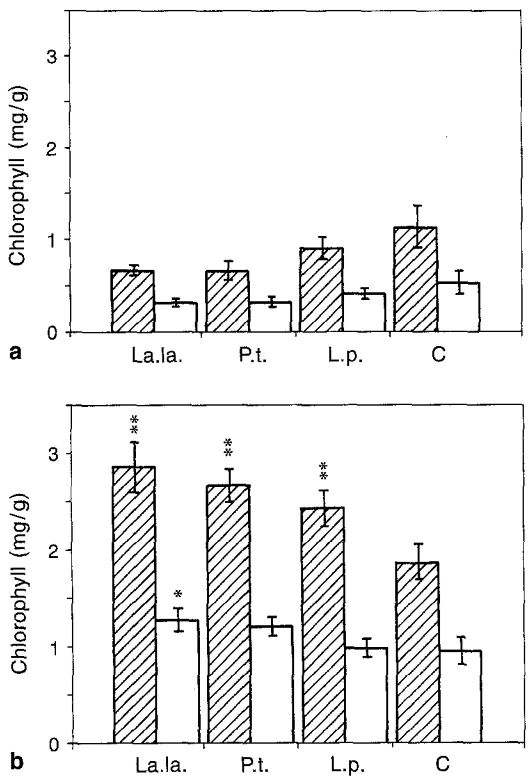

Fig. 3a,b. Chlorophyll concentrations (per g dry weight) in the needles of 1-year spruce seedlings inoculated with various fungi (abbreviations as in Fig. 1). ▨ Chlorophyll a; □ chlorophyll b; **a** May 1992; **b** September 1992

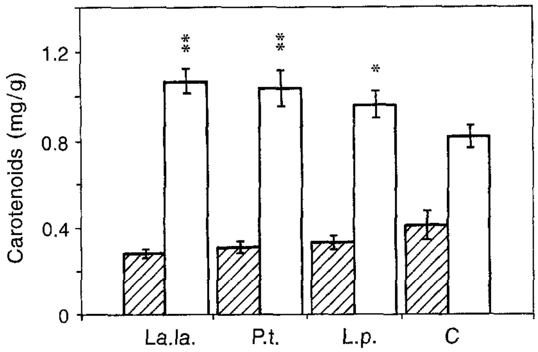

Fig. 4. Carotenoid concentrations (per g dry weight) in the needles of 1-year spruce seedlings inoculated with various fungi (abbreviations as in Fig. 1). ▨ May 1992; □ September 1992

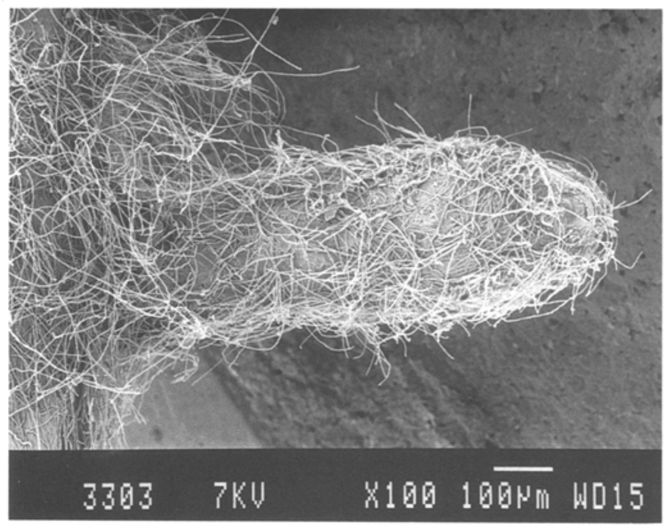

Fig. 5. Roots of a 1-year spruce seedling inoculated with *Lactarius piperatus* showing early infection with a loose hyphal envelope at the apex of a short root

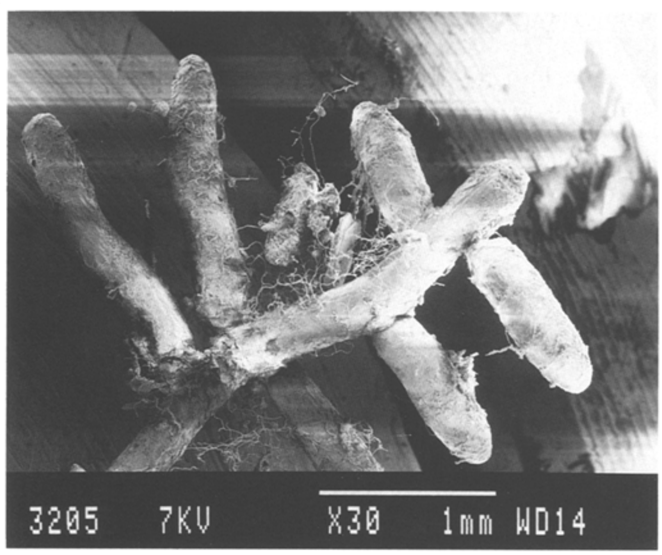

Fig. 6. Roots of a 1-year spruce seedling inoculated with *Laccaria laccata*; the hyphal mantle has formed

The water potential of the 1-year spruce seedlings did not show any significant differences between the test plants and the controls.

SEM studies of the roots proved the presence of mycorrhizae in all three inoculated groups. The earlier stages of mycorrhization with a loose hyphal envelope at the apex of short rootlets were predominant (Fig. 5). However, we often noted more fully developed mycorrhizae with a compact hyphal mantle and a branching root system (Fig. 6). The latter forms were seen predominantly in the seedlings inoculated with *Laccaria laccata* and *P. tinctorius.*

Discussion

The root system showed that 1 year after inoculation the mycorrhizae were well established in all three inoculated groups. Mycotrophy was also present in the controls, but there were atypical forms of root mycorrhization and the colonization was much lower. This was most probably due to the fungus *Thelophora terrestris,* whose fruit bodies are present in extremely large numbers during times of high humidity in our nursery. *T. terrestris* is known to be adapted to growth in nurseries, and it particularly colonizes sterilized substrates (Marx and Bryan 1970). Colonization by an ectomycorrhiza does not occur during the first growing season in the classical method of seedling cultivation or, if it occurs, is a slow infection most frequently due to this fungus (Molina 1982).

Even though we did not determine the precise degree of mycorrhizal colonization in this experiment, our examination of the roots with SEM confirmed the statement that infection with *Laccaria laccata* was successful. Molina (1982) determined that the use of different strains of this fungus was successful in up to 85% of short rootlet infections during containerized cultivation of conifers. Gabrovšek and Gogala (1990) found that the *Laccaria laccata* strain used in this experiment formed a mycorrhiza within 9 months of inoculation in 82% of the examined seedlings. Colonization with *P. tinctorius* was lower, and was markedly less with *Lactarius piperatus.* In fact, these two fungi inoculated in our test seedlings showed less well-developed mycorrhizae.

Explanation of the mycorrhizal effect on photosynthesis is difficult because of possible indirect influences on carbon assimilation. A direct stimulatory effect is seen in the allocation of sugars into the plant roots where they are used by the fungus. But increased carbon assimilation may also occur because of other factors, notably the improved availability of other nutrients, particularly phosphorus. A higher amount of phosphorus in the chloroplasts may be the reason for the net increase in photosynthesis (Sivak and Walker, cited in Jakobsen 1991). Rousseau and Reid (1990) measured the photosynthesis of mycorrhized and nonmycorrhized loblolly pine seedlings which had equal amounts of phosphorus in the leaves and found a stimulation of photosynthesis of up to 17% when the mycorrhizal colonization was high enough. On the other hand, no such effect was seen in plants with poorly developed mycorrhizae. The results of Dosskey and coworkers (1990) show that stimulation of carbon assimilation in Douglas fir occurs only in cases where the root system colonization is intensified. Nylund and Unestam (1987) reported that *Laccaria laccata* increased the photosynthesis of Scots pine but did not increase its growth. Nylund and Wallander (1989) considered that development of mycorrhizal infection on the same symbiont corresponded to a gradually increasing stimulation of photosynthesis, but they also noted that the mycorrhizal effect on photosynthesis may depend on factors not monitored in their experiment.

The most probable cause for the increased photosynthesis of inoculated spruce seedlings is the intensive development of *Laccaria laccata* during the spring season, as the mycorrhizal colonization was faster than that of the other tested fungi. Spring stimulation of photosynthesis was not noticed in the seedlings inoculated with *P. tinctorius* and *Lactarius piperatus,* and their carbon assimilation increased only at the beginning of autumn. This might be explained by the later mycorrhization. Previous experiments have shown that with *Lactarius piperatus* the percentage of mycorrhizal colonization rises towards the end of the growth season, when in *Laccaria laccata* there is no further increase (Gabrovšek and Gogala 1990). In the fall, net photosynthesis of seedlings inoculated with *Laccaria laccata* was significantly lower than in other seedlings but approximately equal to the controls.

The effect of individual fungi on the seedlings can be clearly seen from the analysis of the needle pigments. Because of the earlier growth of shoots, in the spring the needles of inoculated seedlings contained less chlorophyll and carotenoids, but this did not limit photosynthesis. Just at the time when the needles of seedlings inoculated with *Laccaria laccata* contained the least chlorophyll, their photosynthetic activity was greatly increased. At the beginning of autumn, the concentration of pigments was already significantly higher in the inoculated seedlings than in the controls. A possible cause for this accelerated synthesis of pigments during the growth season may lie in the changed hormonal state of the plant. Mycorrhized plants contain more cytokinins (Edriss et al. 1984), and increasing concentrations of these hormones may influence chlorophyll synthesis (Parthier 1979; Kuraishi et al. 1992). Nasr (1993) found more chlorophyll in vesicular arbuscular mycorrhized tomatoes and the level rose further on addition of exogenous cytokinins.

The water content of inoculated and control plants was similar. Adequate watering resulted in no changes that could be attributed to better water absorption or retention. Some strains of the fungi *P. tinctorius* and *Laccaria laccata* were found not to influence water regulation in the Scots pine during water stress (Diebolt and Mudge 1987). Mycorrhizal colonization with *Laccaria laccata* did not influence the water potential of the Douglas fir during drought and in the same plants stomatal conductance and photosynthesis were lower than in unmycorrhized trees (Dosskey et al. 1991).

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