



## Colonization of green roof plants by mycorrhizal and root endophytic fungi



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### ABSTRACT

Green roof plants must survive hot and dry conditions in low nutrient artificial growing media. Although soil microorganisms such as arbuscular mycorrhizal fungi (AMF) can ameliorate these constraints by increasing water and mineral uptake, virtually nothing is known about the microbes associated with the roots of green roof plants. We determined levels of AMF and dark septate endophyte (DSE) colonization of plants grown for four years on an experimental green roof in Halifax, Nova Scotia. Green roof plant species included the forb *Solidago bicolor*, the grasses *Danthonia spicata* and *Poa compressa* and the succulent *Sedum acre*. We also assessed root colonization of these same species, as well as three additional succulents (*Sedum spurium*, *Rhodiola rosea* and *Hylotelephium telephium*), collected from their natural habitats. We further assessed the inoculum potential of a commercial green roof substrate before and after the introduction of host plants. Levels of AMF colonization were similar within plant species, regardless of collecting location (roof or field). All plant species were colonized except for the succulent *S. acre*, which is commonly utilized as a green roof plant. The commercial growing medium contained extremely low levels of viable AMF propagules, but this increased significantly after planting with *Solidago*. Conversely, all species (from roof, field and bioassay) were well colonized by DSE, which appear to differ from the AMF with respect to their pattern of dispersal onto the green roof. Although the widespread use of non-mycorrhizal succulent species such as *S. acre* precludes the ecosystem services provided by the AMF symbiosis, the benefits of both succulent tissue and AMF could be obtained simultaneously with careful green roof plant selection.

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### 1. Introduction

Green roofs are an important component of the integrated strategies towards developing environmentally resilient cities (Oberndorfer et al., 2007). They can ameliorate the negative consequences of urban development by intercepting precipitation and returning a portion back to the atmosphere through evapotranspiration (Onmura et al., 2001), moderating interior building temperatures (Del Barrio, 1998), extending the lifespan of roofing materials (Feng et al., 2010), sequestering carbon (Getter et al., 2009), re-introducing biodiversity (Brenneisen, 2005) and reducing the severity of the urban heat island effect (Getter and Rowe, 2006; Alexandri and Jones, 2008).

The ability of green roofs to perform these functions largely depends on the composition of the growing media, the vegetative layer and their interaction. Critical factors regarding the vegetative layer include the anatomical and physiological characteristics of individual plants, such as root and leaf structure, transpiration rates, stomatal sensitivity and biomass production (Dunnett et al., 2008; Dvorak and Volder, 2010; MacIvor et al., 2011). However, in natural systems, these factors are themselves influenced by complex relationships between the plants and their root-associated microorganisms (Gobat et al., 2004), including the arbuscular mycorrhizal fungi (AMF); symbiotic root-inhabiting fungi that form mutualistic symbioses with more than two thirds of all terrestrial plant families (Wang and Qiu, 2006). As obligate biotrophic organisms, AMF are dependent on compatible plant hosts, from which they receive carbohydrates in exchange for improved access to limiting soil nutrients (Smith and Read, 2010). AMF colonization also improves drought tolerance (Duan

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et al., 1996; Augé, 2001; Allen, 2007), resistance to soil pathogens (Newsham et al., 1995; Sikes et al., 2009), tolerance to heavy metals (Hildebrandt et al., 2007) and increased soil aggregate stability (Rillig and Mummey, 2006).

These effects are facilitated by the formation of a variety of inter- and intracellular fungal structures which interface with the host root for nutrient exchange (arbuscules, hyphal coils), lipid storage (vesicles) and the transport of water and nutrients (intraradical hyphae) (Peterson et al., 2004). Fungal hyphae also emanate from the host root and grow into the surrounding substrate, absorbing and transporting soil nutrients and water directly to the root cortex of the host plant (Allen, 2007). These extraradical hyphae have diameters as small as 2  $\mu\text{m}$  (Friese and Allen, 1991), allowing for access to the smallest of soil micropores. They are also able to access soil water present as water films coating soil aggregates (Allen, 2009). The AMF symbiosis therefore represents an enormous increase in the absorptive surface area available to the plant host, given that a single gram of soil may contain up to 100 m of AMF hyphae (Miller et al., 1995). Plants in water-stressed soils are particularly dependant on AMF, given that as soil dries, water retreats into successively smaller and smaller pores, becoming unavailable to the relatively large-diameter plant roots.

AMF hyphae are also well adapted for accessing and transporting soil nutrients, such as phosphorous (the movement of which is not dictated by mass flow) (Barber, 1962; Allen, 2009) and are able to extend beyond the zones of nutrient depletion which quickly develop around plant roots (Smith et al., 2011). As such, AMF associations are essential for many plant species in nutrient poor soils.

Further, rapid transfer of photosynthates from the host plant into the soil environment by AMF can result in as much as 15% of the soil organic carbon pool being derived from AMF (Johnson et al., 2002; Staddon et al., 2003; Leake et al., 2004; Johnson, 2008). Not only do these organic materials assist in the stabilization of soil aggregates (Rillig and Mummey, 2006), they also supply other soil microorganisms with carbohydrates (Andrade et al., 1998; Artursson et al., 2006) and may therefore improve the quality of the growing medium itself, providing benefits for both mycorrhizal and non-mycorrhizal green roof plants.

Plant roots are also commonly colonized by the “dark septate endophytes” (DSE) a ubiquitous group of fungi that form melanized, septate hyphae, colonizing root tissue without causing any obvious host response (Kernaghan and Patriquin, 2011). There is some evidence that DSE may enhance host tolerance to drought as well as other extreme environmental stresses (Barrow, 2003; Waller et al., 2005; Rodriguez and Redman, 2008), although their ecological function remains largely elusive. It also appears that AMF and DSE fungi may compete for host resources when colonizing the same root system, although competition may be reduced through spatial and temporal separation (Perez-Naranjo, 2009).

Root symbiotic fungi are undoubtedly an important component of green roof ecosystems, but very little information is available concerning the colonization status of plants grown in these environments. Although a recent survey of fungi in urban green roof substrates found significant levels of potentially mycorrhizal and DSE fungi (McGuire et al., 2013), to the best of our knowledge no one has explicitly quantified the mycorrhizal or root endophytic colonization of green roof plants.

The objective of the present research is therefore to determine the level to which the roots of either commonly used or proposed green roof plants engage in the AMF symbioses, both in an experimental green roof system and in their native habitats. We also set out to investigate the AMF inoculum potential of commercially available green roof growing media, both before and after the continuous growth of various species of green roof plants.

## 2. Materials and methods

### 2.1. Collection of green roof samples

At the end of August 2010, *Sedum acre* L. (succulent), *Solidago bicolor* L. (forb), *Poa compressa* L. (C3 grass) and *Danthonia spicata* (L.) Beauv. (C3 grass) were sampled from an ongoing native plant green roof experiment at Saint Mary's University in Halifax, Nova Scotia (Lundholm et al., 2010). This represented a selection of proposed or commonly used green roof succulents, forbs and grasses. Plants had originally been collected from Nova Scotian field populations and transplanted into peat-vermiculite. In some cases, the original plants were maintained in peat-vermiculite, in other cases, new plants were propagated from seeds or cuttings from field collected material. Although plants were grown in a soil-less mixture, the potted plants were kept outdoors on the ground for up to one year before planting into modular self-contained units containing 6 cm of growing medium (Sopraflor X, Soprema Inc., Drummondville, QC, Canada) on the roof. Each module contained a single plant species (monoculture). Plants received no supplementary water or nutrients for four years (between establishment on the roof and collection for the current study).

Roots from 10 individuals of each species were collected; two individuals from each of five modules per species. Roots were washed thoroughly with water and stored in a mixture of 50% ethanol, 5% acetic acid and 45% water (Brundrett et al., 1994).

### 2.2. Collection of field samples

In order to determine if the patterns of mycorrhizal colonization on the green roof differed from those of the same plant species in the field, we collected roots from wild populations of the four green roof species (*S. acre*, *S. bicolor*, *P. compressa* and *D. spicata*). We also collected three other succulent species in the field (*Sedum spurium* M. Bieb., *Rhodiola rosea* L. and *Hylotelephium telephium* L. H. Ohba.) in order to obtain further information on the mycorrhizal status of other local members of the Crassulaceae.

Field collections were made at a variety of locations near Halifax, Nova Scotia in October 2011 and again in June 2012. Roots of each species were collected from two separate locations, except for *H. telephium*, for which only one naturally occurring population could be found. Five individuals were collected per site per sampling time, for a total of 20 individuals per species. Specimens of *S. acre*, *S. spurium* and *R. rosea* were often collected from rocky habitats with very little growing substrate. Roots were stored as above.

### 2.3. Mycorrhizal bioassay

In order to determine if the growing media was a source of mycorrhizal inoculum responsible for the green roof plants, a mycorrhizal bioassay using leek “bait plants” was conducted to test the inoculum potential of a commercially available growing medium, Soprema Sopraflor X<sup>TM</sup>. This material is composed of expanded shale, sand, vegetable compost, perlite and blond peat. Four variations of this material were tested: (1) ‘fresh medium’, received directly from the manufacturer, (2) growing medium that had supported *S. bicolor* for four years, (3) medium that had supported *S. acre* for four years and (4) growing medium that had been left un-vegetated for four years. Additionally, controls were created by autoclaving each of the four treatments at 121 °C for three separate 1 h cycles.

Leek (*Allium porrum* L.) seeds were surface sterilized for 45 min in 0.5% sodium hypochlorite and rinsed in distilled water prior to a 48 h germination on sterile filter paper. Germlings were then planted into sterilized, freely draining trays, each containing three

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