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Can mycorrhizal inoculation stimulate the growth and flowering of peat-grown ornamental plants under standard or reduced watering?

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ABSTRACT

Although the growth of plants is often successfully stimulated by inoculation with arbuscular mycorrhizal fungi (AMF), the question remains whether AMF are beneficial under the specific conditions of peat-based pot cultivation of ornamental plants. A series of two greenhouse experiments aimed on this question. In the first experiment, we tested the effect of inoculation with AMF, applied as a commercial inoculum, on various biometric parameters including the flowering of eight ornamental plant species. *Capsicum annuum*, *Dimorphoteca sinuata*, *Gazania splendens*, *Impatiens hawkerii*, *Pelargonium peltatum*, *Pelargonium zonale*, *Sanvitalia procumbens* and *Verbena × hybrida* were planted in pots with a peat-based substrate. AMF were naturally absent in this substrate. The plant species differed in their mycorrhizal growth response (MGR) evaluated as the effect of inoculation on shoot biomass. The MGR was positively correlated with the level of root colonization, which ranged from 17% to 68% depending on the plant species. Inoculation with AMF also significantly increased other growth parameters important for ornamental plants, namely the number of flowers (*S. procumbens*, *Verbena × hybrida*), flower size (*I. hawkerii*), shoot dry weight (*P. peltatum*, *P. zonale* and *S. procumbens*), root dry weight (*G. splendens*, *P. peltatum* and *S. procumbens*), the number of leaves (*C. annuum*, *G. splendens*, *P. peltatum* and *P. zonale*), plant length (*C. annuum*, *P. zonale* and *S. procumbens*), the number of branches (*P. zonale* and *S. procumbens*) and the total length of branches (*S. procumbens*). In the second experiment, *P. zonale* was used as a model plant grown under two watering regimes: standard and low. A strong positive effect of AMF on plants was observed under both watering regimes for all measured parameters (shoot fresh and dry weight, plant length, leaf area, number of branches and flowers). In all the parameters, inoculated low-watered plants performed significantly better than well-watered plants without AMF. We conclude that (1) inoculation with AMF improved the general vitality and visual quality of ornamental plants, and that (2) for *P. zonale* this stimulation occurred even under the low watering regime.

1. Introduction

Arbuscular mycorrhizal fungi (AMF) establish symbiosis with most terrestrial plant species. Mycorrhiza is mutualistic symbiosis with a wide range of positive effects on host plants. Mycorrhizal plants are more efficient in the uptake of nutrients (Smith and Read, 2008), reproduce more successfully (Koide, 2010), exhibit improved post-transplant survival and growth (Vosátka, 1995), and are considered more resistant to certain pathogens (Dugassa et al., 1996). Inoculation with AMF is often regarded as a reasonable approach to improving plant growth and is being promoted for a wide range of applications (Feldmann et al., 2008; Gianinazzi et al., 2010), including cultivation of ornamental plants (Koltai, 2010; Vosátka and Albrechtová, 2008).

AMF have been observed to increase shoot dry weight (Vosátka et al., 1999; Šrámek et al., 2000), the length or number of branches (Meir et al., 2010) or the number and size of flowers in ornamental plants (AboulNasr, 1996; Perner et al., 2007; Long et al., 2010). They have also been found to accelerate flowering (Gaur et al., 2000; Garmendia and Mangas, 2012). Since ornamental pot-grown plants are occasionally exposed to water deficiency, the reported positive effect of AMF on the water regime of plants (von Reichenbach and Schonbeck, 1995; Asrar et al., 2012) might be another benefit of mycorrhizal symbiosis. However, no definite conclusion regarding this matter has been reached because the exact mechanisms of how AMF affect the efficiency of water use by

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plants are still unexplained (Auge, 2001). Mycorrhizal symbiosis is indeed a very complex relationship, and plants can even respond negatively to AMF inoculation (Johnson et al., 1997; Klironomos, 2003). Neutral or even negative effects of AMF have been reported for some ornamental plants (Koide et al., 1999; Linderman and Davis, 2004; Gaur and Adholeya, 2005). The inconsistent effects of AMF inoculation may be caused by the diversity of host plants and fungal symbionts or by variable cultivation conditions (Fester and Sawers, 2011). Plants can differ in their response to AMF even on the level of cultivars (Linderman and Davis, 2004) or genotypes (Sensoy et al., 2007). Similarly, different AMF species (Klironomos, 2003) or even isolates of the same AMF species (Munkvold et al., 2004) differ in their effects on plants. Obviously, the complex characteristics of the soil environment and of cultivation substrates also contribute to the varied effects of mycorrhizal symbiosis.

Ornamental plants are usually planted in peat-based substrates. Although some ecological concerns about the use of peat have been brought up in recent years, alternative substrates are not becoming prevalent, so peat will arguably remain the key component of substrates for years to come. Peat-based substrates are, however, well-known for the absence of beneficial soil microorganisms including AMF (Linderman and Davis, 2003a; Perner et al., 2007; Koltai, 2010). Even subsequent introduction of AMF into peat-based substrates can be difficult due to possible negative effects of some types of peat on various aspects of AM symbiosis such as germination and early mycelial growth (Ma et al., 2006), colonization establishment (Calvet et al., 1992), the development of intraradical colonization (Linderman and Davis, 2003b; Ma et al., 2007) or the effectiveness of the symbiosis itself (Vestberg et al., 2005).

Inoculation with AMF is nevertheless being seriously considered as a practice for amending substrates. Moreover, a specialized peat substrate for ornamental plants containing AMF has recently appeared on the market. Similar products from other manufacturers can be expected before long. We aimed to find out whether AMF inoculation is an effective means of stimulating the growth of ornamental plants grown in peat-based substrates. In the first greenhouse experiment, we cultivated eight ornamental plant species in a standard commercial peat-based substrate and inoculated them with a commercially available inoculum of AMF, keeping non-inoculated plants as controls. We aimed to answer the question whether mycorrhizal inoculation stimulates various aspects of plant growth perceived together as the visual quality of ornamental plants. In the second experiment, we grew *Pelargonium zonale* (arguably one of the most popular ornamental plant species in the Czech Republic) under two watering regimes. Our goal was to find out whether the effect of mycorrhizal symbiosis changes under limited moisture conditions.

2. Material and methods

2.1. Screening experiment

Round plastic 700 ml pots were filled with a commercial peat substrate (horticultural substrate–type B; manufacturer: Rašelina a.s., Soběslav, Czech Republic). This substrate contained highly decomposed and disintegrated dark peat mixed with a lower quantity of fibrous white peat. The substrate had the following properties: pH 5.3 (according to European Union norm EN 13037), electric conductivity 0.63 mS cm^{-1} (EN 13038), N_{NH_4} 144 mg kg^{-1}, N_{NO_3} 856 mg kg^{-1}, P 45 mg kg^{-1}, K 621 mg kg^{-1}, Mg 498 mg kg^{-1} (all CAT-extractable, EN 13651), Ca 975 mg kg^{-1} (water-extractable, EN 13652). We selected this substrate for the study because it is a universal substrate for horticulture with a good price/quality ratio that is usable in a wide range of applications. Prior to the experiment, the peat-based substrate was tested for the presence of AMF propagules with negative results (the roots of *Zea mays* – a universal host plant used in this bioassay – remained uncolonized after six weeks of cultivation in the tested substrate).

In our experiment, we included eight of common species of ornamental plants: *Capsicum annuum*, *Dimorphoteca sinuata*, *Gazania splendens* (var. Daybreak Red Stripe), *Impatiens hawkerii* (var. Divine), *Pelargonium peltatum* (var. Tornado), *P. zonale* (var. Gizela), *Sanvitalia procumbens* (var. Sprite) and *Verbena* × *hybrida*. The plants germinated from seeds purchased from a local retailer (manufacturer: SEMO a.s., Smržice, Czech Republic) in plastic multipots with 15 ml cells filled with a heat-sterilized (autoclaved twice at 121 °C for 30 min, 24 h apart) sand–zeolite mixture 1:1 (v/v). Young plants were transplanted into experimental pots (one plant per pot) when they had two cotyledons and one true leaf.

The mycorrhizal inoculation comprised two treatments: (1) AM—treatment inoculated with a commercial AMF inoculum and (2) NM—non-mycorrhizal control treatment. The inoculum Symbivit® (manufacturer: Symbiom s.r.o., Lanškroun, Czech Republic) consists of a mixture of zeolite and expanded clay that acts as a carrier of propagules (spores, mycelium and colonized root fragments) of six different *Glomus* species. We used a universal commercial AMF inoculum because it better corresponds to its potential commercial application as far as its quantity and quality is concerned. For the control treatment, a custom-made carrier of the same properties but without AMF propagules was provided by the manufacturer of the inoculum. Eight millilitre of the inoculum or the AMF-free carrier, respectively, per pot were poured into holes below the transplanted seedlings. The experiment comprised 10 replicates per treatment per plant species, AM and NM pots were randomized in blocks of the same species.

The experiment was established in a greenhouse at the beginning of September, and lasted for three months. The ambient light was supplemented with 400 W metal halide bulbs switched on for photoperiods of 14 h. The light intensity measured at the top of the pots reached 6000 lx (measured in the middle of the experiment, i.e. at the end of October; Testo 435-2 with probe 0635 0545, Testo AG, Germany) when no ambient light was present (i.e. at the beginning and end of the set photoperiod). Combined with daylight, the light intensity ranged from 12,000 to 20,000 lx. The temperature was maintained between 27 °C (day) and 15 °C (night) by an automatic ventilation and heating (in later autumn) system installed in the greenhouse. The experiment was watered as necessary to ensure that the plants would not be exposed to drought stress. Manufacturers commonly amend commercial peat-based substrates with significant amounts of fertilizer to cover the nutrient demands of plants for several weeks. Because the study was intended to simulate real-life conditions in which plants receive little maintenance, no additional fertilizer was applied throughout the experiment.

The experiment was harvested at the flowering stage of each species (with the exception of *P. peltatum*, whose flowering was delayed and expected in several weeks). Rigorous quantification of the ornamental plants' visual quality, a highly subjective matter, is quite a challenging task because it is perceived as a combination of multiple plant traits. To accomplish this task as best as possible, we opted to measure the following biometric or flowering-related parameters (optimized on a per-species basis to reflect the morphology of each given plant species) at harvest: number of leaves (*C. annuum*, *D. sinuata*, *G. splendens*, *I. hawkerii*, *P. peltatum* and *P. zonale*), number of branches (*C. annuum*, *D. sinuata*, *I. hawkerii*, *P. peltatum*, *P. zonale*, *S. procumbens* and *Verbena* × *hybrida*),