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SHORT NOTE

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Phosphorus-32 absorption and translocation to host plants by arbuscular mycorrhizal fungi at low root-zone temperature

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Abstract Arbuscular mycorrhizal (AM) mycelia persist in soil over winter. Functioning of the AM symbiosis very early in the spring when the soil temperature is low may be of ecological significance for perennial and biannual plants in cool climates. An indoor experiment was conducted to investigate the effects of low root-zone temperatures on ^{32}P uptake by 10-week-old leek plants (*Allium porrum* L.) inoculated or not with the AM fungus *Glomus intraradices* Schenck & Smith. Plants were grown in a greenhouse at approximately 23°C prior to exposing their roots to 23°C, 15°C or 0°C. Mycorrhizal colonization increased ^{32}P activity of leek leaves at a root-zone temperature of 23°C seven days after injection of ^{32}P into the soil, whereas 14 days after injection, ^{32}P increases were measured at both 23°C and 15°C. The lack of difference in ^{32}P activity between AM and non-AM plants at 0°C, both 7 and 14 days after injection, suggests that the AM fungus is not functional at this low root-zone temperature.

Keywords Cold · Spring soil temperature · Leek · Arbuscular mycorrhiza · Cool climate

Introduction

Phosphorus uptake by arbuscular mycorrhizal (AM) fungi is affected by factors such as length of viable hyphae, spread of hyphae in soil and uptake kinetics of the hyphae (Jakobsen et al. 1994). Each of these components may be affected by environmental conditions including soil temperature. Warm soil temperatures favour AM fungi development. For example, germination of *Glomus coralloidea* and *Glomus heterogama* was optimum at 34°C, whereas 20°C was best for *Glomus mosseae* (Schenck et al. 1975). Maximum arbuscular development of AM fungi in soybean roots occurred near 30°C and mycelial development on the root surface was greatest between 28°C and 34°C (Schenck and Schröder 1974). Percentages of AM root colonization often increase with temperature up to about 30°C and even above (Bowen 1987).

Previous studies were mainly conducted at soil temperatures above 20°C, i.e. at temperatures much higher than those found in temperate climates, in spring (Bowen 1991). The effects of cool soil temperatures on AM fungi have received much less attention. Freezing inocula of two *Glomus* species for one, two and three 14-h periods with 10-h thawing periods between freezes, and a continuous freeze before inoculation did not affect the development of *Glomus intraradices* in roots (Nemec 1987). Below 16°C, both AM fungal development and length of root available for colonization were reduced (Smith and Bowen 1979). Baon et al. (1994) found that barley failed to become colonized by *Glomus etunicatum* when root temperatures were held at 10°C, although it became colonized at 15°C. Allen et al. (1989) found high (40–60%) colonization of *Agropyron* at 12°C, using both field (*Glomus* spp.) and pot-cultured (*Gigaspora margarita*) inocula. A temperature of 15°C could inhibit AM root colonization in greenhouse-grown soybean (Zhang et al. 1995), and reduce sporulation and hypha production by *Glomus intraradices* grown on transformed carrot-root cultures in vitro (Wang 1999). Exposure to 10°C further reduced but did not stop the growth of *G. intraradices*. In spite of the negative influence of low temperature generally observed on AM fungal activity, mycorrhiza formation under a winter soil temperature near 5°C was reported in bulbous Liliaceae species blooming in spring before forest canopy closure (Daft et al. 1980).

We know that cool soil temperatures may slow down or inhibit AM development. We also know that AM hy-

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phae are capable of surviving and retaining infectivity during periods when the vegetation with which they developed is either dormant or actually dead (Jasper et al. 1993; Addy et al. 1997). But we do not know if existing AM mycelia are functional in early spring when the shoots of perennial plants are exposed to sun and drying conditions before the soil has time to warm up. Soil water is abundant after snowmelt but cool soil temperatures restrict plant water uptake and, in turn, photosynthesis rates (Bergh and Linder 1999; Wang and Zwiazek 1999). Cool soil temperatures also reduce nutrient diffusion rate in soil. The AM mycelia, if active at low soil temperature, may hasten growth recovery in perennial or biannual host plants in spring.

Leek is a mycorrhiza-dependent plant. It is also a cold-tolerant biannual plant that can overwinter under the snow and emerge intact the following spring to bloom and produce seeds. Therefore, leek is a plant whose mycorrhiza could be active early in spring and hence, under low soil temperature.

Radioactive tracer (^{32}P) and compartmentalized-system experiments have demonstrated that AM extraradical hyphae can take up P from soil and transport it to roots where it is used by the host plant (Jakobsen et al. 1992a, 1992b; Pearson and Jakobsen 1993). In this study, we tested the ability of a pre-established AM mycelium to function at low temperature, using ^{32}P uptake by leek as an indicator.

Materials and methods

AM and non-AM plants were produced in a greenhouse maintained at temperatures of 23–26°C. A sandy loam soil [pH 4.2; Mehlich-3 (Mehlich 1984) extractable P, K, Ca and Mg, 39, 117, 964 and 61 mg kg^{-1}, respectively] was oven-sterilized at 80°C for 4 h to eliminate indigenous AM fungi. The commercial inoculum, Mycorise (Entreprises Premier, Rivière-du-Loup, Quebec) which contains *G. intraradices* initially isolated from Pont-Rouge (Quebec), was mixed into the sterilized soil of designated pots at a rate of 30 ml 0.4-l pot^{-1}. A portion of the inoculum was autoclaved for 30 min at 123°C, and was thoroughly mixed with sterilized soil at the same rate to form the control treatment. Four leek (*Allium porrum* L.) seeds were surface-sterilized twice with 0.5% NaOCl (2 min), rinsed thoroughly with sterilized de-ionized water, sown in a hole made at the centre of each pot and thinned to two seedlings per pot after emergence. Therefore, each experimental unit included two plants. The root temperature was set at 23°C using a thermostatically controlled water-bath circulation system. The system was as follows: 2-l plastic containers were glued and sealed to the bottom of water baths (68×42 cm) with silicon sealant (GE Silicones Canada, Pickering, Ontario) to form water-free chambers around which temperature-controlled water circulated. A hole, drilled in the base of each container and through the bottom of the water bath, allowed for excess water to drain from the chambers. Pots were randomly placed in the water-bath chambers. The soil was maintained at field capacity by watering as needed with deionized water during the growth period. Pots were not fertilized.

Ten weeks after sowing and inoculation, eight inoculated and eight non-inoculated pots were randomly selected and harvested for biomass and colonization determination in order to verify plant status before the rest of the pots were transferred from the greenhouse to the radioisotope laboratory for ^{32}P injection. Plant roots and shoots were weighed fresh. Shoots were dried and total shoot dry mass was measured. Roots were washed and total root length was estimated using the computerized image analysis system, WinRhizo (Regent Instruments, Quebec). Roots were cut into 1-cm pieces. A subsample of each root system was taken for the determination of AM colonization and the rest of the roots was used for the determination of dry matter content and calculation of total root dry mass. Percentage of AM root colonization was determined under a dissecting microscope using the grid-line intersect method (Giovannetti and Mosse 1980) after roots were cleared in 10% (w/w) KOH and stained with 0.01% acid fuchsin (Kormanick and McGraw 1982) overnight at room temperature.

In the radioisotope laboratory, pots containing AM and non-AM pots were assigned randomly to one of three root-zone temperatures: 0°C, 15°C or 23°C. Designated pots were wrapped in plastic bags and maintained at 0°C in an ice bath in which water was circulated. Water-bath circulation systems at 15°C and 23°C were thermostatically controlled. Pots were long and thin (19 cm long×4 cm upper-end diameter and 2.5 cm lower-end diameter) minimizing the effect of the lamps heating the soil surface on soil temperature. There were eight pots per treatment. All shoots were exposed to 23°C air temperature. Plants were first acclimated for 5 days in their new environment. In the labelling units, plants were exposed daily for 14 h to light at 400 mol m^{-2} s^{-1} from Cool White Sylvania fluorescent tubes. Before ^{32}P application, pots were watered to field capacity, and no watering was performed after labelling. Five millilitres of a ^{32}P-labelled H_3PO_4 (ICN Biochemicals, Costa Mesa, Calif.) solution containing an activity of 715 KBq was applied by injection into each pot, 3 cm away from plant crowns and 7 cm beneath the soil surface. Measurements of ^{32}P activity were made at 1 and 2 weeks after ^{32}P injection. Preparation involved: sampling and weighing young leaf tips (14- to 17-mg samples), mixing leaflet samples with a 1.0 ml aliquot of a 1:2 (v:v) solution of 33% H_2O_2 and 66% $HClO_4$, digesting the samples for 2 h in an oven at 62°C, and adding 12 ml distilled water to each vial. Readings of ^{32}P were taken for 1 min per sample with a liquid scintillation spectrometer, model LKB 1219 Rackbeta. Before statistical analysis, readings were corrected for background radiation and ^{32}P decay.

The general linear model procedure of the SAS statistical software package (SAS Institute 1992) was used for the statistical analysis. Protected least significant difference tests were used to determine the significance of differences between treatment means at the 5% probability level.

Results and discussion

After 10 weeks of growth in the greenhouse at 23°C, i.e. just prior to ^{32}P application, plants inoculated with *G. intraradices* had 85% of their root length colonized. No mycorrhizal colonization was detected in non-AM plants. Plant root length (AM, 1.27 m; non-AM, 1.32 m), and shoot (AM, 0.13 g; non-AM, 0.11 g) and root (AM, 0.015 g; non-AM, 0.017 g) dry mass of inoculated and non-inoculated controls did not differ significantly (P<0.05).

Low root-zone temperature decreased (P<0.05) ^{32}P activity both in AM and non-AM plants. PO_4^{3-} are sparsely soluble and move mainly by diffusion in soil (Tisdale et al. 1993). Temperature is an important variable affecting diffusion rate and, hence, P movement in soil (Brady and Weil 1999). P uptake by plants is metabolism dependent and plant metabolism is temperature sensitive (Grant and Robertson 1997). Therefore, the reduction in ^{32}P uptake observed could be attributable to either a slower ^{32}P diffusion rate or to a slower ^{32}P uptake and loading in phloem at low root-zone temperatures, or to both.

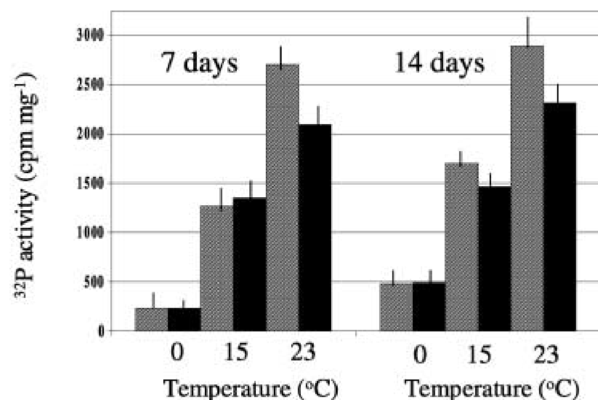

Fig. 1 ^{32}P activity in leek leaves at three temperatures 7 and 14 days after injection, with SEMs. *Grey bars* Arbuscular mycorrhizal (AM), *black bars* non-AM

The length of path for diffusion is another important factor influencing P movement in soil. AM hyphae grow profusely in soil well beyond the zone exploited by roots. They explore extensively a large soil volume, take up PO_4^{3-} ions from the bulk of soil, and transport them to the host roots reducing in this way the effective path for diffusion of the ions. Consequently, mycorrhizae would seem important for P uptake when low soil temperature restricts the diffusion of PO_4^{3-} ions in soil.

A significant (P<0.05) mycorrhiza by temperature interaction at both sampling times indicated that, contrary to expectations, the relative contribution of *G. intraradices* to ^{32}P uptake was smaller at low temperatures. Seven days after injection of ^{32}P into the soil, ^{32}P activity in AM plants whose roots were held at 23°C was significantly higher than that of non-AM plants held at the same temperature (Fig. 1). Fourteen days after injection, mycorrhizal fungi increased the ^{32}P activity of leek leaves at both 23°C and 15°C. No difference between mycorrhizal treatments was found at 0°C, regardless of the date of sampling. Leaf ^{32}P activity at 0°C was always higher than the background level, indicating that P was being taken up even at the lowest root-zone temperature. These observations suggest that leek roots had some activity at 0°C, whereas the contribution of *G. intraradices* to uptake ceased to be significant. Perhaps cold affects the AM fungus more severely than the root.

It is unlikely that AM mycelia freeze at 0°C. In other biological systems, in plants for example, several electrolytes reduce the cytoplasm freezing point. Fungal hyphae can also become cold-hardened (Mazur 1968; Park 1968). Amijee and Stribley (1987) suggested that trehalose compounds inside AM fungi could also lower the freezing point of their cytoplasm. The mucilaginous material coating many soil microorganisms (Park 1968; Stotzky 1972) could also prevent ice crystal formation in the vicinity of vulnerable AM fungal tissues. Addy et al. (1994) described the cytoplasm of AM mycelia extracted from frozen soil as being included in beads separated from each other by voids, within hyphal tubes, presumably as a result of drying. Drying is a strategy used by plants to avoid frost damage during winter, which could also be used by fungi. Discontinuity of the cytoplasm within hyphal tubes would prevent the transport of P through cytoplasmic streaming as well as through diffusion or bulk flow. For safety reasons, we did not verify the state of the AM cytoplasm in soil maintained at 0°C.

Leek is a biannual plant. As such, its mycorrhiza is expected to be active in spring under low soil temperature conditions. The strain of *G. intraradices*, initially isolated from cold-temperate climate, Quebec, Canada, should have potential for cold tolerance. Nevertheless, we did not observe in our artificial system mycorrhizal activity at 0°C in leek plants. However, we cannot conclude that this is also the case in nature. We cannot rule out that gradual cold hardening in fall or overwintering alter mycorrhizal metabolism and can condition AM mycelia to be functional before soil warming in spring. This hypothesis needs to be tested in the field.

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