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Carbon-sink stimulation of photosynthesis in Douglas fir seedlings by some ectomycorrhizas*

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SUMMARY

Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] seedlings responded differently regarding rate of photosynthesis when inoculated with three different ectomycorrhizal fungi. *Rhizopogon vinicolor* FSL788-5 caused a significant increase in net photosynthesis rate compared to non-mycorrhizal controls, while *Hebeloma crustuliniforme* HeCr2 and *Laccaria laccata* S238-A had no effect. Colonization by *Rhizopogon* and *Hebeloma* caused increased osmotic potential in the leaf symplast compared to controls, while *Laccaria* did not. Colonization levels for *Rhizopogon*, *Hebeloma* and *Laccaria* were 36, 93 and 73 % of root tips, respectively. *Rhizopogon* and *Hebeloma* produced abundant extramatrical hyphae and/or rhizomorphs, while *Laccaria* was smooth-mantled. *Hebeloma*-colonized seedlings were significantly smaller than non-mycorrhizal controls; *Rhizopogon* seedlings were smaller, but significantly so only at $P < 0{\cdot}10$. *Laccaria* did not affect seedling size. Only smaller *Hebeloma* seedlings exhibited elevated concentrations of N, P, K, and Ca over non-mycorrhizal controls. These data demonstrate a non-nutritional basis for increased rate of photosynthesis caused by some ectomycorrhizal fungi that can be explained by the increased photosynthate sink generated by extensive fungal growth associated with the mycorrhizas.

Key words: Ectomycorrhizas, photosynthesis, Douglas-fir, C-allocation, nutrition

INTRODUCTION

The presence of vesicular–arbuscular (VA) ectomycorrhizas on root systems of plants is correlated with higher rates of net photosynthesis (Allen *et al.*, 1981; Paul & Kucey, 1981; Parke, Linderman & Black, 1983; Reid, Kidd & Ekwebelam, 1983; Brown & Bethlenfalvay, 1987; Nylund & Unestam, 1987). However, mechanisms responsible for this effect have not been clearly identified.

The suggestion has been made that diversion of carbohydrates to the mycorrhizal fungus stimulates photosynthesis rate (Reid *et al.*, 1983). This hypothesis is based on the concept that rate of photosynthesis is related to demand for carbohydrates (Sweet & Wareing, 1966; Herold, 1980). Harley & Smith (1983) suggest mycorrhizal root systems require more photosynthate than non-mycorrhizal root systems. Reid *et al.* (1983) and Koch & Johnson (1984) showed greater percentages of assimilated ^{14}C from labelled CO_2 translocated to ectomycorrhizal root systems and to VA mycorrhizal root systems, respectively, than to non-mycorrhizal root systems. Photosynthate diversion to the fungus has appeared large enough to cause decreased plant growth (Bethlenfalvay, Brown & Pacovsky, 1982; Bethlenfalvay *et al.*, 1982).

Although mycorrhizas establish a sink for photosynthate by utilizing it for fungal biomass and metabolic energy, a clear experimental link to stimulated photosynthesis has not been demonstrated because interacting factors are involved. A prevailing view attributes higher net photosynthesic rate in mycorrhizal plants to improved mineral nutrition (Paul, Harris & Fredeen, 1985). Mycorrhizas enhance nutrient uptake, particularly P, under most circumstances (Bowen, 1973; Harley & Smith, 1983). Enhanced N, P and K nutrition have each been correlated with higher rates of net photo-

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synthesis (Brix, 1971; Keller, 1972; Nátr, 1972; Longstreth & Nobel, 1980). Mycorrhiza-induced changes in plant hormone balance might also be a factor (Slankis, 1973; Allen, 1985).

Investigations designed to test the occurrence of non-nutritional stimulation of net photosynthesic rate by mycorrhizas have failed owing to inability to eliminate nutritional effects of mycorrhizas as a reasonable case (Reid *et al.*, 1983). In this paper, we report results of a study in which ectomycorrhizas stimulated net photosynthesic rate of Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] seedlings in the absence of nutritional effects.

Materials and Methods

Plant and fungal culture

Douglas fir seedlings were grown from seed initially in a mixture of vermiculite and peat moss. Plants were selected for uniformity at ages 6 and 8 weeks and transplanted into 250 cm^3 plastic tubes containing a mixture of 3 parts soil [sandy loam; organic matter 0·8 %; CEC 19 $cmol_c\ kg^{-1}$; available P (Bray) 10 mg kg^{-1}; pH 6·5] and 1 part mycorrhizal inoculum substrate (v/v). The soil substrate mixture was settled to a bulk density of 1060 kg m^{-3}.

Inoculum substrate of *Laccaria laccata* S238-A and *Hebeloma crustuliniforme* HeCr2 consisted of mycelium cultures grown for three months on a mixture of 30 parts vermiculite, 1 part peat moss, and 12 parts modified Melin–Norkrans (MMN) solution (v/v/v; Marx & Kenney, 1982). Fungus-free control substrate was identically prepared but contained no fungal culture. The substrate was rinsed in cool tap water prior to mixing with soil, to remove residual MMN solution.

Rhizopogon vinicolor FSL788-5 inoculum was prepared by macerating sporocarps in distilled water with a blender, making a spore suspension of an estimated concentration of 6×10^5 spores ml^{-1}.

At age 6 weeks, one group of 400 seedlings was transplanted into tubes with the soil mix containing fungus-free substrate. Half of the seedlings in this group were inoculated by injecting 5 ml of *Rhizopogon* spore suspension into each tube. At age 8 weeks, 600 additional seedlings were transplanted into tubes with the soil mix containing either *Laccaria*, *Hebeloma* or fungus-free substrate.

Seedlings were maintained in a glasshouse for four more months under a 15 h lighter period, obtained by supplementing natural light with high-pressure sodium-vapour lamps (150 μmol $m^{-2}\ s^{-1}$ in the 400–700 nm waveband) during 4 h periods in the morning and evening. Seedlings were watered as needed to maintain soil water potential higher than about −0·05 MPa. With every fifth watering, each seedling received 15 ml of a nutrient solution containing (in mg kg^{-1} solution) 120 N (7:1, NO_3^-:NH_4^+), 20 P, 100 K, 100 Ca, 36 Mg, 64 S, and Long-Ashton micronutrient solution (Hewitt, 1966). After this 4-month period, leaf expansion had ceased and terminal buds had begun to form. Randomly selected seedlings were transferred to a growth chamber for measurement of net photosynthesis rate and osmotic potentials.

Rate of net photosynthesis

Seedlings were transferred to a walk-in growth chamber 3 days prior to measurements. Growth chamber conditions were: 15 h daylength from a bank of fluorescent and incandescent lamps; light intensity 350 μmol $m^{-2}\ s^{-1}$ in the 400–700 nm waveband for 13 h per day with 1 h gradual on and off periods in morning and evening, respectively; 26 °C day/22 °C night; 50 % r.h. day/80 % night; windspeed 0·15 m s^{-1}; CO_2 concentration 13–15 mmol m^{-3} depending upon ambient outdoor concentration. All seedlings were thoroughly watered the evening before measurements were begun.

Net photosynthesic rates were measured periodically during the course of one day using a 2 l cuvette (designed to enclose an entire shoot without the leaves touching the walls) in series with the LI-6000 Portable Photosynthesis Meter (LI-COR, Lincoln, NE). Measurements were made on each seedling at 06.45 (predawn), 07.15, 07.45, 08.45, 14.15, 20.45, 21.45, and just after dark at 22.15 h. Measurements were replicated on 12 seedlings from each treatment.

Net photosynthesic rates were computed by the LI-6000 using an assumed leaf area of 100 cm^2. These values were subsequently adjusted for actual leaf area, estimated from leaf dry mass using the conversion function

$$\text{Area} = -2{\cdot}3 + 120{\cdot}9\,(\text{mass}) - 35{\cdot}0\,(\text{mass}^2),$$

where area is in cm^2 and mass in g. The function was derived using 40 plants selected from among all treatments to represent the normal range of shoot sizes. Leaf area of each seedling was measured by placing all of its needles side-by-side, with minimum overlap and vacant area, on adhesive 1 mm grid paper. The corresponding dry mass of these leaves was determined after oven drying (65 °C, 48 h). Data from all seedlings were pooled to derive the function, since there were no apparent differences between treatments.

Osmotic potential

A different set of seedlings was transferred to the growth chamber and treated in an identical manner to those used for measurement of net photosynthesis. Leaf sap osmotic potentials were measured periodically through the course of one day at 06.45 (predawn), 07.45, 08.30, 11.00, 20.30, and just after dark at 22.45. Cell sap for measurement of osmotic potential was derived using 20–30 needles pulled

from each seedling. These were sealed immediately in a Tygon capsule, frozen on dry ice and then thawed, and squeezed to express the sap. Measurements were made with a vapor pressure osmometer (WESCOR, Logan, UT). Osmometer values were adjusted to estimate osmotic potential of the leaf symplast, assuming apoplastic water was a constant 20% of total leaf water content (Joly, 1984). One measurement was made on needles from each seedling. Osmotic potential was determined on 6–10 plants from each treatment at each measurement time.

Nutrition, colonization and growth

Nutrient concentrations were determined from leaves of each plant used for measurement of net photosynthesis rate. All leaves were removed the midday following measurement. Total N and P concentrations were determined colorimetrically (RFA Method, ALPKEM Corp., Clackamas, OR) on Kjeldahl-digested leaves. Total K and Ca concentrations were determined by atomic absorption spectrophotometry on perchloric acid-digested leaves.

Root systems of all plants were thoroughly washed of soil and microscopically examined to estimate the percentage of root tips which had formed well-developed mycorrhizas.

Plant dry mass (65 °C, 48 h) was determined for each seedling used in this study. No attempt was made to separate fungal tissue from root tissue.

Statistical analysis

Parameters were examined by analysis of variance. Where significant differences ($P < 0{\cdot}05$) were found in comparisons of three treatment means, the Least Significant Difference (L.S.D.) was used to identify dissimilar treatment means.

RESULTS

Rate of net photosynthesis

Figures 1 and 2 indicate *Rhizopogon* had an effect on net photosynthesic rate of Douglas fir seedlings while *Hebeloma* and *Laccaria* did not. *Rhizopogon* stimulated net photosynthesic rate significantly ($P < 0{\cdot}05$) compared to non mycorrhizal controls during the early part of the day. The effect decreased gradually with time and disappeared toward the end of the day. On a daily basis, *Rhizopogon*-colonized seedlings fixed about 15% more CO_2 than non-mycorrhizal controls. Neither *Laccaria* or *Hebeloma* had any effect on net photosynthesic rate of seedlings at any time of day.

Osmotic potential

Figures 3 and 4 show that *Rhizopogon* and *Hebeloma*

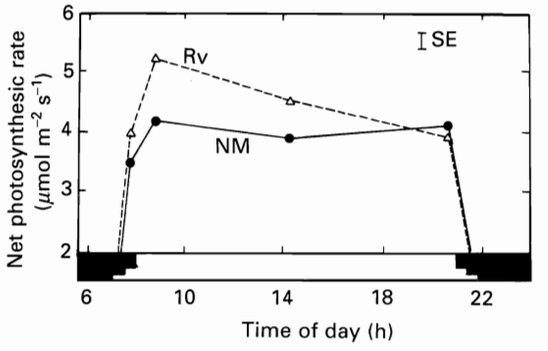

Figure 1. Rate of net photosynthesis measured periodically over the course of one day under well-watered soil conditions or 6-month-old Douglas fir seedlings: non-mycorrhizal (NM); colonized by *Rhizopogon vinicolor* (Rv).

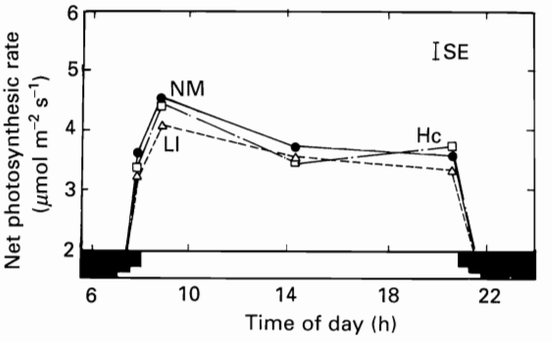

Figure 2. Rate of net photosynthesis measured periodically over the course of one day under well-watered soil conditions for 6-months-old Douglas fir seedlings: non-mycorrhizal (NM); colonized by *Laccaria laccata* (Ll); colonized by *Hebeloma crustuliniforme* (Hc).

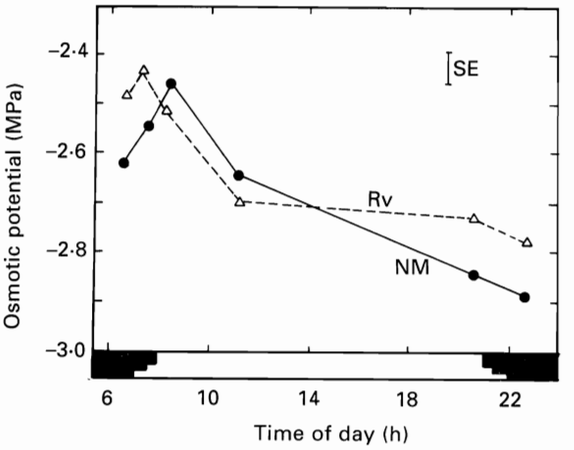

Figure 3. Leaf osmotic potential measured periodically over the course of one day under well-watered soil conditions for 6-month-old Douglas fir seedlings: non-mycorrhizal (NM); colonized by *Rhizopogon vinicolor* (Rv).

colonization led to higher osmotic potentials in the leaf symplast compared to their respective non-mycorrhizal controls. Statistical comparisons were made at times of day when sample sizes were large (10 observations); at 6.45 (predawn), 20.30, and just after dark 22.45. *Hebeloma*-colonized plants had

Table 1. *Amount of mycorrhizal colonization, plant dry mass, and leaf nutrient concentration of 6-month-old Douglas fir seedlings : non-mycorrhizal, and colonized by* Rhizopogon vinicolor, Laccaria laccata *or* Hebeloma crustuliniforme

Comparison group no.	Fungal treatment	Colonization (%)	Plant dry mass	Concentration of nutrients in leaves (%)			
				N	P	K	Ca
1	Non-mycorrhizal	0	2·08 ± 0·06 a	0·64 ± 0·03 a	0·06 ± 0·01 a	0·66 ± 0·04 a	0·33 ± 0·02 a
	Rhizopogon vinicolor	36 ± 2	1·92 ± 0·06 a	0·71 ± 0·03 a	0·06 ± 0·01 a	0·71 ± 0·05 a	0·33 ± 0·02 a
2	Non-mycorrhizal	0	2·44 ± 0·06 a	0·63 ± 0·03 a	0·08 ± 0·01 a	0·76 ± 0·08 a	0·31 ± 0·02 a
	Laccaria laccata	73 ± 3	2·53 ± 0·05 a	0·57 ± 0·02 a	0·09 ± 0·01 a	0·69 ± 0·04 a	0·31 ± 0·02 a
	Hebeloma crustuliniforme	93 ± 2	2·01 ± 0·06 b	0·74 ± 0·04 b	0·11 ± 0·01 b	0·82 ± 0·04 a	0·41 ± 0·02 b

Values are means ± 1 SE. For each comparison group, values not followed by the same letter are significantly different at the $P < 0·05$ level.

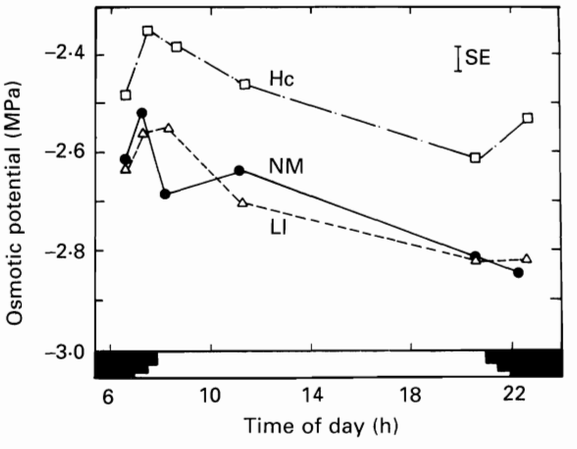

Figure 4. Leaf osmotic potential measured periodically over the course of one day under well-watered soil conditions for 6-month-old Douglas fir seedlings: non-mycorrhizal (NM); colonized by *Laccaria laccata* (Ll); colonized by *Hebeloma crustuliniforme* (*Hc*).

significantly higher osmotic potentials ($P < 0·05$) than either *Laccaria* or non-mycorrhizal control plants at all of these times of day. *Rhizopogon* plants had significantly higher osmotic potential than non-mycorrhizal controls at predawn (06.45, $P < 0·05$) and just after dark (22.45, $P < 0·10$), but not at 20.30 h. Osmotic potentials in *Laccaria*-colonized seedlings were not different from non-mycorrhizal controls at these times of day.

Colonization

Hebeloma and *Laccaria* colonized root systems at high levels (93 and 73 % of root tips, respectively, Table 1), forming thin-mantled mycorrhizas. *Rhizopogon* developed thick-mantled mycorrhizas, but only on 36 % of the total number of seedling root tips. *Rhizopogon* and *Hebeloma* produced abundant extramatrical hyphae and/or rhizomorphs throughout the soil volume, while there was little evidence of hyphae in the soil around *Laccaria*-colonized seedlings.

Plant growth and nutrition

The effect of mycorrhizas on plant growth was determined by comparing plant dry mass at the end of the experiment. *Hebeloma*-colonized plants were significantly smaller than non-mycorrhizal controls at age 6 months (Table 1). *Rhizopogon*-colonized plants also tended to be smaller, but this was significant only at the $P < 0·10$ level. *Laccaria* did not affect seedling growth.

Neither *Rhizopogon* or *Laccaria* had any significant affect on leaf concentrations of N, P, K and Ca at the end of the experiment (Table 1). However, *Hebeloma*-colonized plants had significantly greater concentrations of N, P and Ca ($P < 0·05$) than non-mycorrhizal controls.

DISCUSSION

Stimulation of net photosynthesic rate by *Rhizopogon* is consistent with the concept that photosynthesis is stimulated by increased demand for its products (Sweet & Wareing, 1966; Herold, 1980; Bagnall, King & Farquhar, 1988). It has been shown that mycorrhizal root systems represent a greater photosynthate sink than non-mycorrhizal root systems (Reid *et al.*, 1983; Koch & Johnson, 1984). In this study, *Rhizopogon* colonization reduced plant biomass over 4 months, despite evidence that net photosynthesis rate was higher than for non-mycorrhizal controls over at least part of this period. These data suggest that *Rhizopogon* developed a substantial sink for photosynthate. The additional photosynthate which was produced by *Rhizopogon*-colonized plants appears to have been exported to support the abundant fungal growth observed in the soil.

Increased demand for photosynthate, represented here by *Rhizopogon* fungal growth, may stimulate photosynthesis through mass action by reducing concentration of soluble photosynthetic products,

especially sucrose, in leaves (Herold, 1980). In this study, generally higher osmotic potential of *Rhizopogon*-colonized plants is consistent with this mechanism. Lack of correspondence between higher net photosynthesic rate and higher leaf osmotic potential at specific times of day may reflect a weak correlation between osmotic potential and sucrose concentration brought about by shorter-term dynamics of photosynthate partitioning within leaves. Therefore, this mechanism remains to be tested through more rigorous examination of the carbon economy of mycorrhizal plants.

For *Rhizopogon*-colonized plants, altered plant nutrition cannot account for stimulated net photosynthesis rate since there were no nutritional differences compared to non-mycorrhizal controls. The hypothesis that mycorrhizas stimulate photosynthesis by inducing a change in plant hormone balance remains unconfirmed. However, if mycorrhizal colonization had stimulated photosynthesis directly through enhanced nutrition or plant growth regulators, it would likely have led to greater plant dry mass and lower leaf osmotic potentials, a result which was not observed in this experiment. For *Rhizopogon*, our results indicate that net photosynthesic rate was stimulated by a mechanism not related to nutrition and consistent with the photosynthate sink-source concept.

Hebeloma also showed evidence of acting as a sink for photosynthate. *Hebeloma*-colonized plants were significantly smaller and had much higher osmotic potential than non-mycorrhizal controls, in spite of a similar rate of net photosynthesis. This correlated with the visual observation of abundant *Hebeloma* hyphal growth. These observations, however, are not altogether consistent with the sink-source concept, since net photosynthesic rate was not stimulated. Furthermore, significantly higher leaf nutrition, apparently due to growth suppression, also did not stimulate photosynthesis. The reason for this behaviour is not clear. Perhaps this represented an interactive effect of photosynthate sink and altered nutrition on photosynthesis and translocation.

There was no evidence that *Laccaria* enhanced photosynthate demand. *Laccaria* colonization had no effect on plant growth, net photosynthesic rate, or leaf osmotic potential, nor did *Laccaria* produce abundant hyphal growth, as *Rhizopogon* and *Hebeloma* did. *Laccaria* also had no effect on plant nutrition. These observations are consistent with the sink-source concept insofar as *Laccaria* did not effect photosynthesis or photosynthate demand.

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