

ASSESSMENT OF ARBUSCULAR MYCORRHIZAL FUNGI IN A GREEN ROOF
SYSTEM

by

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Submitted in partial fulfilment of the requirements
for the degree of Master of Environmental Studies

at

Dalhousie University
Halifax, Nova Scotia
August 2013

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ABSTRACT

The selection of plant species for use on green roofs has been based primarily on their ability to cope with the harsh conditions of the urban rooftop environment. Green roof plants must also survive in artificial growing media with low organic content and a lack of soil microorganisms. This includes the arbuscular mycorrhizal fungi (AMF), which improve host nutrient and water uptake, water-holding capacity and aggregate stability. A literature survey was first conducted in order to determine which aspects of green roof functioning might be enhanced through the integration of AMF. Levels of AMF colonization were then determined for seven plant species, either commonly used or proposed for green roof use. Plants were sampled from the experimental green roof at Saint Mary's University, Halifax, and from naturally occurring communities in the Nova Scotia coastal barrens. A commercially produced growing medium was also tested for AMF inoculum potential both before and after the introduction of green roof plants, in a mycorrhizal bioassay experiment. For green roof root samples, the abundance of fungal structures involved in nutrient exchange (arbuscules and hyphal coils), storage (vesicles and microsclerotia) and transport (hyphae and melanized hyphae) were assessed individually. Colonization was poor in both field and rooftop samples of the commonly utilized green roof succulent, *Sedum acre*, but much higher in other more recently proposed species, such as *Solidago bicolor*. The commercial growing medium was found to contain extremely low levels of viable AMF propagules, but this increased significantly after cultivation of an AMF-host plant in the medium. Although the apparent lack of mycorrhizal dependency of *S. acre* helps to explain its popularity as a green roof plant, its use precludes the important ecosystem services potentially provided by AMF symbioses.

LIST OF ABBREVIATIONS AND SYMBOLS USED

AM	Arbuscular mycorrhiza
C	Celsius
Cd	Cadmium
cm	Centimeter
Cu	Copper
E	Einsteins
ECM	Ectomycorrhiza
Fe	Iron
H	Hydrogen
h	Hour
K	Potassium
m	Meter
O	Oxygen
P	Phosphorous
Pb	Lead
sec	Seconds
Spp.	Species
μ	Micrometer
Zn	Zinc

ACKNOWLEDGEMENTS

Documenting all the individuals whose help or encouragement I have received during the course of this thesis would be the task for another, less publishable, thesis. Instead, and in no particular arrangement (other than the importance of our relationship), I will try to list those individuals whose actions have most significantly impacted the creation of this document.

I would like to begin by thanking Dr. Jeremy Lundholm for providing the impetus for my research; without his interest in the microbiology of green roofs, my project ideas would have been left adrift and unattended. Further, through his gainful employment, I was able to continue to pay rent, feed myself, and learn a great deal about the green roof industry. I must also thank my committee member, Dr. Leanne Philip, for helping to refine the otherwise broad and unanswerable research questions I initially wished to pursue, and for providing me with many of the technical skills I would need to complete this work.

However, it is to my supervisor, Dr. Gavin Kernaghan, that I likely owe the largest debt. His willingness to take me into his lab with virtually no references, sources of funding, or personal necessity on his part, demonstrates his great wealth of generosity (and maybe a little foolhardiness). Regardless, his support has been essential for both the completion of this thesis and my personal development as a researcher.

Finally, there are all those friends whose continuing support was essential for both the completion of my thesis, and the maintenance of what little remains of my sanity. To my lab partners, Mike and Tyler, without your almost-constant distractions I may have actually finished my thesis on-time, thereby missing-out on a wealth of offensive internet content and abusive volumes of alcohol. To the Roommates I love, by treating me like the reclusive, socially-awkward uncle you all already have, I've come to see the world as a cold and scary place I want no part of. To my love Michele, the completion of this thesis wouldn't be an occasion worth celebrating if you hadn't persevered through my social ineptitude and asked me to dinner some time ago. And finally, to Carl, for everything.

CHAPTER ONE

INTRODUCTION

1.1 Preface

Urbanization gives rise to large areas of impervious surfaces, as construction materials such as asphalt and concrete are deployed in place of respiring soils and vegetation. This substitution has not been without consequences for both the local environment and for urban residents in that reduced health of urban streams (Arnold Jr et al., 1996), wider temperature fluctuations and extremes (Yuan et al., 2007) and greater airborne particulate matter (Lam et al., 2005) are all hallmarks of urbanization. By preventing precipitation from percolating through the soil profile, impervious surfaces simultaneously reduce groundwater recharge and increase the volume of urban stormwater runoff (L. Wang et al., 2001). Stormwater is considered an unwanted consequence of urban development, and is quickly removed by drainage systems and transported to receiving water bodies. However, any pollutants intercepted by stormwater as it traverses the drainage systems may be deposited directly into receiving waters. Further, in municipalities where stormwater drains are combined with grey-water drains (combined drainage system), there is the potential for drainage systems to overflow during particularly intense precipitation events (Walsh et al., 2009). Both factors can lead to significant decreases in the health of aquatic ecosystems, erosion, and damage to municipal infrastructure and water-treatment facilities (Meyer et al., 2005). The cost of maintaining sewer systems, and of purifying stormwater runoff, represent substantial economic investments for the municipalities responsible for their upkeep, providing both economic and environmental

incentives for reducing the volume of stormwater entering urban drainage systems (Rahman et al., 2005).

Additionally, urban construction materials are capable of absorbing substantial amounts of solar radiation (Kato et al., 2005). While vegetation is able to dissipate heat generated by solar radiation through various mechanisms such as evapotranspiration and albedo, urban construction materials (asphalt, concrete, roofing) absorb heat readily and re-radiate it via conduction (Kim, 1992). This can lead to increased temperatures within urban centers, as compared to surrounding vegetated areas, as heat accumulated during the day is re-radiated during the evening. Increased urban temperatures require extensive energy expenditures in order to moderate indoor environments.

While urbanization has necessitated large expenditures of energy and labour to mitigate various unwanted consequences such as stormwater and elevated temperatures, removal of respiring soils and vegetation has also had significant negative impacts on urban biodiversity (Brenneisen, 2005; Moore et al., 2005). Although the previously described problems may be addressed with innovations in infrastructure, the only method for improving urban biodiversity is through increasing available habitat for flora and fauna. Sustainable urban development must therefore include plans for reducing the volumes of energy required to maintain city centers, as well as remedial actions to ensure habitat creation within urban areas.

One technology that promises to address many of the issues surrounding sustainable urban development are green roof systems (Getter et al., 2006). Green roofs, which are essentially engineered terrestrial ecosystems, utilize the comparatively unexploited space

of urban rooftops to reintroduce soil (technically engineered growing media) and vegetation to the urban sphere. Proponents of this technology have cited a variety of potential benefits (termed ‘ecosystem services’) at both the scale of individual buildings and at the city level. These include moderation of indoor temperatures (cooling in the summer, warming in the winter), stormwater capture, habitat provisioning, amelioration of the urban heat island effect, extended lifespan of roofing materials, carbon sequestration and improved aesthetics (Oberndorfer et al., 2007; Rowe, 2011).

While improving the ability of green roofs to provide these ecosystem services has been the focus of recent research, maximizing the scope of green roof application has also received attention (Friedrich, 2005; Getter et al., 2006). Improving green roof application (ensuring construction atop as many buildings as possible) requires that they be designed within the context of the load-bearing capacity of pre-existing structures. While new buildings may be designed to sustain heavy loads atop their roofs, particularly in areas that receive regular snowfall, older buildings often lack extensive rooftop load-bearing capacities (Castleton et al., 2010). Green roofs are therefore divided into two gross categories: extensive and intensive. While both varieties tend to use lightweight growing media composed of coarse porous mineral aggregates which provide low bulk-density and rapid drainage, extensive green roofs are composed almost entirely of these materials ($\geq 70\%$) at shallow depths ($2 \geq 20$ cm), while intensive roofs use greater amounts of organic material and coarse-sand aggregates and enhanced media depth (≥ 20 cm) (Oberndorfer et al., 2007; Ampim et al., 2010). These weight-limiting modifications allow extensive green roofs to be deployed atop a greater proportion of buildings, and as

such are viewed as having the greater application potential of the two varieties, leading to their involvement in the bulk of green roof research.

While extensive green roofs enjoy heightened applicability, the lightweight design of the growing media has created harsh conditions for associated vegetation. Given that extensive green roof growing media are less than 20 cm in depth, composed largely of inorganic materials and utilize large pore sizes (Friedrich, 2005), water content can fluctuate drastically between precipitation events (Jacks, 1954). Under these conditions, growing media can quickly shift between saturation and the permanent wilting point, with both circumstances preventing normal root functioning (Campbell et al., 1991). Accordingly, green roof species selection seems to be most influenced by factors of plant drought tolerance, ease of propagation, and the ability of plants to rapidly produce ground cover, with the majority of green roofs utilizing easily-propagated, shallow rooted, drought tolerant succulents and semi-succulents from the family Crassulaceae (although some drought-tolerant grasses are also utilized) (Monterusso et al., 2005; Rowe et al., 2006; Snodgrass et al., 2006; Durhman et al., 2007; Oberndorfer et al., 2007; Getter et al., 2008). While highly durable within the drought stressed, shallow and low organic growing media typical of green roofs, members of the Crassulaceae and their relatives are highly water-conservative (Schuber et al., 1981; Gravatt et al., 1992; Borland, 1996; Fioretto et al., 1999; Sayed, 2001), with some species demonstrating transpiration rates an order of magnitude lower than similarly sized non-succulent herbaceous species (Körner et al., 1979; Gravatt et al., 1992; Compton et al., 2006). However, although they exhibit drought tolerant and avoidant adaptations, the low transpiration rates of succulent plant species may reduce their ability to effectively perform services related to

evaporative cooling and stormwater mitigation in comparison to non-succulents (Compton et al., 2006; Lundholm et al., 2010). Further, the use of species from a single plant family could preclude the ability of green roofs to substantially improve urban habitat provisioning, given that this function requires a diverse array of plant species.

In response to a perceived over-reliance on the Crassulaceae, research into the feasibility of using locally adapted plants in green roof design has recently been initiated (Monterusso et al., 2005; Lundholm et al., 2010; Butler et al., 2012). While the term often goes undefined in green roof research, locally adapted plants are typically recognized as being inhabitants of the local biome within which an individual green roof operates (Butler et al., 2012). A related strategy has been the use of ‘habitat templates’, i.e. identifying natural habitats which resemble the green roof environment in hopes that the targeted plant community will be ‘pre-adapted’ to green roof conditions (Lundholm, 2006). Template research has included attempts to mimic maritime coastal barrens and alpine slopes and arid grasslands, but this approach has been met with limited success (Monterusso et al., 2005; Williams et al., 2010; MacIvor et al., 2011). The theorized benefits of employing locally adapted species within green roofs include improved durability, adaptation to local climate, reduction of invasive species and relevance for local fauna (Oberndorfer et al., 2007). However, experiments with locally adapted non-succulents has often encountered difficulties, with locally adapted plants experiencing decreased survival rates when grown under green roof conditions compared to their wild counterparts and to green roof members of the Crassulaceae (Monterusso et al., 2005; Martin, 2007; Dvorak et al., 2010; Thuring et al., 2010; Butler et al., 2012). The most readily apparent limiting factors in these cases have been water availability

(Nagase et al., 2006; Wolf et al., 2008) and limited available rooting volume, exposing roots to extreme temperatures and drought (Tyler, 1996; Durhman et al., 2007). However, the disparity in survival rates between members of the Crassulaceae and locally adapted plants may potentially be the result of multiple design factors other than the water-holding capacity of the growing media.

While harsh conditions resulting from media design are limiting the variety of plants that can be utilized within extensive green roofs, the lack of attention given to soil microbiology in the green roof literature may be hampering the development of more diverse species assemblages. In fact, soil microbiology in general, and arbuscular mycorrhizal (AM) fungi specifically, have been largely absent from green roof design.

This is a significant oversight considering the importance of AM fungi for proper plant function under natural conditions (Smith et al., 2010). AM fungi are critical for the survival of many plants, with approximately two thirds of all terrestrial plants, representing some 200,000 species, capable of hosting them (B. Wang et al., 2006; Smith et al., 2010). Many of these species are deemed to be obligate hosts and are heavily dependent upon AM fungi to enhance their survival under natural conditions, while many others depend on them to endure critical periods of stress such as drought. For obligate plant species, AM fungi are essential for: acquisition of soil nutrients (Tu et al., 2006; Smith et al., 2011) and water (Augé, 2001; Allen, 2007); protection from soil pathogens (Newsham et al., 1995; Sikes et al., 2009); and resistance to heavy metal toxicity (Hildebrandt et al., 2007). While AM fungi are ubiquitous in most natural soils, the synthetic nature of the growing media production process may preclude the occurrence of AM fungi in green roofs, while their location high above ground level may prevent their

entry into green roofs via natural dispersal mechanisms. Moreover, the potential absence of AM fungi within green roofs may prevent the establishment and viability of plant species that depend on them, as they experience decreased stress tolerance when prevented from symbiotically engaging with AM fungi.

An absence of AM fungi from green roof systems may not only filter out AM-dependent plant species, but may also favour species with minimal dependence, potentially resulting in an over-representation of non-mycorrhizal species within green roof plant assemblages. Interestingly, most species of the Crassulaceae are included within the 20% of plant species currently deemed to be non-mycorrhizal (B. Wang et al., 2006). It may be the case, therefore, that by ignoring AM fungi, green roof designers are reducing the potential plant selection to the minority of plants that do not engage in the AM fungal symbiosis, such as species from the Crassulaceae, and excluding the vast majority of plants which require AM fungi for their continued survival. This scenario may represent a substantial limitation to the ability of green roofs to act as caches of urban biodiversity and to maximize the provisioning of ecosystem services.

1.2 Objectives

Given the integral status of AM fungi within natural ecosystems, their incorporation into green roof systems may be necessary in order to maximize their ability to provide ecosystem services and enhance the diversity of their plant assemblages. This thesis was conducted within this context and aims to ascertain the status of AM fungi within an extensive green roof system. The precise purposes of the project were to:

1) Determine to what extent the roots of plant species, of common or proposed use within green roofs, were colonized by AM fungi both in the field and within a green roof system.

2) Determine the AM fungal colonization potential of a commercially produced green roof growing medium.

Furthermore, a thorough review and synthesis of literature pertaining to both green roofs and AM fungi was conducted in order to determine the potential benefits associated with the integration of AM fungi into green roof design. Given that the integration of AM fungi into green roof design will certainly require capital expenditures by producers, it seems necessary to justify this through conceptualizing the material benefits that AM fungi are likely to provide, beyond the more academic arguments surrounding biodiversity.

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CHAPTER TWO

POTENTIAL BENEFITS OF MYCORRHIZAL COLONIZATION OF GREEN ROOF PLANTS

2.1 Introduction

The utilization of green roof technology is becoming an increasingly popular method for addressing the challenges involved in sustainable urban development (Getter et al., 2006). Proponents assert that the multi-functionality of green roofs results in great improvements over conventional roofs, as well as other environmentally-minded roofing technologies, such as high-albedo white roofs or gravel-ballast roofs, which are capable of addressing only single issues (Getter et al., 2006; Oberndorfer et al., 2007; Gaffin et al., 2010). While these technologies do augment internal heat accumulation and stormwater capture, green roofs also improve the moderation of indoor temperatures, the amelioration of the urban heat island effect, urban biodiversity and carbon sequestration (Oberndorfer et al., 2007). Green roofs are able to provide these services due to their mimicry of natural ecosystem processes, such as evapotranspiration and plant biomass accumulation (Dunnett et al., 2008).

The most well-researched of these ecosystem services are the moderation of indoor temperatures and stormwater capture (Getter et al., 2006; Schmidt, 2006). By capturing precipitation and evacuating it from the roof via evapotranspiration, green roofs are able to cool the rooms below them as well as reduce the amount of water that runs off the building and into urban catchments. These ecosystem services can result in substantial reductions in energy consumption. Jaffal et al. (2012) found a 32% decrease in annual energy consumption resulting from the cooling action of a green roof. Green roofs have

also been observed to reduce stormwater runoff up to 100%, compared to conventional roofs (Mentens et al., 2006). However, attempts to maximize the provisioning of these ecosystem services have been at odds with the realities of green roof implementation.

Green roof design has been guided not only by attempts to maximize per capita ecosystem service provisioning, but also maximization of total provisioning through designs that increase the range of buildings upon which green roofs can be constructed. Difficulties associated with this endeavour stem from the pre-existing conditions of urban infrastructure, namely the weight-bearing capacity of roof-tops (Panayiotis et al., 2003). Roofs may be designed with substantial weight-bearing capacity in areas which receive regular snowfall, but building codes in most jurisdictions do not require roof-designs to accommodate the significant weight additions inherent to green roof construction (Kosareo et al., 2007). As such, green roofs are categorized at the most gross level, based on the depth/weight of growing media utilized (Getter et al., 2006). This differentiation is the basis of much green roof research; while a change in media depth will increase the overall weight of the green roof, it also impacts the hydrology and therefore the variety of vegetation deemed suitable (Monterusso et al., 2005).

Based on growing media depth, green roofs have been divided into intensive and extensive varieties. Intensive green roofs typically have greater than 20 cm of growing media, while extensive green roofs utilize between 2 and 20 cm (Oberndorfer et al., 2007). Although the greater depth of growing media associated with intensive green roofs allows for enhanced diversity of plant species, it also necessitates more maintenance, irrigation and structural integrity enhancement of the underlying roof, restricting the number of buildings upon which they can be constructed. Conversely, extensive green

roofs are designed to be low maintenance, and avoid the need for added rooftop structural supports, although the use of thin growing media causes rapid fluctuations in moisture content and extreme temperatures within the rooting zone, resulting in extremely harsh conditions for plant growth (Nagase et al., 2006). As such, extensive green roof design has become heavily reliant upon species of succulents and semi-succulents from the family Crassulaceae, as well as drought-tolerant grass species (Dunnett et al., 2005).

2.2 Green Roof Cross-Section

Although there is a variety of extensive green roof designs, they are generally composed of five distinct layers: vegetation, growing media, filter membrane, drainage membrane, and a waterproofing/root barrier membrane (Oberndorfer et al., 2007). While the filter, drainage and waterproofing/root barrier membranes prevent moisture and plant biomass from damaging the associated building, it is the growing media and vegetation layers that are responsible for providing the bulk of the ecosystem services. As such, these two layers have been designed to achieve the goals of extensive green roof implementation.

2.2.1 Growing Media

In order to minimize their associated weight loads, green roof growing media have been designed to reduce bulk density and facilitate rapid drainage (Beattie et al., 2004; Friedrich, 2005). This has been achieved largely through the utilization of coarse-diameter lightweight mineral aggregates within growing media, which is often almost entirely composed of such materials, instead of organic materials, which typically comprise less than 20% of media volume (Beattie et al., 2004; Friedrich, 2005). While there is a breadth of materials classified under the term *coarse-diameter lightweight*

mineral aggregate, some of the most common include construction waste-materials, such as crushed clay bricks, roofing tiles and cement, natural aggregates like scoria, pumice, sand and artificial or modified materials such as heat-expanded shale, clay and slate (ESCS) (Ampim et al., 2010). ESCS is of common use given its minimal weight (560-1120 kg m⁻³) (Friedrich, 2005), resistance to decomposition, and in the case of heat-expanded shale, high porosity that affords water holding capacity; between 9 and 35% water absorption per unit weight (expanded shale can absorb 380 kg tonne⁻¹) (Sloan et al., 2008). These materials are graded to various particle sizes and mixed in order to produce a growing medium containing a range of pore sizes to provide at least 10% air-filled pore space and 35% space for plant-available water; this composition is deemed amenable to plant growth (Ampim et al., 2010). However, pore-size distribution in green roof media is designed to favour macropores in order to increase the proportion of gravitationally-held water. While this facilitates rapid drainage, it reduces media capillary-space and plant-available water (Gobat et al., 2004).

2.2.2 Vegetation

The vegetation used in extensive green roofs has been selected largely in reference to growing media design (White et al., 2003; Snodgrass et al., 2006). The abundance of macropores and coarse aggregates, as well as the shallow depth, results in periodic conditions of drastically reduced water supply (Williams et al., 2010). The rooftop environment contains stresses apart from water limitation, such as high winds, complete insolation and temperature extremes within the rooting zone (Oberndorfer et al., 2007). Species that have proven tolerant to these conditions have been drawn almost entirely from the family Crassulaceae, although some drought-tolerant grasses are used as well.

The Crassulaceae have evolved a variety of xerophytic adaptations that make them suitable for use in green roofs: succulent leaves and woody stem tissues, shallow fibrous root-mats or tubers, facultative crassulacean acid metabolism (CAM) or CAM cycling, and deeply set small stomata (Clausen, 1975; Denton, 1982; Gurevitch et al., 1986; 't Hart, 1997; 't Hart et al., 2003; Byalt, 2011). Further, they are capable of clonal propagation and forming mats of growth, both important characteristics for achieving rapid coverage of the green roof (Gurevitch et al., 1986; Gravatt et al., 1992; Getter et al., 2008). Of additional importance is the well-established commercial cultivation of many Crassulaceae species, which ensures that plant materials are readily available for green roof construction (Alm, 2004; Abiven et al., 2009; Byalt, 2011).

2.3 Ecosystem Services

While a variety of ecosystem services is provided by green roofs, such as habitat provisioning, carbon sequestration, improved durability of roofing membranes, and enhanced aesthetics, the two most commonly discussed as being the impetus for green roof construction are evaporative cooling and stormwater mitigation (Ayata, Tabares-Velasco, & Srebric, 2011; Barrio, 1998; Bengtsson, Grahn, & Olsson, 2005; Compton & Whitlow, 2006; Czemieli Berndtsson, 2010; DeNardo, Jarrett, Manbeck, Beattie, & Berghage, 2005; Oberndorfer et al., 2007). This is likely in part due to the tangible nature of these benefits, their ability to be measured in economic terms, and their significance within urban planning. As such, these two services have been described here in some detail.

2.3.1 Evapotranspirative Cooling

The roof of a building is typically the portion which receives the greatest amount of solar radiation; in Mediterranean climates, the surface of a conventional bituminous roof can reach temperatures upwards of 90°C (Jaffal et al., 2012). Solar radiation reduces the durability of roofing membranes through direct exposure to ultraviolet radiation and diurnal fluctuations in temperature that result in their expansion and contraction. Heat is conducted through the roofing membrane from areas of high temperature to low temperature, the direction of conduction being dependent on temperature differences between indoor and outdoor spaces. In seasonal climates, this typically results in the radiation of heat into buildings during summer months and heat flow out of buildings during winter. Heat flow into buildings can result in significant indoor temperature increases requiring energy-intensive climate control systems to maintain indoor temperatures at comfortable norms. In many regions, this requires significant energy expenditures and expensive infrastructure investments; lack of necessary indoor climate controls can represent a severe health hazard (Wargocki et al., 2002). To address these issues, green roofing technologies have been developed, in part, to passively cool indoor spaces without requiring large energy expenditures, thereby reducing the carbon footprint of urban areas.

Green roofs are able to reduce air temperatures at the roof surface as well as the thermal loads received by underlying rooms (Barrio, 1998; Getter & Rowe, 2006; Jaffal et al., 2012; Kumar & Kaushik, 2005; Lin & Lin, 2011; Liu & Baskaran, 2003; Parizotto & Lamberts, 2011; Teemusk & Mander, 2010; Theodosiou, 2003; Wong, Chen, Ong, & Sia, 2003). This is a function of the green roof's insulative capacity, imparted by increased

shading and albedo provided by the vegetation, with as much as 27% of solar radiation being reflected by the plants (Eumorfopoulou & Aravantinos, 1998). Another potentially significant aspect is the ability of a green roof to perform passive cooling through evapotranspiration (Lundholm, MacIvor, MacDougall, & Ranalli, 2010; Onmura, Matsumoto, & Hokoi, 2001; Wong et al., 2003), the removal of water from the green roof growing media through both evaporation and transpiration from the green roof vegetation. Through evapotranspiration, latent heat is absorbed and dispersed, leading to a reduction in rapid temperature escalation of the roof surface and inside associated buildings. Evapotranspiration is also responsible for the reversal of heat flow, cooling the interior by passively transferring heat to the building's exterior (Ayata et al., 2011; Niachou, Papakonstantinou, Santamouris, Tsangrassoulis, & Mihalakakou, 2001; Theodosiou, 2003). Implementation of a green roof also protects roofing materials from direct exposure to ultraviolet radiation and daily temperature variations that damage roofing membranes and reduce their longevity.

In previous studies, the thermal advantages provided by green roofs compared to conventional roofs have been significant. A Greek case study by Jaffal et al. (2012) observed that a $13.9 \text{ kWh m}^{-2} \text{ year}^{-1}$ decrease in building cooling requirements was achieved through the implementation of a *Sedum spp.* based green roof, resulting in an annual energy consumption reduction of 32%. In their study, it was determined that increasing the leaf area index (LAI) of green roof vegetation helped to reduce cooling loads during the summer, when installed on both insulated and uninsulated buildings. In India, Kumar and Kaushik (2005) found a 7.2°C reduction in indoor air temperatures under a green roof, as compared to beneath a conventional roof. The study determined

that increasing LAI reduced both the maximum daily air temperature and the diurnal air temperature variation within the canopy of green roof vegetation, preventing 4 W/m² of solar radiation from entering the growing media of the green roof. In Brazil, Parizotto and Lamberts (2011) determined that a green roof was able to reduce surface temperature in ceramic-tiled roof from 57.5°C to 39.4°C, while diurnal temperature variation was also reduced from 37.2°C to 17.7°C. In the temperate climate of Ottawa, Canada, Liu (2003) found maximum temperature reductions of the roof surface of up to 45°C compared with a conventional reference roof. In their study, the reference (conventional) roof reached temperatures above 30°C on more than half of the study days while the green roof reached 30°C on only 3% of days. Also, the green roof experienced median diurnal temperature fluctuations of ±6°C while the conventional roof had a median diurnal fluctuation of ±45°C. In that study, the green roof resulted in a 75% reduction in energy demand for temperature moderation within rooms underneath the green roof. A study of green roofs in Toronto, Canada, demonstrated both a reduction and delay in peak surface temperatures; a conventional roof reached 66°C by 2:00 pm while the green roof reached only 38°C by 6:30 pm. The same study found that heat flow through the green roof was decreased by 70-90% in comparison with the conventional reference roof over the duration of the study (Liu & Minor, 2005). The study determined that the most significant factor in providing cooling benefits was the water-holding capacity and water availability within green roof substrates, as opposed to the absolute volume of the substrate utilized.

Green roofs in tropical Singapore showed a 30°C reduction in maximum daily temperature and a 27°C reduction in diurnal temperature variation (57°C vs. 26.5°C and 30°C vs. 3°C, respectively) (Wong et al., 2003). Emphasizing the importance of the

vegetative layer within their green roof system, Wong et al. (2003) observed greater heat flow through bare-soil roofs (i.e. growing media without vegetation), where surface temperatures reached 42°C and experienced a 20°C diurnal fluctuation, compared to a green roof where surface temperatures reached 26.5°C and experienced a 3°C fluctuation. The greatest cooling benefits were derived from green roofs utilizing plants with high LAI, increasing surface area for transpiration and shading (Wong et al., 2003). Furthermore, measuring diurnal differences in cooling potential, Wong et al. (2003) found that while bare roofs continued to radiate stored heat into underlying rooms during evening hours, the green roof maintained a cooler air column and continued passive cooling. In Taiwan, Lin and Lin (2011) observed significant benefits from the vegetation layer of their green roofs. When comparing a range of substrate and vegetation types, they found that the best plant combination reduced roof surface temperature by 25.7°C, compared to a bare-soil roof. The study also observed indoor temperature reductions of 7.3°C when comparing bare-soil roofs to green roofs; according to the researchers, this reduction translated into a 22-24% reduction in annual energy costs.

Theodosiou (2003) also found that of the measured components relating to cooling potential, foliage density was the most important, as it influenced both shading and amount of potential evapotranspiration. Additionally, a higher canopy layer was able to trap cool air and create a thermal mass at the roof surface. This, in addition to the insulating properties of the green roof, further reduces temperature fluctuations and delays the onset of peak daytime temperatures by several hours. Onmura et al. (2001) determined that heat gain by rooms underneath a green roof were reduced by half due to increased evapotranspiration as influenced by the moisture content and moisture retention

of green roof growing media. The importance of the vegetative layer is further highlighted in a study by Feng, Meng, and Zhang (2010), who found that 58.4% of heat removed from the system was done through evapotranspiration and a further 9.5% through the net photosynthesis of vegetation.

Besides their ability to cool buildings through transpiration and shading, the vegetative layer of a green roof is able to prevent solar radiation from reaching the roof's surface through the reflective properties of their foliage (i.e. albedo) (Alexandri & Jones, 2008). Solar radiation is reflected away from the roof in amounts dependent on both the albedo value and total canopy cover of the vegetation. A conventional bituminous roof is very poor at reflecting solar radiation due to its very low albedo. Plants with broad, flat leaves typically have higher albedo values than plants with high volume to surface-area ratios, as do plants with lighter coloured foliage (Lundholm et al., 2010). Accordingly, the effectiveness of a green roof in reflecting solar radiation is dependent upon leaf morphology and biomass levels. Recorded albedo values for green roofs utilizing a variety of different plant species have ranged, on average, from 0.18 to 0.23 (measured as reflected radiation/incoming radiation) (Lazzarin, Castellotti, & Busato, 2005; Lundholm et al., 2010) as compared to a conventional bituminous roof which display albedo values of approximately 0.066.

It is apparent that a majority of studies have found potential evapotranspiration to be among the chief mechanisms behind the cooling benefits of green roofs (Lazzarin et al., 2005; Onmura et al., 2001; Ouldboukhitine, Belarbi, Jaffal, & Trabelsi, 2011; Wanphen & Nagano, 2009). Additionally, some studies have found that green roofs utilizing mixtures of plant species can display significant improvements in evaporative potential

(Lundholm et al., 2010; MacIvor, Ranalli, & Lundholm, 2011). It would appear, therefore, that greater emphasis should be applied to the selection of plant species that are capable of greater rates of transpiration while still being able to tolerate the harsh conditions presented by the green roof environment.

2.3.2 Stormwater Mitigation

Urbanization has resulted in the replacement of large areas of natural and agricultural lands with impervious surfaces such as asphalt and concrete. This modification prevents normal functioning of the hydrologic cycle; stormwater which would have otherwise percolated into the soils and been transpired by vegetation or recharge groundwater reserves instead becomes runoff (Paul & Meyer, 2008). This increase in runoff has necessitated the construction of expansive drainage systems designed to prevent flooding by capturing and transporting stormwater away from urban areas. In natural systems, stormwater would take one of a variety of pathways within the hydrologic cycle, either returning to the atmosphere via evapotranspiration, moving vertically into soils or becoming runoff and moving horizontally through soils (Meyer, Paul, & Taulbee, 2005). In a heavily vegetated natural ecosystem, the amount of stormwater that becomes runoff is roughly 10%; anywhere from 50% to 80% is returned to the atmosphere through evapotranspiration (Meyer et al., 2005). However, in highly developed urban areas, where the amount of impervious surface area may be 50-100% of the total area, the amount of stormwater that becomes runoff may increase to 55%, while the amount that is removed via evapotranspiration declines to below 30% (Paul & Meyer, 2008). This is of concern as overland flow of stormwater runoff transports debris, sediments, nutrients and contaminants downstream, polluting local bodies of water and damaging infrastructure

(Badin et al., 2011; Barraud, Gibert, Winiarski, & Bertrand, 2002; Hogan & Walbridge, 2007). Adequate management of stormwater is necessary to maintain the ecological integrity of local bodies of water and to reduce the erosion of urban infrastructure (Hatt, Fletcher, Walsh, & Taylor, 2004). Treating stormwater after it has acquired potentially damaging solutes is difficult and expensive and has prompted the development of technologies able to intercept stormwater before becoming problematic (i.e. intercepting stormwater as close to the source as possible).

The use of infiltration technologies (bioswales, rain gardens, holding ponds etc.) in attempts to minimize the impacts of stormwater runoff is limited due to the general paucity of urban construction space. Alternatively, green roofs have shown potential utility in the mitigation of stormwater runoff while taking advantage of the ample available construction space represented by rooftops, which in dense urban areas often account for 40-50% of total impervious surface area (Dunnett & Kingsbury, 2004). Several studies have established the ability of green roofs to significantly reduce the amount of stormwater runoff in addition to delaying peak flows after rainfall events (Bengtsson et al., 2005; Berghage et al., 2009; Carter & Rasmussen, 2006; Czemieli Berndtsson, 2010; DeNardo et al., 2005; Getter, Rowe, & Andresen, 2007; Gregoire & Clausen, 2011; Hutchinson, Abrams, Retzlaff, & Liptan, 2004; Larcher, 1996; Liu, 2003; Mentens et al., 2006).

Green roofs mitigate stormwater through temporary detention of precipitation, portions of which are evaporated, transpired, incorporated into plant tissue or released as runoff. Some precipitation is intercepted and stored on the canopy surface of green roof vegetation where it may evaporate or flow downwards to the base of the plant and

growing media. Typically, the larger portion of precipitation permeates into growing media where it evaporates from the media surface, is transpired by vegetation or is incorporated into plant tissue. Green roofs modify stormwater dynamics in various ways compared to conventional roofs; volume and peak flow-rates of runoff are reduced and the runoff period is extended so that smaller volumes of runoff leave the roof for up to three hours after a precipitation event has ended (Czemiel Berndtsson, 2010; DeNardo et al., 2005; Gregoire & Clausen, 2011; Liu, 2003; Mentens et al., 2006; Moran, Hunt, & Jennings, 2004; VanWoert, Rowe, Andresen, Rugh, Fernandez, et al., 2005).

The ability of a green roof to provide these services is dependent upon a number of design factors, such as species-specific vegetative characteristics, growing media composition and depth as well as the slope of the roof (Getter & Rowe, 2006; Getter et al., 2007; Lundholm et al., 2010; Oberndorfer et al., 2007). Additionally, stormwater mitigation potential is dependent upon factors independent of design, chiefly the nature of the weather period preceding a precipitation event (Stovin, 2010). Therefore, the stormwater mitigation potential of a specific green roof will be dependent on factors unique to the engineering and the climatic variables within which it operates (Bengtsson et al., 2005; Czemiel Berndtsson, 2010; Villarreal & Bengtsson, 2005).

During a precipitation event, initiation of runoff is delayed and maximum flow rates will not be triggered until the green roof has surpassed its maximum retention capacity (Mentens et al., 2006; Spolek, 2008; Teemusk & Mander, 2007). The maximum retention capacity of a green roof is deemed to be the difference between total volume of a precipitation event and the final volume of runoff; the amount retained is the sum of retention volumes for the growing media, vegetation and any moisture-retention layers

present within the green roof design (DeNardo et al., 2005). The bulk of this capacity, however, is typically accounted for by the growing media (VanWoert, Rowe, Andresen, Rugh, & Xiao, 2005). A green roof utilizing a growing media with a high field capacity and plants with high transpiration will be able to mitigate greater volumes of stormwater.

Soil field capacity is the volume of water remaining in a freely draining soil after all surplus water has been removed by gravity (Hillel, 1998). Given that a green roof is required to support a vegetation layer, the minimum soil moisture limit is deemed to be the permanent wilting point, the point at which vegetation can no longer access soil water. While the permanent wilting point is typically described as 15% of soil volume or -1.5 MPa, it is technically a relative value dependent upon specific plant traits governed by their ability to access soil water (Gardner, 1960). Plant species differ in their wilting points due to factors including root morphology, symbiotic relationships and potential transpiration rates. Regardless, the wilting point is the lowest allowable volume of water required to maintain the vegetation layer and represents a firm lower limit to green roof functioning (Bengtsson et al., 2005). The maximum retention capacity of a green roof is therefore considered to be the difference between the field capacity and the permanent wilting point. While high soil field capacities would be preferable for plant growth and maximum water retention, growing media design is constrained by the weight restrictions necessary for roof-top construction; a saturated growing media can weigh between 70 and 170 kg/m² (Oberndorfer et al., 2007). Additionally, weight restrictions prevent increasing maximum retention through simple increases in media volume. Moreover, this does not seem to provide substantial increases in retention capacity after a given volume of medium (Jarrett, Hunt, & Berghage, 2006). Accordingly, the most popular growing

media are those, which optimize retention capacity and weight through a combination of large pore spaces, large particle size and low organic content.

While a review of green roofs in Germany (Mentens et al., 2006) described increased stormwater mitigation as a function of increasing media depth, a model green roof simulation by Jarrett et al. (2006) found that the maximum retention provided by growing media experiences a plateau. Roofs with a maximum retention capacity of 40 mm were able to retain 50% of annual precipitation while roofs with only 6 mm of maximum retention capacity retained 40%; increasing storage capacity beyond 40 mm produced only marginal benefits (Jarrett et al., 2006). However, this result may only be pertinent in certain climates; in areas where precipitation events typically do not exceed a certain maximum, shallow growing media may be sufficient to deal with the majority of events while the extra storage capacity of deeper green roofs would remain underutilized. Furthermore, the increased surface-area to volume ratios within green roofs utilizing shallower growing media may result in a medium that can dry more thoroughly between precipitation events, allowing for a greater restoration rate of retention capacity.

Following a precipitation event, the amount of water that can be retained by a green roof is lowered as the growing medium has been brought closer to field capacity; runoff will be triggered earlier during a subsequent precipitation event if the growing medium is unable to return to its maximum retention capacity. Consequently, the rate at which a green roof is able to restore its retention capacity (how quickly water is removed from the system) will help determine the roof's effectiveness in mitigating stormwater issues during periodic precipitation events. Given that the only desirable avenues for moisture removal from a green roof are tied to either evaporation from vegetative surfaces and the

growing medium or transpiration by the vegetation layer, it would seem beneficial to optimize these aspects of green roof design. While much attention has been paid to aspects of growing media (Beattie & Berghage, 2004; Berghage et al., 2007; Friedrich, 2005), less research has focused on selection of novel plants and their microbial symbionts with the goal of increasing transpiration rates (Lundholm et al., 2010; MacIvor et al., 2011; Wolf & Lundholm, 2008).

These arguments – the importance of growing media versus vegetation - are not necessarily mutually exclusive, but are instead scale-dependent. At the level of individual rain events, the majority of retained water appears to be accounted for by the growing medium (although this may be related more to medium structure than total medium volume) (Jarrett et al., 2006), whereas a significantly smaller fraction is intercepted by the canopy or stored in plant tissue (Dunnett et al., 2008). The utility of the vegetative component becomes apparent when multiple precipitation events occur over a short period of time. The moisture content of the growing medium after a precipitation event will determine retention ability during subsequent events. A vegetation layer that is able to efficiently remove water from growing media, via transpiration, will be more capable of restoring a green roof to its maximum retention capacity between precipitation events. Stovin (2010) discusses the antecedent dry weather period (ADWP) in reference to a UK study of green roofs. It was determined that the ability of the green roof to retain stormwater over multiple precipitation events was most related to preceding weather conditions. When the ADWP was conducive to high rates of evapotranspiration (i.e. several days of no precipitation, low humidity, high solar radiation) the green roof was able to restore the majority of its retention capacity before subsequent precipitation

events. When precipitation events were closely spaced, preventing the restoration of retention capacity, the ability of the green roof to mitigate stormwater was significantly reduced. In their study, a green roof was able to retain 65% of an initial 24.8 mm precipitation event, but was only able to retain 27% of a 24.8 mm event that occurred several days afterwards. It was determined that this was due to weather conditions that were not conducive to high potential rates of evapotranspiration, which limited the ability of the green roof to restore its retention capacity. Berghage et al. (2007) found that planted roofs using *Sedum spurium* lost twice as much water for the first 5 to 6 days after a precipitation event than equivalent non-planted roofs; after which time the two roof types had relatively equal rates of water loss.

Analyzing a 70 m² *Sedum* spp.-based green roof with 75 mm of growing media, Hathaway, Hunt, and Jennings (2008) observed a 64% average reduction in runoff and a 77% reduction in runoff rate over a 12-month study period. Variation in runoff reduction was observed to be greater within the month than between months and was dependent on the duration and potential evaporation rates of dry periods between precipitation events. During the month of July, rainfall events were more frequent and the percent of precipitation retained by the green roof was only 53%. The *total volume* of retained precipitation, however, was higher in July than in any other month of the experiment. A storm event on the 23 of July, preceded by five days of dry weather, resulted in retention of 30 mm precipitation, roughly 40% of total precipitation. A storm event on the 24th of July deposited less than 10 mm of precipitation, and the green roof was able to retain approximately 6 mm before runoff was triggered. Generally, the green roof had better retention capabilities when precipitation events were separated by 3 to 5 days.

In Toronto, Canada, MacMillan (2004) observed the performance of a green roof with 140 mm of growing medium planted with a variety of wild flowers over the spring, summer and autumn months. Over the duration of the study period, the roof reduced runoff volumes by 55% and runoff rates by 46-85%. However, precipitation retention was maximal during summer months (76% runoff reduction). Summer precipitation events were fewer, typically separated by several days of dry weather, and potential evapotranspiration rates were high. In comparison, precipitation events during early spring and autumn months occurred frequently and often on consecutive days while temperatures were considerably lower than during summer months. This left the growing medium damp, cold and unable to be restored to maximum retention capacity, resulting in runoff retention of only 37% (MacMillan, 2004).

During a 13 month study of a 76.3 mm deep green roof in Athens, USA, Carter and Rasmussen (2005) calculated a monthly range of precipitation retention from 39 to 100%, and a 13 month average of 76%. While the green roof was able to retain nearly 100% of all smaller (<25.2 mm) precipitation events, approximately 50% was retained for larger storm events (> 76.2mm). Further, delays in peak flow rates were typically 10 minutes, but in some cases where the preceding dry period was substantial, two-hour delays were observed. Two uncommonly large precipitation events in September, separated by a three-week dry period, deposited 82.3 and 84.3 mm of precipitation of which 54% was retained by the green roof on both occasions. For comparison, considerably smaller precipitation events in November, occurring on consecutive days and depositing 38.4 and 11.9 mm, resulted in only 21% of precipitation retained on both occasions. Such an

outcome would seem to indicate that the distribution of precipitation events might be as predictive of stormwater runoff as the absolute size of events.

Similarly, Moran et al. (2004) observed 62% precipitation retention over a 9-month study period, while per-month retention ranged from 44% to 88%. A diversity of studies have found similar retention rates for a variety of green roofs: a study by Hutchinson et al. (2004) using a 110 mm green roof was able to retain 69% of precipitation over a 15-month period; an 89 mm green roof designed by DeNardo et al. (2005) retained 45% of precipitation over seven observed storm events; while assessing a 30 mm *Sedum* green roof, Bengtsson et al. (2005) found 46% of precipitation was retained; Köhler et al. (2002) found 60% annual runoff reduction from a 50 mm green roof; runoff was reduced 78% and peak flow delayed 18 minutes by a 70.62 mm green roof over a 13-month period (Carter & Rasmussen, 2006); Prowell (2006) observed that for light precipitation events (0-5mm), green roofs were able to reduce runoff by 85%, but this was limited to 37% for heavy events (>23mm). When measuring individual rain events, Bliss, Neufeld, and Ries (2009) found maximum flow-rate reductions of 70%, with peak runoff not being triggered until three hours after the beginning of some precipitation events. Green roofs were able to retain greater percentages of precipitation during low-duration precipitation events (70% retention) compared with high-duration events (20% retention) (Bliss et al., 2009).

Recurring themes within studies analyzing the ability of green roofs to mitigate stormwater indicate that retention is enhanced in climates where precipitation events are preceded by several days of dry weather and when potential evapotranspiration rates are high. Additionally, retention capacity can be increased through the use of growing media

and synthetic components with elevated water-holding capacity, as well as the selection of vegetation with high transpiration rates. While a green roof may be tailored to best suit local climatic conditions, weight restrictions prevent greater reliance on increased volumes of growing media. Consequently, research which aims to improve the ability of green roofs to mitigate stormwater may achieve greater gains were it to be focused on the selection of vegetation.

2.4 Potential Effects of Arbuscular Mycorrhizal Colonization on Green Roof Ecosystem Provisioning

2.4.1 Introduction

Mycorrhizas – meaning fungus root – are symbiotic structures formed between diverse soil fungi and the roots of most plant species. In this mutualistic association the plant trades a portion of its photosynthetically derived carbon for soil-water and mineral nutrients scavenged by the mycobiont. This is accomplished through the formation of fungal nutrient-exchange structures within the host root, as well as outward growth of fungal hyphae into the surrounding soil environment. This extraradical mycelium (mycelium outside the root) acts as a highly expanded and refined root-network with very high surface area, capable of exploring additional volumes. The extraradical hyphae also have the enzymatic potential to mineralize organic nutrient sources, which would otherwise be unavailable to the host plant. The ubiquity of this relationship has led some researchers to claim that “...the majority of plants, strictly speaking, do not have roots—they have mycorrhizas” (Stephen Wilhelm). As many as 95% of all terrestrial plant families are colonized by some form of mycorrhizal fungus (Smith & Read, 2010). While

the symbiosis is ancient – over 460 million years old – it has only been a topic of scientific study since the late 19th century (Koide & Mosse, 2004; Redecker, Kodner, & Graham, 2000). Since its discovery however, the complexity and importance of the symbiosis has been thoroughly demonstrated, if not entirely understood, within almost every biome on the planet. While the most thoroughly studied effects of AM fungal colonization are those occurring within individual host plants, AM fungi (and mycorrhizae in general) can have biome-scale significance (van der Heijden, Boller, Wiemken, & Sanders, 1998). While their importance within natural systems is well-recognized, research in the field of engineered ecosystems has typically not included the influence of mycorrhizal fungi. This oversight has surely had consequences in the selection of plant species for engineered ecosystems such as green roofs, given the high levels of mycorrhizal dependence exhibited by many plant species. The mycorrhizal literature is rife with the consequences of depriving highly mycorrhizal-dependent plants from their root symbionts; decreased fitness being the general observation (Janos, 2007; Smith, Facelli, Pope, & Andrew Smith, 2010; Smith & Read, 2010).

Colonization by AM fungi has myriad well documented influences on the survival and growth of the host: pathogen resistance (Norman, Atkinson, & Hooker, 1996; Sikes, Cottenie, & Klironomos, 2009); improved water relations (Allen, 2007; Augé, 2001); increased nutrient uptake (Gashaw Deressa & Schenk, 2008; Leigh, Hodge, & Fitter, 2009; Pearson & Jakobsen, 1993; Smith, Jakobsen, Grønlund, & Smith, 2011); mediation of interspecific competition (Allen, Allen, & Friese, 1989; Rinaudo, Bàrberi, Giovannetti, & van der Heijden, 2010) and improvement and protection of soil structure

(Bearden & Petersen, 2000; Burri, Gromke, & Graf, 2011; O'Dea, 2007; Rillig, Wright, Nichols, Schmidt, & Torn, 2001). Given that extensive green roofs are intended to behave as a functioning ecosystem, any or all of the various functions of AM fungi would technically be advantageous within a green roof setting. However, the most important benefits of AM fungal colonization within a green roof ecosystem involve improvements to osmotic stress tolerance, evapotranspirative cooling, stormwater capture, leachate quality, and erosion control. Specifically, the improved access to soil water and nutrients (Allen, 1982, 2007; Smith & Read, 2010), increased transpiration rates (Allen, 1982; Cui & Nobel, 2006; Koide & Schreiner, 1992; Marulanda, Azcon, & Ruiz-Lozano, 2003; Newman & Davies, 1988), and maintenance of soil structure (Rillig & Mummey, 2006) are AM-mediated mechanisms which have the most potential to improve the functioning and longevity of green roofs.

2.4.2 Water Relations

Extraradical mycelia can develop to lengths of 125 cm and have a diameter of 2 to 5 μm , compared with root hairs, which typically have diameters between 10 and 15 μm (Marulanda et al., 2003; Allen, 2009; Lehto et al., 2011). This allows AM-colonized host plants to explore greater volumes of soil more effectively, as mycelia have the ability to exploit soil pores which root hairs are too large to access. The importance of this ability becomes apparent when soil approaches the permanent wilting point (soil water potential less than -1.5 MPa) and soil moisture retreats into micro and ultramicropores, rendering it unavailable to most plant root systems (Allen, 2007). During such periods of low soil moisture, AM colonized plants have an additional pool of soil water to rely on.

The ability of AM mycelia to rapidly transport water to the host is related to their cellular structure. Fungal hyphae are composed of linear chains of cells and when an AM fungus colonizes a plant root, its hyphae make contact with, and eventually penetrate, the epidermis of the plant root. This process is not a simple invasion however, as the plant helps mediate fungal entry through inter and intracellular spaces (Bonfante et al., 2000). The fungal hyphae then colonize the root cortical cells, but enervate, rather than penetrate, the plasma membrane, forming a plant-fungus interface termed the periarbuscular membrane. Within the host cell, the fungus then produces vesicles for lipid storage and arbuscules (“little trees”) or hyphal coils for nutrient transfer. From these structures, the interior hyphae (intraradical hyphae) are connected to the exterior hyphae (extraradical hyphae) growing into the soil. From the point of colonization, thick-walled hyphae (~20 μm in diameter) are produced which give rise to dichotomously branching absorptive networks, with each subsequent branch narrowing in diameter until there are eight divisions forming approximately 128 mycelial tips, each roughly 2 μm in diameter (Friese et al., 1991; Bago et al., 1998; Allen et al., 2003). In colonized sections of host roots, colonization points occur in approximately 1 mm intervals, producing between 1 and 100 m of extraradical mycelium within a single gram of soil (Miller, Jastrow, et al., 1995; Smith, Facelli, et al., 2010), depending on colonization density. The dichotomous process of mycelial branching, coupled with narrow-diameter hyphae, ensures that very little pore-space is unexplored within the mycelial growth region. This substantially increases the amount of contact that root systems have with the soil and significantly improves potential for water and nutrient uptake.

Further, while the exterior length of a mature mycelium is shielded by hydrophobic membranes, hyphal tips are hydrophilic and readily absorb soil water (Allen, 2007). Given that AM mycelia are coenocytic (lacking cross-walls except at branching points), the only membranes that water has to pass through when traversing hyphal cytoplasm (symplastic water transport) or intercellular space (apoplastic water transport) are at the hyphal tips and arbuscules. This allows for rapid movement of water from mycelial tips to host root cortical cells (flow is bidirectional and dependent on hydrostatic gradients). This arrangement is in contrast to absorption directly through the host's root network, which accounts for at least 50% of hydraulic resistance within the vascular system (Nelson et al., 1982; Vandeleur et al., 2005; Tataranni et al., 2012). Delivery of water directly to root cortical cells helps decrease host hydraulic resistance (resistance to water flow) by increasing the relative fraction of apoplastic water flow. This is one mechanism by which AM fungi are able to increase the water conductivity of the host root system (Bárzana et al., 2012; Tataranni et al., 2012). The amount and rate of water transport along hyphal gradients has been estimated by several researchers to be ecologically relevant and capable of modifying plant water relations, especially under drought conditions (Allen, 1991; Khalvati et al., 2005; Ruth et al., 2011).

In an early experiment to determine whether AM fungal hyphae make direct and significant contributions to host-plant water uptake, Hardie (1985) compared water uptake of AM and non-AM clover and leek plants. Initially, the AM plants had improved water uptake as compared to non-AM controls. Upon severing of all AM colonization points, water uptake was depressed to the level of non-AM controls. Similarly, in a split-pot experiment by Faber et al. (1991), hyphae of AM-colonized cow pea were allowed to

explore volumes of soil from which host roots were restricted by dividing the pot with a fine mesh that only allowed hyphae to pass through. Severing the ties between the host and AM hyphae resulted in a 35% reduction in water uptake and a cessation of soil drying from the hyphae-only compartment, indicating that hyphae, not evaporation, was accounting for the reduction in soil-water content from this compartment (Faber et al., 1991). Using high-resolution water-content sensors, Ruth et al. (2011) found that the AM contribution to water uptake of colonized barley plants accounted for roughly 20% of the total. Additionally, AM-colonized plants consumed 31% more water per day than non-mycorrhizal controls (Ruth et al., 2011).

Ruiz-Lozano et al. (1995) used a vertically stratified pot-experiment to observe the effects of colonization by two different species of AM (*Glomus deserticola* and *Glomus fasciculatum*) on water-uptake and gas exchange rates of *Lactuca sativa*. In their experiment, pots were divided into three sections, with the roots of the host plants confined to the top section (the fungus-root section), while the fungal mycelia were free to explore the soil volume of the bottom 2 sections. Water was supplied only to the bottom sections of the container in order to observe the ability of both the mycelia and mycelia-root networks to absorb water. The AM fungi were able to influence water-relations under both well-watered and drought conditions, but in a species-specific manner. *G. fasciculatum* improved water-use efficiency under well-watered conditions while *G. deserticola* increased gas exchange rates and stomatal conductance under both drought and well-watered conditions. Under well-watered conditions, transpiration was increased by just over 100% in plants colonized by *G. deserticola*

(Ruiz-Lozano et al., 1995). Under conditions simulating drought, the fresh weight of AM-colonized plants was increased by up to 215% compared with uncolonized plants.

The velocity of soil water translocation through AM hyphae to the host plant is typically between 0.31 and 16.1 cm min⁻¹, the large range being due to mycobiont species, hyphal architecture, phase of plant development and experimental conditions (Faber et al., 1991; Allen, 2009; Ruth et al., 2011). Estimated volumes of flow have varied from 0.1 to 0.76 $\mu\text{l H}_2\text{O hr}^{-1}$ for each hyphal entry point depending on mycobiont/host species and soil moisture conditions (Allen, 1991; Faber et al., 1991). In a growth chamber experiment Marulanda et al. (2003) found that, compared with non-mycorrhizal controls, AM-colonized plants were able to increase the rate of water uptake by as much as 4.75 ml plant⁻¹ day⁻¹. They also found a strong positive correlation between the amount of mycelium and host water uptake; more extraradical mycelium within the soil allowed for greater water uptake. AM-host plants with the greatest water uptake also produced the most mycelium, approximately 50 cm g⁻¹ of soil. This is in contrast to natural soils in which mycelia can average 81 to 111 cm g⁻¹ (Miller, Jastrow, et al., 1995; Marulanda et al., 2003), implying that the amount of water translocated by AM hyphae in naturally occurring soils is much greater than estimates based on artificial systems.

Direct uptake of soil-moisture by AM mycelia has not been the only explanation for the improved water-relations seen in host plant roots. There has also been considerable research comparing the transpiration rates, water throughput rates, osmotic regulation abilities and decreased stomatal resistance of mycorrhizal versus non-mycorrhizal plants (Augé, 2001). It appears that AM-colonized plants experience greater rates than their non-mycorrhizal counterparts. However, these are often difficult to predict and appear to

be dependent on host/mycobiont species and climatic conditions (Augé, 2001; Augé, Moore, et al., 2004). Osmotic adjustment also seems to be enhanced through AM-colonization, allowing host-plants to maintain gradients favourable for water-flow from the soil into roots through modified proline production (Porcel et al., 2004; Valentine et al., 2006; Manoharan et al., 2010) and the increased absorption capabilities of other osmotic solutes important for osmoregulation such as K^+ , Ca^{2+} and Mg^{2+} (Wu et al., 2006). A further modification of host water relations by AM fungi is their modification of stomatal resistance, which is typically lower in AM colonized plants (Dixon et al., 1994; Augé, 2000, 2001; Wu et al., 2006). All of these modifications have the benefit of enhancing plant photosynthetic rates.

An important confounding factor with regards to the effects of AM-colonization on gas exchange rates is the fact that the nutritional status of the host plant is also improved by colonization. Because AM-colonized plants are often larger and have higher nutrient concentrations than non-colonized plants (Smith & Read, 2010), they may also display heightened stomatal conductance and gas exchange rates due to greater water demand (Augé, 2000). Many early studies of AM-host gas exchange relationships have assumed that the improved nutrition provided by AM is the mechanism that is primarily responsible (Koide et al., 2004). However, it has become clear that even when AM-host plants and controls are of equal size and nutrition status, significant differences in gas exchange rates can occur (Augé, 2001). Currently, no agreed-upon mechanism exists for these nutrition-independent modifications of gas-exchange.

Significant increases in transpiration rates have been observed under both laboratory and field conditions as well as under well-watered and drought conditions (Augé, 2001).

Allen (1982) found a 100% increase in transpiration rates of greenhouse propagated *Bouteloua gracilis* plants that had been colonized by the AM species *Glomus fasciculatus*. Although AM colonization can result in species-specific modifications to plant root systems, sometimes resulting in the production of greater proportions of finely branched absorbing roots, no increases in specific root length were observed. Instead, Allen (1982) proposed that the increased soil-fungus contact provided by the fungal mycelium allowed for greater through-flow and transpiration rates in AM-colonized plants.

In contrast, Goicoechea et al. (1997) observed increased transpiration rates in *Medicago sativa* plants under drought conditions, but found no evidence of increased water uptake. Instead, lower levels of abscisic acid (ABA) production were observed in AM-colonized roots under all moisture conditions. ABA is a phytohormone produced during periods of osmotic stress. When soil moisture is low, ABA is produced in the roots and translocated to the leaves, triggering stomatal closure and reducing water loss through transpiration. Although the mechanism is unknown, by reducing ABA production in the host roots, AM fungi were able to elicit higher transpiration rates (as well as CO₂ exchange rates), under all measured levels of soil moisture (Goicoechea et al., 1997).

Green et al. (1998) conducted a study of both *Vigna unguiculata* and *Rosa hybrida* to determine the mycorrhizal influence on plant hormones, transpiration rate and stomatal conductance. In both species, inoculation with *Glomus intraradices* resulted in increased transpiration rates and stomatal conductance under well-watered conditions; a result that had been previously established by Augé et al. (1986). Interestingly, Green et al. (1998) found that some of the mechanisms by which AM fungi influence host transpiration rates

and stomatal conductance may reside in the plant leaves. Leaves from AM host-plants were detached and their transpiration rates and stomatal conductance measured. After detachment, the leaves of *R. hybrida*, but not *V. unguiculata*, still displayed the increased transpiration rates and stomatal conductance (Green et al., 1998). This result would seem to indicate that AM fungi are capable of modifying some fundamental aspects of leaf physiology which result in permanently modified gas exchange rates.

In an experiment analyzing the effect of AM colonization of *Citrus tangerine* during periods of drought stress, Wu et al. (2006) found that the transpiration rates of plants colonized by *Glomus versiforme* were increased by 30% under well-watered (-0.09 MPa) conditions and 27% when plants experienced drought (-0.4 MPa) when compared to non-mycorrhizal controls. These increases were attributed to 18% and 29% reductions in stomatal resistance which were facilitated by increased uptake of K^+ , Ca^{2+} , and Mg^{2+} ; allowing for greater gas exchange and significantly enhanced biomass accumulation under both drought and well-watered conditions.

While observing potential temperature-dependent effects of host-colonization by the AM fungus *Glomus etunicatum* on *Zea mays*, Zhu et al. (2011) also found significant increases in transpiration rate and stomatal conductance. Transpiration rates of AM-colonized plants were increased by 17.08%–22.47% and stomatal conductance by 25.44%–61.03% compared to non-mycorrhizal controls. Augé, Moore, et al. (2004) measured the effects of irradiance and temperature on the gas exchange rates of AM-colonized *Sorghum bicolor* plants and found that the stomatal conductance was substantially increased. Conductance was increased by up to 70% during periods of intermediate temperature and irradiance levels up to $156 \text{ mmol m}^{-2} \text{ sec}^{-1}$.

As noted above, transpiration rate and water throughput can significantly augment the ability of green roofs to mitigate stormwater and cool the underlying structure. By increasing host transpiration rates, providing access to a greater proportion of pore-space and enhancing overall water throughput, introduction of AM fungi into green roofs should enhance the ability of the roof to provide evapotranspirative cooling and stormwater capture; the two ecosystem services which have prompted widespread interest in green roofs (Oberndorfer et al., 2007). By increasing the rate at which water is removed from the growing media, it may be returned more quickly to its maximal water retention capacity, allowing it to capture greater volumes of water during subsequent precipitation events. Increased transpiration may also help to disperse greater amounts of energy stored within media-solution, increasing the efficacy with which evapotranspirative cooling is performed. Transpiration may be an especially relevant factor with respect to these two ecosystem services when the vegetation layer achieves full coverage of the green roof surface. The development of a full canopy may thoroughly shade the growing media, reducing the rate of evaporation (Lundholm et al., 2010). As water leaving the roof through evaporation decreases, increasing the rate of transpiration may help to account for the difference.

While the influence of AM fungi on host water relations may improve green roof functionality, there are also benefits for host drought tolerance (Bárzana et al., 2012). Of the limiting factors preventing the utilization of a wider range of plant species for green roofs, drought stress is among the most significant (Thuring et al., 2010). Drought stress can result in decreased growth rate (or complete cessation of growth), reduced fertility and stomatal closure, leading to loss of transpiration and gas exchange

(Farooq et al., 2009). Moreover, green roof growing media is particularly prone to drought, due to the minimal depth utilized (anywhere between 3 and 15 cm) and the high abundance of macropores, leading to a high surface area to volume ratio and larger proportions of gravitationally-held water. As a result, evaporative processes can quickly reduce soil water content to the point that average evaporation is greater than precipitation, leading to arid conditions within the green roof environment, irrespective of surrounding ground-level climatic conditions. Further, while some of the water holding capacity of green roof media is within aggregates of ESCS, only about 50% is available to plants (Sloan et al., 2002).

To cope with this situation, green roof plant selection has become reliant upon species within the family Crassulaceae (e.g. *Sedum* spp.), many of whose genera are arid-adapted and typified by shallow rooting systems, both drought avoidant and tolerant features, and the species-dependant utilization of CAM or CAM-cycling photosynthesis (Teeri et al., 1981; Lee et al., 1987; Byalt, 2011). However, the low transpiration rates displayed by *Sedum* spp. may lessen the effectiveness of green roofs to provide stormwater capture and evaporative cooling benefits (Compton et al., 2006). Additionally, reliance upon a handful of genera limits potential increases in urban plant biodiversity.

As discussed previously, due to their small diameters (2-20 μm) and high density (1-100 m g^{-1} soil), AM extraradical mycelium have access to a greater proportion of soil pores than the comparatively coarse roots of their hosts (Allen, 2007; Smith, Facelli, et al., 2010). This morphology allows plants colonized by AM fungi to access water-filled pores at lower water potentials, increasing the pool of plant-available water. When soil water potentials drop below the permanent wilting point, estimated at -1.5 MPa for most

mesic plant species, most, if not all, of the pore space available to plant roots will no longer contain water (Larcher, 1996). As such, the ability of AM extraradical mycelium to access pores as small as 2µm becomes highly significant during periods of drought (Allen, 2007, 2009; Lehto et al., 2011). This extra pool of water made available to colonized plants may be sufficient to prevent permanent damage to hosts during periods of drought, making AM fungi especially ecologically relevant during this time (Allen et al., 1986). Furthermore, in structured soils with low bulk density and large proportions of macro-aggregates, such as those often used in green roofs, the entirety of a plant's root system does not make contact with the soil, leaving gaps surrounding roots (Van Noordwijk et al., 1992). By bridging the gaps adjacent to host roots, the extraradical mycelium of AM fungi can maintain an uninterrupted flow of water and nutrients into host cortical cells. This function may be especially important in green roof media which favour macropores and coarse particle sizes, meaning that proportions of the plant root system will certainly experience a lack of contact with the growing media, although this may be mediated if colonized by AM fungi.

In a potentially analogous situation, Allen et al. (1992) found that on the pumice-laden slopes of post-eruption Mount St. Helens, *Lupinus lepidis* experienced significantly improved growth and survival rates when colonized by AM fungi; even though this species is normally unresponsive under mesic conditions. Individual root systems of *L. lepidis* plants were exposed to an extreme water deficit as the pumice stone had minimal water-holding capacity. The water that was present was held within micropores inside the individual fragments of pumice, making it unavailable to roots. When colonized by AM

fungi however, the extraradical hyphae were able to bridge the gaps surrounding roots and translocate water held within micropores of the aggregate interior (Allen et al., 1992).

Similar to volcanic materials, the porous nature of the various lightweight aggregates used in green roof construction, especially heat expanded shale, means that the aggregates themselves contain water within their interior pore space. Aggregates of expanded shale can absorb $380 \text{ kg tonne}^{-1}$ of water, or about 36% of their weight, although only half of this is plant-available water (Sloan et al., 2002; Sloan et al., 2008). Aggregates of heat expanded slate and clay on the other hand seem to have minimal water-holding capacity as their exterior pore space is not connected with interior pores, preventing efficient absorption of water (Castro et al., 2011). The extraradical mycelia of AM fungi may be able increase the proportion of water within shale aggregates that is available to plants due to their small diameter, allowing them to access interior micropores and substantially increase the pool of plant available water within aggregates (Friese et al., 1991; Allen, 2007).

Comparable phenomena have been observed in plant communities occurring within thin soils atop weathered bedrock in seasonal Mediterranean climates experiencing months of low to non-existent precipitation. In these situations, granite and feldspar parent materials can act as important reservoirs of water for nearby plant communities. Hubbert et al. (2001) found that during the dry season in southern California, plants relied almost entirely upon water held within the weathered granite bedrock, although it was unknown how the plants were accessing this water given that their roots were unable to penetrate the micropores in which most of the water was stored. Given that they calculated the rate of passive water flow through the rock matrix at approximately $10^{-3} \text{ cm h}^{-1}$, it seemed

highly unlikely that water was reaching plant roots through capillary actions. In a later study of a related ecosystem, Borneyasz et al. (2005) observed the extraradical mycelium of ectomycorrhizae penetrating the granite matrix to a depth of approximately 4 metres, although the study did not attempt to determine if these mycelia were actively translocating water from the granite to the host plant. In a further study by Egerton-Warburton et al. (2003), the extraradical hyphae of both AM and ECM (ectomycorrhizal) fungi were found growing to depths of up to 200 cm into weathered bedrock. It was estimated that the bedrock held as much water as the overlying soil, which was rapidly depleted of water during the dry season and hypothesized that mycobionts were translocating water stored in the bedrock to the hosts, preventing them from displaying signs of drought stress during prolonged dry season.

Given that growing media should contain between 80 and 100% inorganic content, such as expanded shale, AM fungi could dramatically increase the amount of water that is available to plants by accessing water stored inside aggregates. Further, through their ability to cross-link aggregates (Degens et al., 1996) extraradical mycelia are capable of not only bridging air gaps adjacent to roots, but preventing gaps from forming in the first place.

Further evidence of the importance of AM within coarse, shallow, water-stressed soils comes from a study by Doubková et al. (2013), who found that *Knautia arvensis* was better at maintaining growth and recovering from drought when colonized by AM fungi within a serpentine soil (low Ca, high Mg, low water holding capacity) . Under drought stress, plants colonized by AM fungi displayed increased biomass production, nutrient acquisition and enhanced photosynthetic and transpiration rates when compared with

uncolonized plants. Notably, AM-colonized plants had significantly lower root:shoot biomass ratios compared with uncolonized plants, indicating that the extraradical hyphae were making significant contributions to water uptake, reducing the amount of host-derived resources required for root growth and water uptake.

A study by Birhane et al. (2012) observed the water relations of *Boswellia papyrifera* seedlings under two watering regimes mimicking naturally occurring wet and dry seasons (continuous and pulsed water supply respectively). They found that under pulsed water supply, where seedlings were denied water for 15 consecutive days between watering, AM-colonized plants displayed greater biomass, water use efficiency, stomatal conductance and carbon assimilation. Interestingly, the greatest biomass accumulation was seen in the coarse root fraction, which in *B. papyrifera* aids in water storage. This pattern of investment in water storage organs was also seen in the uncolonized plants, but was increased 10% when colonized by AM fungi (Birhane et al., 2012).

A similar scenario was observed by Goicoechea et al. (2004), where seedlings of *Anthyllis cystoides* were exposed to severe drought. Part of *A. cystoides* response to drought stress is the production of a wax that coats the exterior of the leaf cuticle. When severe drought was initiated in their study, plants colonized by the AM fungus *Glomus fasciculatum* were observed to produce significantly greater amounts of wax on healthy leaves and more quickly shedding chlorotic leaves (Goicoechea et al., 2004), leading to reduced drought damage and enhanced post-drought recovery. Essentially, colonization by *G. fasciculatum* enhanced the drought responsiveness of its host.

In addition to increasing the volume of water accessed by the host, AM fungi have also been hypothesized to enhance host root permeability (Uehlein et al., 2007). As the intraradical structures of AM fungi are restricted to the apoplast, there must be bidirectional transport of water and nutrients across the periarbuscular membrane which envaginates the arbuscules before water or nutrients can reach xylem vessels (Peterson et al., 2004). Facilitating this transport are aquaporins, a group of proteins that increase the permeability of membranes within and between cells. Recent literature reviews (Uehlein et al., 2007; Ruiz-Lozano et al., 2010) have summarized the observed effects of AM symbiosis on the regulation of genes coding for a variety of aquaporins. These effects seem to vary with AM fungal species and soil moisture levels. Ruiz-Lozano et al. (2006) found that under conditions of drought, the AM fungus *Glomus intraradices*, which improved the drought tolerance of its host, increased the expression of aquaporins, resulting in greater permeability of the periarbuscular membrane and allowing greater flow of water into the host. Under the same conditions and with the same host, *Glomus mosseae* also improved the ability of its host to survive drought, but resulted in a decrease in aquaporin formation. The authors hypothesized that this represented two different AM fungal strategies for mitigating drought, one in which water uptake was increased (*G. intraradices*) and another that conserves water (*G. mosseae*). Hydraulic conductivity in relation to aquaporin formation and AM fungal colonization has not apparently been studied under well-watered conditions, but has been done for ectomycorrhizal (ECM) fungi (Marjanović et al., 2005). In a study of ectomycorrhizal-colonized *Populus tremuloides*, it was found that under well-watered conditions colonization by ECM fungi increased the formation of aquaporins in colonized root tissue, leading to increased

hydraulic conductivity of the host root system and an improved ability to transport water from the soil to the host. It is possible that AM fungi may have a similar effect on host root hydraulic conductivity, although given the species specific nature of the AM fungal interaction, speculation must be limited.

The passive absorption of soil water by plants requires that they maintain a potential energy within their root systems that is lower than that of adjacent soil water. A lower potential energy within plant roots results in suction causing water to enter into the plant (Larcher, 1996). However, as soil moisture content decreases, the suction force exerted by the soil also increases (Gobat et al., 2004). As a result, plants must be able to modify their internal suction force to adapt to changing soil moisture conditions. This is accomplished through a process known as osmoregulation, whereby cells actively accrue various osmotic solutes which act to decrease the potential energy within the cell, thereby maintaining absorption of water into the roots (Morgan, 1984). Osmotic adjustment becomes more important, and difficult, as the potential energy of soil water decreases. Plants which have evolved in response to arid conditions tend to have much lower permanent wilting points than those which have evolved under more mesic conditions (Larcher, 1996). However, one factor that seems to improve the ability of plants to osmoregulate is AM fungal colonization. Wu et al. (2006) found that under drought conditions, *Citrus tangerine* seedlings that had been colonized by *Glomus versiforme* displayed better drought tolerance, which they attributed to improved osmoregulatory abilities. They determined that the enhanced access to Ca^{2+} and Mg^{2+} provided by *G. versiforme* resulted in a net accumulation of Ca^{2+} and Mg^{2+} of 23.4 and 2.6% respectively, compared with only 6.1 and 1.8% for uncolonized seedlings. Additionally, the leaves of

AM-colonized seedlings had higher levels of K^+ and Ca^{+2} than uncolonized seedlings, providing enhanced osmoregulation resulting in improved photosynthetic rates 50% greater than that of uncolonized seedlings during drought stress. Similarly, Porcel et al. (2004) found greater drought tolerance in *Glycine max* plants which had been colonized by *Glomus intraradices*, as indicated by improved leaf water potential and a 27% increase in biomass production, compared with uncolonized controls. This was attributed to a 14% increase in proline levels in the roots of colonized plants. As a solute, proline is able to lower the potential energy of a solution; this decrease may have been sufficient to maintain a flow of water into the root cells of *G. max*. Wu et al. (2007) observed that increased drought resistance in *Citrus tangerine* was again attributed to improved osmoregulation. This was the result of increased Mg^{2+} and Ca^{2+} within colonized root cells, but also due to increased levels of soluble sugars within the roots. This was likely the result of increased carbohydrate demand from the mycobiont, leading to greater sink strength within colonized roots and a corresponding transfer of carbohydrates to the root system which would allow for improved osmoregulation (Wu et al., 2007; Kaschuk et al., 2009).

Augé (2004) found that the leaves of *Phaseolus vulgaris* plants that had been colonized by either *Glomus intraradices* or *Glomus margarita* were able to maintain full turgor at lower soil moisture when compared to uncolonized controls. The authors found that this was the result of greater accumulation of osmotic solutes within the leaves of plants colonized by AM fungi and enabled them to maintain turgor pressure within leaves and continue carbon assimilation during drought. Overall, the literature points to a better ability of AM-colonized plants to resist drought through osmotic regulation in the roots

or leaves of host plants, allowing for continued flow of water into root tissue or a maintenance of leaf turgor pressure (Bethlenfalvay et al., 1988; Duan et al., 1996; Marulanda et al., 2003; Augé, Moore, et al., 2004). However, the magnitude and character of mycorrhizal effects on osmotic solute accumulation tend to be dependent on the mycobiont and host species, as well as the degree of drought (Augé, 2001).

Another way in which AM fungi further improve host water relations is through mycorrhization of soil (Augé et al., 2003; Augé, 2004; Augé et al., 2007). As the extraradical hyphae of AM fungi grow into soils surrounding their host, they modify its structure and influence soil biology (Aspiras et al., 1971; Beare et al., 1997; Andrade et al., 1998; Bearden et al., 2000; Miller et al., 2000; Bearden, 2001; Chenu et al., 2001; Drew et al., 2003; Augé, 2004; Rillig et al., 2006; Bedini et al., 2009; Daynes et al., 2012). This is accomplished through the release of both labile and recalcitrant hyphal exudates, physical pressure exerted on soil particles by growing hyphae and through the enmeshment and entanglement of soil aggregates (Rillig et al., 2006). While extraradical hyphal growth is enabled by host photosynthates, these benefits are also available to non-hosts growing in the same soil. Augé, Sylvia, et al. (2004) found that non-AM plants growing in a soil which had previously supported AM-colonized plants experienced improved drought tolerance as evidenced by their maintenance of higher stomatal conductance during both periods of ample watering and drought, allowing for greater gas exchange. Furthermore, in order to reach a moisture potential of -0.02 MPa the AM soils had to decrease to a lower soil water content than corresponding non-AM soils, suggesting that the two soils had different water retention qualities (Augé, Sylvia, et al., 2004).

While the ability of AM fungi to enhance gas exchange rates and stomatal conductance of their hosts seems to be dependent upon host-mycobiont identity and affected by climatic conditions, it is clear that there are often significant benefits to the host. Colonization by AM fungi can improve the ability of host plants to survive drought by increasing the pool of plant-available water, augmenting the permeability of root systems, improving the sensitivity of leaf cells, and allowing for greater osmotic adjustment. Furthermore, by modifying the soil in which they are growing, AM fungi can improve growing conditions for both host and non-host plant species. Employing AM fungi within green roofs may increase the drought tolerance of both host and non-host plants, allowing designers to include a greater variety of species and reducing their reliance upon members of the Crassulaceae. Further, green roofs with AM inoculum potential may require less irrigation, reducing maintenance costs. Consequently, given their effects on host water relations, design of the vegetative layer should be amended to include AM fungi. However, since the effects of AM fungi are species specific, care must be taken to select the proper combinations of hosts and mycobionts as some combinations may be better suited to the conditions of the green roof than others.

An alternative to relying solely on *Sedum* spp. is to introduce AM-colonized species into the green roof environment. Colonization by AM-fungi has been reported by many researchers to reduce the incidence, severity and recovery time from drought stress (Augé, 2001; Allen, 2007, 2009). This is largely accomplished through more efficient and expansive exploration of the soil by extraradical mycelium, modified regulation of aquaporins with the host root system, and enhanced osmotic regulation.

2.4.3 Nutritional Improvements

The most thoroughly researched aspect of the AM fungal symbiosis are the nutritional benefits afforded to the host plant (Kothari et al., 1990; Smith & Read, 2010). It is well established that AM fungi are able to enhance the host's supply of nutrients such as P, N, K, Ca, Cu, Zn and Fe, especially under conditions of low nutrient abundance (Faber et al., 1990; Marschner et al., 1994; Liu et al., 2000; Chen et al., 2003; Cheng et al., 2006; Smith & Read, 2010; Veresoglou et al., 2012). The best-defined mechanism implicated in AM mediated nutrient improvement is the increased absorptive surface area provided by AM extraradical mycelium. In many environments however, P is the limiting nutrient and the ability of AM fungi to improve its availability is often cited as their most important function (Larcher, 1996; Gobat et al., 2004; Smith et al., 2011), although their ability to absorb N has recently been examined as well (Govindarajulu et al., 2005).

Traditionally, the provisioning of P has been viewed as the major resource traded by the mycobiont for host photosynthates (Koide et al., 2004). The metric used to gauge the equity of this trade has been host growth response; increased growth being regarded as a sign of mutualism and growth depression signalling parasitism. Recently however, this concept has begun to change, as experimental techniques have been refined (Smith et al., 2011).

Using isotope labelling, researchers have identified two separate pathways through which AM colonized plants absorb P; either a direct path through high affinity P transport proteins in the host root system, or through the extraradical mycelium (Pearson et al., 1993; Gashaw Deressa et al., 2008; Smith et al., 2011). Whether these two pathways

interact is uncertain. Earlier research assumed that the AM pathway simply added to the amount of P absorbed through the direct path (Koide et al., 2004; Smith et al., 2012), while current research indicates that colonization by AM fungi may lead to a reduction in P absorption by the host root system, potentially through a down-regulation of genes coding for P transporters in the direct path (Smith et al., 2012). The result is that plants, even those which experience a growth depression through AM colonization, may be completely dependent upon their mycobiont for P uptake, since their root system has reduced (in some cases completely) its ability to absorb P (Pearson et al., 1993; Janos, 2007).

While studying the effects of AM fungal colonization on the P dynamics of *Cumcumis sativus*, Pearson et al. (1993) found that colonization by *Glomus caledonium* led to the complete inhibition of P uptake through the hosts root system. However, the P delivered to the host by the mycobiont resulted in greater production of host biomass in comparison with uncolonized controls. Similarly, Neumann et al. (2010) found that under conditions of low soil moisture, *Sorghum bicolor* plants which had been colonized by *Glomus mosseae* had twice the P content of uncolonized plants.

Regardless of the absorption pathway, plants colonized by AM fungi are often observed to produce significantly greater amounts of biomass than uncolonized controls, due in part to the mycobiont mediated enhancement of host P uptake (Johnson et al., 1997; Read et al., 2003; Hoeksema et al., 2010), although this can depend upon host and mycobiont identities. In some host-mycobiont interactions, host biomass accumulation is significantly reduced compared with uncolonized plants (Klironomos, 2003). The cause of these growth depressions may not stem from excess carbon drain imposed by the

mycobiont (i.e. parasitism), but because the down-regulation of P absorption by the host is not adequately compensated for by mycobiont P uptake. This lack of compensation may simply result from reduced P supplies in the soil, the inability of the mycobiont to absorb sufficient amounts of P, or it may be an artefact of experimental design (Smith et al., 2011). In an experiment observing biomass accumulation in tomato plants, growth depression was observed in AM colonized plants when grown individually (Facelli et al., 2010), but the biomass differences were reversed when colonized and uncolonized plants were grown within the same pot (Facelli et al., 2010). The explanation given was that the mycobiont species involved was particularly good at accessing P at the expense of uncolonized tomato, thereby giving the host a significant competitive advantage (Facelli et al., 2010). The enhanced plasticity of AM fungal mycelia in comparison to plant roots likely allows them to more quickly locate and exploit P patches within the soil matrix.

The ability of AM fungi to access P is based on the physiognomy of their extraradical mycelia, and the behaviour of P within the soil solution. P is absorbed by plant roots in the form of orthophosphate (H_2PO_4^-), an ion with extremely limited mobility within the soil solution (Tinker et al., 2000). As such, plants typically absorb orthophosphate at a faster rate than which it is replaced within soil pores occupied by their roots, resulting in its depletion around roots and preventing subsequent uptake (Gobat et al., 2004). AM fungi ameliorate this situation by bridging these zones of depletion through the extension of their extraradical mycelium, thereby maintaining the connections between the root network and P sources in the soil (Peterson et al., 2004). Furthermore, the minimal diameter of the extraradical hyphae allows AM fungi to access P located within soil

micropores, much in the same way that they are able to absorb water from them (Friese et al., 1991; Pearson et al., 1993; Drew et al., 2003).

There is mounting evidence that AM fungi also make significant contributions to the N uptake of host plants (Schroeder et al., 2005; Leigh et al., 2009; Veresoglou et al., 2012). In an early study, Johansen et al. (1992) found that *Cucumis sativus* plants colonized by *Glomus intraradices* were able to access ^{15}N labeled NH_4^+ located in an adjacent compartment partitioned by a $20\mu\text{m}$ mesh. AM-colonized plants recovered up to 30% of applied NH_4^+ whereas non-mycorrhizal plants were unable to significantly deplete this resource. N absorption by AM fungi may be particularly relevant when nitrogen is released through the decomposition of organic matter. The relative speed with which AM extraradical mycelia can be deployed towards nutrient patches may give AM colonized plants a competitive advantage when nitrogen patches are transient.

Hodge et al. (2001) grew *Plantago lanceolata* plants in order to determine their ability to capture N from decomposing organic matter which had been ^{15}N labelled. Plants colonized by *Glomus hoi* where extraradical mycelia were allowed to access the patch captured approximately 15% of released N compared to colonized plants that only had access to organic patches via solute diffusion and could only capture 5%. In a similar experiment, Hodge et al. (2010) found that 3% of N in *Plantago lanceolata* was scavenged from patches of organic matter by its associated mycobiont *G. hoi*, whereas the mycobiont satisfied 31% of its own N through exploitation of organic patches. However, while the ability of AM hyphae to absorb and transport N from the soil to the plant has been demonstrated

(Govindarajulu et al., 2005; Whiteside et al., 2009; Whiteside et al., 2012), the ecological relevance of this mechanism is still being debated (Veresoglou et al., 2012).

The greatest evidence for the relevance of N absorption comes from periods of low soil moisture. NO_3^- and NH_4^+ are normally quite mobile within the soil solution, causing them to readily diffuse towards plant root systems (Marschner, 1995). Under conditions of low soil moisture however, they cease to move via mass flow, at which point the contribution of AM fungi to N uptake may become more important (Tobar et al., 1994; Ruiz-Lozano, 2003).

The nutrient-scavenging benefits of colonization by AM fungi appear to be strongest when nutrients are limiting, either due to scarcity, or low mobility within the soil matrix (Neumann et al., 2010), whereas their benefit is questionable in environments such as agro-ecosystems, where nutrients occur in abundance (Miller, McGonigle, et al., 1995; Titus et al., 2000; Corkidi et al., 2002).

The enhanced nutrient scavenging abilities of plants colonized by AM fungi could enhance the functioning of green roofs in three distinct ways; either through reduced fertilizer requirements, enhanced ability of green roof plants to scavenge for nutrients during drought, or reducing the amount of nutrients lost through leaching.

2.4.4 Leachate

While one of the foremost concerns for green roof designers is the quantity of stormwater retained during a precipitation event, the quality of stormwater leaving a green roof is of additional consideration. Reducing the pollutant load within green roof leachate is of extremely important given the prevalence of contaminants found on urban rooftops

(Walsh, 2000). Runoff from urban areas is one of the most significant vectors facilitating the movement of pollutants into surface waters (Paul et al., 2008). Atmospheric contaminants intercepted by rooftops, as well as contaminants contained within roofing materials, can cause severe damage to the biotic communities of local water bodies (Booth, 2005). Contaminants are washed from rooftops during precipitation events and travel through urban stormwater management systems before being deposited within terminal water bodies (Ammann et al., 2003; Van Metre et al., 2003). The quantity of stormwater runoff resulting from a single precipitation event is correlated with the proportion of urban space occupied by impervious surface areas. Materials such as asphalt and concrete also prevent the regular percolation of stormwater into soils resulting in overland flow of stormwater (Arnold Jr et al., 1996). As urban surface area becomes increasingly dominated by impervious materials, the rate and volume of stormwater runoff increases (Paul et al., 2008), leading to amplified delivery of pollutants to surrounding water bodies and their subsequent deterioration. It is hoped that implementation of green roof technology will improve the quality of urban stormwater runoff through both retention and filtration of precipitation within the growing media and vegetation layer (Getter et al., 2006). However, research indicates that green roofs can be both a contaminant source and sink, depending upon green roof components and location, severity of precipitation events and the contaminant in question.

Studies of green roof leachate have produced a complex set of results concerning runoff quality and variation among green roof types

(Czemiel Berndtsson et al., 2006; Hunt et al., 2006; Berghage et al., 2008; Hathaway et al., 2008; Retzlaff et al., 2008; Czemiel Berndtsson et al., 2009; Aitkenhead-Peterson et al., 2011; Alsup et al., 2011; Buccola et al., 2011; Gregoire et al., 2011; Mendez et al., 2011; Morgan et al., 2012). Research has focused on quantities of heavy metals, total P, and N content in green roof leachate (Czemiel Berndtsson, 2010). Cases of green roofs acting as both pollutant sinks and sources have been described, with green roof parameters relating to leachate quality including growing media composition and depth, plant species identity, plant coverage, intensity/periodicity of precipitation events' green roof age and local air quality (Monterusso et al., 2002).

P has almost universally been found in higher concentrations in green roof leachate when compared with both rainwater and conventional roofs (Czemiel Berndtsson, 2010; Rowe, 2011), with P being as much as 20 times higher in green roof leachate (Moran et al., 2005; Czemiel Berndtsson et al., 2006; Van Seters et al., 2009; Vander Linden et al., 2009). The greatest levels of P found in leachate tended to correlate with how recently the roof had been fertilized and with the age of the roof, with newer roofs releasing more P (Köhler et al., 2002; Czemiel Berndtsson et al., 2006; Emilsson et al., 2007; Hathaway et al., 2008; Czemiel Berndtsson et al., 2009; Aitkenhead-Peterson et al., 2011).

Conversely, in older green roofs, phosphorous retention increases and leached P appears in insoluble forms (Köhler et al., 2002; Teemusk et al., 2007). Köhler et al. (2002) determined that total P levels in the leachate of an extensive green roof decreased over four years by 54%. This was ascribed to increased vegetation demand for P and to declining levels of P present in the growing media due to previous leaching (Köhler et al., 2002; Emilsson et al., 2007). Differences in the nature of precipitation events can also

influence leachate quality, with heavier events leading to greater P release (Teemusk et al., 2007). Given that P tends to be a limiting nutrient within aquatic ecosystems, the eutrophying effects of anthropogenic phosphorous addition can lead to severe declines in water quality (Carpenter et al., 1998). Widespread application of green roof technology could therefore lead to increases in P levels entering urban waterways unless measures are taken to prevent excessive leaching.

The effect of green roofs on N content of leachate is less straightforward, with studies indicating green roofs can act as both sources and sinks of N. Both Aitkenhead-Peterson et al. (2011) and Czemiel Berndtsson et al. (2006) found that vegetated green roofs retained more N than conventional and unvegetated (growing media only) roofs.

However, Aitkenhead-Peterson et al. (2011) determined that dissolved organic N was leaching from their green roof in higher concentrations than inorganic N. Conversely, Monterusso et al. (2002), Moran et al. (2005) and Hathaway et al. (2008) observed increased N concentrations in green roof leachates compared with control roofs and rainwater, likely due to the use of fertilizers. Retzlaff et al. (2008) found N concentrations between 10 and 50 ppm in green roof leachate, depending on utilized growing media composition. Further, the amount of N leaching from green roofs, as with P, seems to be linked with age of the roof and plant species identity. Older green roofs with an established layer of locally adapted plant species produced leachate with lower concentrations of N (Köhler et al., 2002) while roofs utilizing *Sedum* spp. displayed lower N retention performance than roofs planted with locally adapted species (Monterusso et al., 2002).

Studies examining the heavy metal content of green roof leachate have provided results that vary according to substrate composition, timing and intensity of precipitation events, as well as total stormwater retention (Alsup et al., 2010; Czemieli Berndtsson, 2010; Alsup et al., 2011). Through simple reduction in stormwater runoff, green roofs are able to reduce the amount of heavy metals leaving the roof in leachate. However, beyond its ability to reduce runoff, media depth has not been found to influence metal content of leachate (Alsup et al., 2011).

Of particular interest within studies of green roof leachate have been Cu, Pb, Cd and Fe. Green roofs have been both sources and sinks for all elements, depending on precipitation characteristics and media composition. Alsup et al. (2011) found that their green roof acted as major source of Pb, leaching concentrations of Pb twice as high as described acute toxicity levels. This is in contrast to studies by Gregoire et al. (2011) and Czemieli Berndtsson et al. (2009) where Pb was retained by the green roof. Cu was found more often in the leachate of green roofs compared with both rainwater and runoff from conventional roofs, likely as a result of Cu-containing fertilizers used within growing media (Czemieli Berndtsson et al., 2009; Gregoire et al., 2011). While Zn was both retained (65% retention: Gregoire et al. (2011)) and leached by green roofs (Czemieli Berndtsson et al., 2009).

While the factors influencing the quality of green roof leachate are highly varied, there are a number of broad deductions that can be drawn from the research. Foremost, the maintenance regime dictating the level of fertilizer application can have a substantial impact on leachate quality, with greater applications of fertilizer leading to nutrient laden leachate. Additionally, components of the growing media may also influence leachate,

with some materials containing poorly bound metal ions and nutrients. Precipitation dynamics are also of importance, with low-intensity events leading to greater losses of total N while high intensity events produce leachate with high concentrations of N or P. Of further importance is the identity of the vegetation. Many *Sedum* spp. seem to be poor at nutrient interception and produce nutrient-rich leachate, while species with higher nutrient demands and transpiration rates seem to be more efficient at reducing nutrient loss through leaching. Finally, local climate variables also need to be considered, given that atmospheric deposition of nutrients and contaminants will differ between regions depending on factors such as proximity to agricultural and industrial production. Regardless, care must be taken when designing and implementing green roofs so as to limit the loss of nutrients and pollutants through leaching, given their potential to damage the health of local water bodies.

Leaching from green roof systems contributes to both diminished plant biomass production and decreased leachate quality. An overlooked, but potentially significant benefit of colonization by AM fungi is their ability to intercept nutrients and prevent this leaching. As AM fungi produce extraradical mycelia at a density up to 81 to 111 cm g⁻¹ (Miller, Jastrow, et al., 1995; Marulanda et al., 2003), growing up to 25 cm from host roots (Drew et al., 2006) and penetrating pores as small as 2 µm in diameter (Friese et al., 1991), they can substantially increase the absorptive area available to hosts. Host plants especially benefit from the high affinity phosphate transporters utilized by their mycobionts (Smith et al., 2012) which enhance the efficiency of P uptake compared to host roots (Cress et al., 1979). Not only does this allow for increased host access to

nutrients, but helps to more effectively intercept nutrients as they flow vertically and horizontally through the soil profile (van der Heijden, 2010).

Attempting to replicate a leaching prone habitat, van der Heijden (2010) used a sandy dune soil planted with one of three AM-host species to test whether colonization by AM fungi reduced nutrient content in leachate. When nutrients were applied to the soil in low amounts, concentration of PO_4^{3-} in leachate was as high as 0.052 mg L^{-1} in uncolonized plants and as low as 0.017 mg L^{-1} in plants colonized by AM fungi. On average, PO_4^{3-} concentration in leachate was reduced by 60% through colonization by AM fungi and NH_4^+ was reduced by 7.5% (van der Heijden, 2010). Similar results were obtained by Asghari et al. (2005), observing that colonization by AM fungi reduced total P and N content in leachate through the foraging capabilities of their extraradical mycelia.

While the importance of AM fungi in N absorption has been limited to its ability to intercept NH_4^+ , recent research has also shown that extraradical mycelia can significantly reduce losses of NO_3^- in leachate (Asghari et al., 2012). The high mobility of NO_3^- increase the rate at which it moves through soil towards plant root systems, likely reducing the importance of AM fungi in delivering it to host. The ability of AM fungal hyphae to absorb NO_3^- has been demonstrated however, although the relevance to the host is questionable (Tanaka et al., 2005; Cavagnaro et al., 2006). In their study, Asghari et al. (2012) found that tomato plants colonized by AM fungi reduced NO_3^- concentration in leachate by approximately 40 times compared with uncolonized tomato plants. They found concomitant losses in soil NO_3^- and increases in plant tissue concentration, indicating that NO_3^- was being intercepted by AM fungi and delivered to hosts. Similar results have been obtained by Cavagnaro et al. (2012). Somewhat contradictory results

were observed by de Vries et al. (2011) who found that while a soil with high fungal biomass actually increased nitrogen leaching compared to a soil with low fungal biomass. This result was explained as potentially stemming from the reduced activity of AM fungi during the winter months, which was when the study was conducted.

Literature on the impacts of AM fungi on leachate quality is indicative of significantly improved nutrient retention within soil systems. By improving the volume of soil explored and the rate at which certain nutrients are absorbed, AM fungi may be able to both reduce the nutrient addition requirements and leachate loss within green roof systems. While improving nitrogen retention is incredibly important for reducing the negative impacts of green roofs on the health of aquatic systems that receive their run-off, the reduction of P loss is potentially even more important. While N resources can be naturally replenished in soils through the action of nitrogen-fixing bacteria, P must be added manually to green roof growing media (Gobat et al., 2004). Therefore, any P lost through leaching represents a fraction that must be replaced in order to maintain proper growing conditions within the green roof. Therefore, by reducing P content in leachate and tightening nutrient cycles, AM fungi may significantly increase the sustainability of green roof systems.

2.4.5 Enhancement of Soil Structure

A major impediment restricting wide-scale implementation of green roofs is the load-bearing capacity of the buildings upon which they are constructed (Scrivens, 1990). Attempts to maximize application potential have led to the development of extensive green roofs which use shallow (<20 cm) applications of light-weight mineral substrates

with reduced bulk density and minimal (<20%) organic matter (Beattie et al., 2004). Media constituents often include crushed clay tiles, perlite, vermiculite, sand, peat moss and heat-expanded slate, clay, and shale (ESCS). The primary function of media however, is to support the growth of a vegetation layer, therefore growing media must provide conditions capable of supporting sustained plant growth. Growing media must be designed to include optimal water retention and infiltration, aeration, nutrient content and sufficient lateral conductivity to assure that excess water is effectively drained to prevent water-logging (Beattie et al., 2004; Hunt et al., 2006; Berghage et al., 2008) (except in the case of zero discharge roofs, see Compton et al. (2006)). However, attempts to optimize weight reduction and growing conditions have been conducted with minimal recognition of the roles played by AM fungi in engineering and maintaining soil quality. The focus has instead been placed solely on the physical aspects of soil.

The soil component of terrestrial ecosystems is of fundamental importance for plant survival. Soil structure, a measurement of the dimensions, positioning and abundance of aggregates and pores within three-dimensional space, mediates conditions of water (Bronick et al., 2005) and nutrient distribution, accumulation and accessibility to plants as well as soil aeration, erosion rates and patterns of root growth and development (Larcher, 1996). Structural factors influence these aspects based on the varying interacting constituents that make up soil, including the organic, inorganic, biotic and abiotic components. Development of soil structure is therefore an emergent process that reflects the combination of these activities. Within this framework, the various enmeshment and exudate producing activities of plants, fungi and microbes enhance and

protect the structure of the soils in which they are active (Six et al., 2004; Lützow et al., 2006; Rillig et al., 2006).

Of the biotic factors influencing characters of soil structure, AM fungi may be among the most influential (Bearden et al., 2000; Hallett et al., 2009). In some soils they represent up to 50% of total microbial biomass (Olsson et al., 1999; Cheng et al., 2006), where they act to enhance soil structure through the formation of new soil aggregates while strengthening bonds between those that already exist (Rillig et al., 2006). AM fungi are a liaison between the soil and their plant hosts, delivering immobile or otherwise inaccessible soil nutrients and water to their hosts (Smith & Read, 2010). AM fungi also assist in mediating nutrient flows in the opposite direction by delivering up to 30% of host photosynthates to other microorganisms in the rhizosphere through deposition of hyphal exudates and sloughing of senesced hyphae (Douds Jr et al., 2000; Graham, 2000). Given the varied and important roles played by AM fungi in maintaining soil quality as well as mediating relationships between soil organisms, it is essential that they be integrated into green roof design. Challenges facing green roof designers, such as reducing erosion, preventing excessive nutrient loss through leaching, providing plants with accessible soil nutrients and finding ways to sequester greater amounts of carbon, can all be addressed, at least in part, through effective utilization of AM fungi.

The positive effects of AM fungi on soil quality have typically been linked to their influence on aggregate formation and stabilization (Bearden et al., 2000; Piotrowski et al., 2004; Driver et al., 2005; Rillig et al., 2006; O'Dea, 2007). Where organic matter is available as a binding agent, soil aggregation is a hierarchical process in which primary soil particles and inorganic compounds are bound into clusters via electro-chemical

forces (Tisdall et al. (1982) (Greenland, 1977). Clusters are then organized into varying classes of microaggregates (<250 μm diameter) and macroaggregates (>250 μm diameter), through interaction with organic matter. Microaggregates are thought to be more enduring given the stability of the agents that bind them; recalcitrant humic substances that acts as nuclei around which clay particles are able to bind and protect from degradation (Gobat et al., 2004). The forces that bind macroaggregates (ensnaring actions and exudates of fungal hyphae and plant roots) result in comparatively less permanence given the transience of these structures (Jastrow et al., 1998). Aggregates are often the main focus of studies observing soil structure given the range of effects they have on plant growth including protection of soil carbon, influence on aeration and water infiltration, nutrient availability and soil stability (Six et al., 2004). These benefits are interpreted through the hierarchical process of soil development, whereby larger aggregates are formed at the expense of smaller aggregates, thereby reducing bulk soil density (Tisdall et al., 1982). This reduction is due to the formation of pores between aggregates as well as the formation of micropores within aggregates (the porosity exclusion principle) (Dexter, 1988)).

The presence of AM colonized plant roots and AM mycelia have been correlated with greater levels and increased stability of soil macroaggregates, typically within the >250 μm diameter size fraction; although the mechanisms responsible are not fully recognized (Miller et al., 2000; Rillig et al., 2006). However, the current hypothesis is that AM fungi influence soil structure in several critical ways, including the physical entanglement and enmeshment of soil particles through the actions of external hyphae and the ‘gluing’ together of soil particles via hyphal exudates and other AM fungal products such as the

protein glomalin, as well as mucilages and polysaccharides (Jeffries et al., 1994; Augé, 2004; Rillig et al., 2006).

AM fungi physically interact with soil particles through the actions of their extraradical hyphae. As they expand through the soil matrix, these mycelia must grow around, and through, soil particles of varying sizes and densities. In doing so, the force exerted against adjacent soil particles and organic materials can be sufficient to bind them into microaggregates (Tisdall et al., 1997; Rillig et al., 2006). Additionally, the external hyphae can align clay particles along their length through the combined action of exuded polysaccharides and hyphal water uptake (Tisdall, 1991; Chenu et al., 2001). Hyphae can also produce polysaccharide exudates which are capable of dislodging fine clay particles from the surface of microaggregates (Cairney et al., 1989). As hyphae absorb water from surrounding soil pores, the clay particles are organized along the hyphae and sorbed to their surface via the polysaccharide exudates (Aspiras et al., 1971; Tisdall, 1991). When this process is applied over three-dimensional space with a dense network of external AM fungal hyphae, clay particles, polysaccharide exudates and hyphae are entangled to form macroaggregates, resulting in reduced bulk soil density. Even after host death and hyphal senescence, fragments of sloughed AM hyphae continue to engage in aggregate formation (Tisdall et al., 1982). This process has been viewed directly with electron microscopy by Gupta et al. (1988), while Beare et al. (1997) found correlative evidence through experimental inhibition of fungi which reduced the number of soil aggregates greater than 2 mm in diameter by 40%. Using mesh bags which excluded roots but allowed AM fungal hyphae to grow through, Bearden et al. (2000) found similar increases in soil aggregation of the ≥ 0.5 mm diameter size range both inside the mesh

bags (where AM-colonized plant roots were present) and outside the mesh where only AM fungal hyphae were active. This suggests that the actions of the hyphae, not plant roots, were the major contributors to soil aggregation. Similar results were found by Bethlenfalvai et al. (1999) where significant increases in water-stable aggregates were strongly correlated with the development of AM hyphal networks but not to total root volume. Degens et al. (1996) found that in a soil composed largely of coarse sand particles (83% of particles > 0.25 mm), aggregates > 2 mm increased from 7 g kg⁻¹, when *Lolium rigidum* plants were uncolonized, to 16 g kg⁻¹ after inoculation with the AM fungus *Scutellospora calospora*. The result was attributed almost solely to the cross-linking action of AM fungal hyphae, as opposed to hyphal exudate production, as the researchers were unable to detect any fungal polysaccharides. However, this result may have been unique due to the high proportion of large aggregates. A number of other studies have attributed increases in macroaggregate formation to the combination of direct AM hyphal action and the production of AM fungal exudates and proteins such as glomalin.

In addition to the physical entanglement actions of their hyphae, AM fungi affect soil structure through the release of a variety of hyphal exudates into the soil. While some of these include labile carbon sources, the most thoroughly researched compound is glomalin, a recalcitrant, hydrophobic glycoprotein produced in large amounts by members of the Glomeromycota (Wright et al., 1998; Driver et al., 2005; Gadkar et al., 2006). Previously thought to be a hyphal exudate related directly to soil aggregation (Wright et al., 1998), glomalin is currently hypothesized to be tightly bound within the hyphal cell wall, only being released into the soil upon hyphal senescence

(Driver et al., 2005). The role of glomalin inside the hyphae remains speculative, although it is potentially reactive to environmental stresses such as suboptimal temperature, pH and grazing by microarthropods (Steinberg et al., 2003; Purin et al., 2008). Interestingly, Rillig and Steinberg (2002) found that glomalin production was seven times greater in poorly structured soils (average pore size < 106 μm) than in highly aggregated soils (pore size 710-1180 μm), even though external hyphal length was reduced by 80%.

While the direct mechanisms have yet to be definitively described, there is ample correlative evidence for the positive impact of glomalin on soil aggregation (Wright et al., 1999; Rillig et al., 2006). It is thought that once in the soil, glomalin forms a residue that acts as 'glue', binding microaggregates into macroaggregates and as a hydrophobic coating which prevents aggregates from being broken down through hydration (Rillig, 2004). The recalcitrant nature of glomalin results in long residence times within the soil, anywhere from 6 to 42 years (Rillig et al., 2001), suggesting long term effects on soil stabilization. The production of glomalin seems to be influenced by multiple factors, including the specific species of AM fungi and plant host involved, the amount of external mycelium produced and the amount of 'stress' experienced by both AM fungi and host plants (although what constitutes 'stress' is still poorly defined). Plants that are highly dependent on AM fungi, such as those with magnoloid root anatomy, may allocate more resources to their AM mycobiont in order to compensate for the coarseness of their root systems (Peat et al., 1993). As a result, these plants may have more resources to spend on the production of external hyphae and if these hyphae are produced under

stressful conditions, more resources may be allocated to the production of glomalin.

However, this generalization is still hypothetical and based only a few studies.

Various authors have noted the stabilizing effect of glomalin on soil aggregation (Wright et al., 2000; Rillig, 2004; Purin et al., 2008; Emran et al., 2012; Singh, 2012). A pot study by Bedini et al. (2009) utilizing *Medicago sativa* plants inoculated with two species of the AM fungus *Glomus*, found a strong positive correlation between extractable soil glomalin and root volume colonized by the mycobiont. Extractable glomalin was significantly higher in the soils of colonized *M. sativa* plants, as was the abundance of stable aggregates within the 1 to 2 mm diameter size class.

In any case, depending on specific interactions between the AM fungi and plant host, the effect of glomalin on soil aggregation may be as strong as that of plant roots themselves (Rillig & Steinberg, 2002; Rillig, Wright, et al., 2002). Consequently, the effect of glomalin on soil aggregation is both significant and enduring, making it an especially important consideration within any discussion of soil stability.

Organic matter besides glomalin was also measured in Bedini et al. (2009), where colonized root volume was also a good predictor of soil stability, while total root volume was not. Importantly, it was determined that aggregate stability was more highly correlated with soil organic matter of fungal origin than with total soil organic matter, an observation also made by Jastrow et al. (1998). This is a significant result as prior research has linked total soil organic matter to aggregate stability (McCoy, 1998; Chan et al., 2002; Abiven et al., 2009), whereas in the studies by both Bedini et al. (2009) and Jastrow et al. (1998), most of the variation in aggregate stability was explained by the

plant-fungus interaction, as opposed to gross root metabolism. From this, it would appear that AM fungi are capable of altering the quality of organic matter reaching the rhizosphere, with organic matter of AM fungal origins having significant impacts on macroaggregate formation (Rygiewicz et al., 1994). Further, work by Daynes et al. (2012) and Miller et al. (1990) found that fine and very fine plant roots had only a minimal positive effect on aggregate stability, and that much of this effect was accounted for by the AM fungi which were colonizing them. Similarly, Wilson et al. (2009) found that biomass of external mycelium was strongly positively correlated with macroaggregate formation, but that adding plant-root biomass to this model did not explain a significant amount of variation. Wilson et al. (2009) also found that not only was the presence of external mycelium associated with the formation of macroaggregates, but also that destruction of mycelium through fungicide applications resulted in significant macroaggregate degradation.

Given the potentially central role of organic matter within soil development, AM fungi may be especially relevant in soils or growing media in which organic matter is limited. Organic matter can improve soil structure and texture, as well as increase soil water retention (Abiven et al., 2009), but the use of organic matter in green roofs has been a controversial subject however due to its tendency to decay over time (Friedrich, 2005). If organic matter is not replaced upon its decomposition, it can lead to soil compaction and suboptimal growing conditions (Beattie et al., 2004; Friedrich, 2005). Given that extensive green roofs are designed for reduced-maintenance, component replacement is not desirable, making large additions of organic matter an unattractive prospect. Instead, growing media has been designed using a variety of mineral substrates

of varying aggregate sizes in order to effectively replicate the bulk density and water holding capacity of ideal soils while at the same time minimizing weight. As such, organic matter within green roofs is quite limited, suggesting yet another role for AM fungi in mediating carbon flow into growing media. In a semi-arid savannah, where organic matter composed only 1.4% of soil volume, O'Dea (2007) found that AM fungi, as opposed to soil organic matter, were the main drivers of soil structural stability. The amount of live AM fungal biomass present in soil, not plant presence, was the strongest predictor of soil susceptibility to erosion; more extraradical mycelia resulted in less erosion. Similarly, while studying a semiarid shrub-land, Chaudhary et al. (2009) observed that AM extraradical mycelia were strongly correlated with soil stability, both within the soil surface and subsurface. The abundance of organic matter, which was only 1%, was not found to directly enhance soil stability. These results suggest that in soils where organic matter is low, the importance of AM fungi in maintaining soil structure may be heightened. This case is further strengthened by the fact that a large proportion of soil organic matter is of fungal origin (Bedini et al., 2009).

AM fungi are also able to effectively enhance structure in soils with organic matter abundance similar to that prescribed for green roofs. When compost levels were between 6 and 18% of total soil volume, Daynes et al. (2012) found that *Dodonaea viscosa* plants colonized by 8 isolates of AM fungi significantly increased the number of water stable aggregates compared to that of uncolonized plants. Carbon input to the soil was increased 5-8% by AM colonized plants compared with uncolonized controls. Additionally, both the pool of plant available water and the water holding capacity were maximized by 12% compost addition and colonization by AM fungi, but not by compost alone. The cause

was deemed to be the increase in fine pore space caused by the AM extraradical mycelium. Given that AM fungi rapidly transfer recent photoassimilates from their host to the soil (Staddon et al., 2003; Johnson, 2008), they may be useful in maintaining a moderate level of organic matter within the growing media. Integrating AM fungi into green roofs may be an efficient method for enhancing soil structure through the various physical actions of extraradical mycelia, as well as their ability to transfer large amounts of organic material into the soil.

Related to their effects on soil structure is the potential for AM fungi to reduce growing media losses to erosion (Andrade et al., 1998; Caravaca et al., 2002; Bedini et al., 2009; Chaudhary et al., 2009; Burri et al., 2011). Erosion is a serious problem in any environment as the removal of nutrients and the destruction of soil structure have adverse consequences for plant growth (Gobat et al., 2004).

Erosion is especially problematic in green roof environments, given the already harsh edaphic conditions, limited media depth as well as the high cost of replacing growing media. Green roof growing media may be susceptible to erosion if the roof is oriented on a steep slope, exposed to high winds and if vegetation has not yet completely established over the roof surface. AM fungi can be useful in reducing the negative effects of aeolian erosion, although little research has been carried out on the subject. In a study on the effects of AMF colonization on soil erosion, Burri et al. (2011) conducted a wind tunnel study to determine if the rate of erosion in root balls of *Lolium perenne* and *Anthyllis vulneraria* was effected by AM fungal colonization.. For uncolonized samples of *A. vulneraria*, aeolian erosion resulted in a 38.8% loss of soil. This was reduced to only 14.7% soil loss for plants colonized by AM fungi. Erosion losses for *L. perenne* were

lower for colonized versus uncolonized plants, although the differences were not significant. AM fungal colonization also had a significant influence on host biomass production; AM colonization of both plant species reduced the root:shoot ratio and decreased total plant biomass. This indicates that for *L. perenne*, the effects of AM colonization can outweigh those of the host root system with respect to soil stabilization, as colonization overcompensated for the reduction in groundcover and root system biomass. However, as is often the case, these effects were dependent upon the host and mycobiont species involved.

For sloped green roofs, the erosive effects of water may also be an important consideration. While growing media is designed to be extremely fast draining and employs an underlying synthetic water retention layer with high lateral conductivity, large precipitation events can cause soil water content to rise above retention capacity, resulting in overland flow of media aggregates. There is ample research to suggest that AM fungi are capable of reducing soil susceptibility to the disruptive wetting instigated by large precipitation events (Degens et al., 1996; Augé, 2001; Bearden, 2001; Rillig et al., 2006; Graf, 2011). Studying the effects of soil pore disruption resulting from water infiltration, Bearden et al. (2000) found that the presence of AM colonized *Sorghum bicolor* produced the greatest stability of medium (1000-100 μm) and large ($>1000 \mu\text{m}$) diameter soil pores. In their model, plant roots were responsible for stabilizing the large pores while AM extraradical hyphae maintained the integrity of medium sized pores. Maintenance of pore space is integral for good soil structure, but also results in primary particles being bound within aggregates, preventing them from being easily eroded. Similarly, Degens et al. (1996) found that coarse sand grains were stabilized by the cross-

linking action of AM fungal hyphae. In their experiment they observed that as single hyphae grew into the soil, they would coil around multiple grains of sand. As a result, these grains were held within the rhizosphere as water-stable aggregates and their susceptibility to erosion was minimized. This action might be particularly relevant in green roofs given the high abundance of coarse mineral aggregates within many commercially available growing media.

2.4.6 Synthesis

There is substantial evidence to suggest that the integration AM fungi into green roof systems has the potential to significantly enhance their capacity to provide ecosystem services and reduce required maintenance requirements. Some of the major benefits conferred through their integration may include increased host-plant tolerance of stresses such as low soil moisture and low nutrient availability; improved leachate quality; facilitation of greater plant diversity; and stabilization of growing media.

While their utility of AM fungi has not been explicitly tested within the highly artificial substrates of green roof growing media, there have been a variety of studies conducted within habitats with similar edaphic conditions, such as the post-eruption slopes of Mount St. Helens (Allen et al., 1992; Allen, 2007), semi-arid regions with low aggregate stability and less than 1% total organic matter (Chaudhary et al., 2009), mine tailings (Daynes et al., 2012), substrates composed of coarse aggregate sand (Degens et al., 1996; Drew et al., 2003), and post-fire semi-arid savannah (O'Dea, 2007). In these studies, AM fungi were found to be important in determining soil aggregate stability and

water/nutrient availability to host plants, resulting in an overall enhancement of host survival rate and often biomass accumulation.

Furthermore, when considering the potential benefits of AM fungi for green roof systems, one should consider the sheer number of plant species which host AM fungi under natural conditions; approximately 200 000 plant species (Smith & Read, 2010), 68% of which may be highly mycorrhizal-dependent hosts (Wang et al., 2006) and may not survive outside of the symbiosis. The exclusion of AM fungi from green roof design may therefore substantially reduce the number of plant species available for utilization. This also clearly represents a severe limitation to the enhancement of urban biodiversity as well as the ability of green roofs to provide ecosystem services (Lundholm et al., 2010).

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CHAPTER THREE

MYCORRHIZAL COLONIZATION OF GREEN ROOF PLANTS

3.1 Introduction

Green roofs are recognized as an important component of integrated strategies aimed at the development of environmentally resilient cities (Oberndorfer et al., 2007). The negative consequences of urban development are ameliorated through reducing urban stormwater loading by intercepting and retaining precipitation, while returning a portion back to the atmosphere through evapotranspiration (Onmura et al., 2001). Additionally, green roofs moderate interior building temperatures (Del Barrio, 1998), extend the lifespan of roofing materials (Feng et al., 2010), sequester carbon (Getter et al., 2009), reintroduce biodiversity into urban habitats (Brenneisen, 2005) and reduce the severity of the urban heat island effect (Getter et al., 2006; Alexandri et al., 2008).

The ability of green roofs to perform these functions largely depends on the composition of the growing media, properties of the vegetative layer, interactions between these factors, and interactions between the green roof and its environment. Critical components of the vegetative layer include anatomical and physiological characteristics of individual plants such as root and leaf structure, transpiration rate, stomatal sensitivity and biomass production (Dunnett et al., 2008; Dvorak et al., 2010; MacIvor et al., 2011). However, in natural systems, these qualities are themselves influenced by complex relationships between the plants and root-associated microorganisms (Gobat et al., 2004).

One of the most important groups of soil microbes are the arbuscular mycorrhizal (AM) fungi, a diverse group of symbiotic root-inhabiting fungi that form mutualistic symbioses

with more than two thirds of all terrestrial plant families (Wang et al., 2006). As obligate biotrophic organisms, AM fungi are dependent on their interaction with compatible plant hosts, from which they receive carbohydrates in exchange for providing improved access to limiting soil nutrients (Smith et al., 2010). AM colonization also improves drought tolerance (Duan 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 et al., 2006). These effects are related to the formation of a variety of inter- and intracellular fungal structures which interface with the host root cortex and exchange nutrients (arbuscules, hyphal coils), store lipids (vesicles) and transport water and nutrients (intraradical hyphae) (Peterson et al., 2004) (Fig. 3.1).

Differing abundances of these structures produced within host plant root can affect how an AM colonized plant will interact with its environment. Emanating from these interior structures are coenocytic extraradical hyphae which grow into the surrounding soil matrix, absorbing and transporting soil nutrients and water directly to the host's cortex with no cellular cross-walls or membranes to diminish the rate of uptake (Allen, 2007). These extraradical hyphae have a diameter of as little as 2 μm (Friese et al., 1991), allowing them to access the smallest of micropores. This 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 hyphae (Miller et al., 1995). Plants in water-stressed soils are therefore particularly dependant on AM fungi, given that as soil dries, water retreats into successively smaller and smaller pores, becoming unavailable to the relatively large-

diameter plant roots. AM fungal hyphae are also able to access soil water present as water films coating soil aggregates (Allen, 2009).

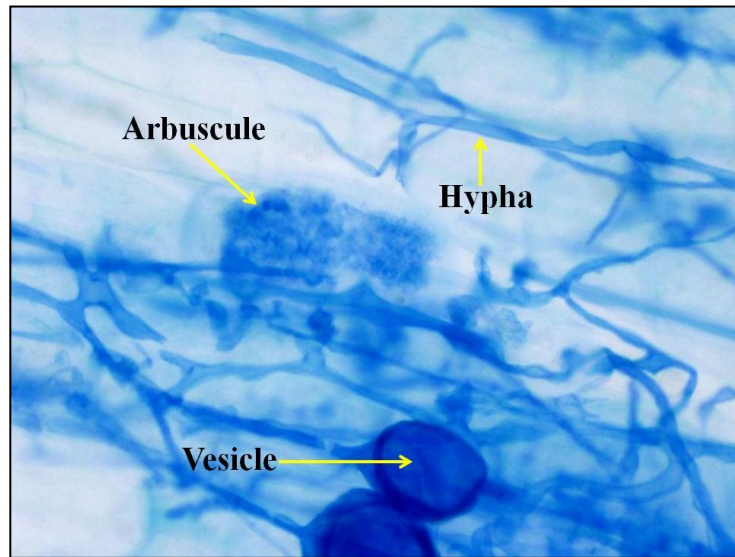


FIGURE. 3.1: Root section colonized by an AM fungus

Extraradical 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). Hyphae are also able to extend beyond the zones of nutrient depletion which quickly form around plant roots (Smith et al., 2011). As such, AM fungal associations are essential for many plant species in xeric habitats, phosphorous-poor soils and competitive plant assemblages.

Bearing in mind the goals of green roof construction, the colonization of plant roots by AM fungal is likely to be an important component of the system. To maximize their impact on urban sustainability, green roofs are designed to be both long lasting and efficient. AM fungi can improve the effectiveness of green roofs by enhancing their ability to retain stormwater and moderate indoor temperatures mainly through their

effects on plant-available soil water and transpiration rates. Further, AM fungi can increase the longevity of green roofs through their influence on the structure of the growing media. The growing medium of an extensive green roof is designed to maximize the opposing objectives of water retention and weight reduction (Beattie et al., 2004) resulting in the use of engineered media utilizing lightweight aggregates of large sizes with minimal (<10%) organic content (Thuring et al., 2010). Further, to minimize nutrient loss through leaching, slow-release fertilizers are applied (Friedrich, 2005) resulting in a highly stressful edaphic environment for vegetation which must tolerate periodic conditions of drought and consistently low nutrient availability.

In nature, AM hyphae represent a conduit through which photosynthates are rapidly transferred from the host to the mycobiont and into the soil environment (Johnson et al., 2002), with up to 15% of the soil organic carbon pool being composed of AM fungal materials (Leake et al., 2004). AM fungi are supplied largely with recent photoassimilates from their host (Johnson, 2008) and when this is coupled with the high turnover rates of AM extraradical hyphae (Staddon et al., 2003), it results in a steady flow of both labile and recalcitrant organic materials into the soil. Not only do these organic materials assist in the stabilization of soil aggregates (Rillig et al., 2006), they also supply other soil microorganisms (such as phosphorous solubilizing bacteria) with a ready supply of carbohydrates (Andrade et al., 1998; Artursson et al., 2006). The introduction of AM fungi could improve both the ability of plants to access soil nutrients and water and the quality of the growing medium itself, providing benefits for both mycorrhizal and non-mycorrhizal roof plants.

Although AM fungi are likely to be important for green roofs, no one has yet quantified the mycorrhizal colonization of green roof plants. The objective of the present research is therefore to determine the level to which plants, either commonly used or proposed for use in green roof systems, engage in the AM symbioses, both *in situ* and in the field as well as how AM fungal inoculums may be entering green-roof growing media. This will be accomplished by quantifying AM structures within host plant roots, as well as determining the AM fungal inoculum potential of commercially available green roof growing media before and after the continuous growth of various species of green roof plants.

3.2 Materials and Methods

3.2.1 Sample Collection

3.2.1.1 Field sampling

Mycorrhizal colonization was quantified for field populations of *Sedum acre* L., *Sedum spurium* M. Bieb., *Rhodiola rosea* L., *Hylotelephium telephium* L. H. Ohba., *Danthonia spicata* L., *Solidago bicolor* L., and *Poa compressa* L. Plants were sampled at a variety of locations within the Halifax Regional Municipality, Nova Scotia, during October, 2011 and June, 2012. Attempts were made to sample plant species from mixed stands where possible and at two separate locations, sampling each location both in the autumn (October) and late spring (June), although this was not possible for *H. telephium* as only a single naturally occurring population could be found. For field samples, twenty

individuals of each species were used; five individuals were collected from each site at each sampling period (two sites, two sampling periods).

S. acre was collected at the base and in the cracks of a stone wall (+44°66'88.67", -63°57'20.01") co-occurring with *P. compressa* and *Hieracium flabellovis*. Further samples were obtained from locations near Peggy's Cove, Nova Scotia, growing alone atop a boulder in less than 5 cm of soil within 35 m of the high-water mark of the Atlantic shoreline, and growing within 15 cm of soil, 15 m from the shoreline, co-occurring with *Distichlis spicata*. *S. spurium* was collected from a roadside stone outcrop (44°62'69.31", -63°57'63.36") co-occurring with *Festuca filiformis* Pourr. and *D. spicata*. Further samples were retrieved from an ocean-side sand dune near Summerville, Nova Scotia (+43°95'11.04", -64°81'80.52") within 15 m of the high-water mark; there were no other plants within 10 m of the samples. *R. rosea* was collected from roadside locations along Chebucto Head Road (+44°50'18.77", -63°53'37.85") co-occurring with *Iris versicolor* L., *Festuca rubra* L., *Solidago sempervirens* Michx. and *Plantago maritima* L. Further samples were retrieved from rock cracks within 25 m of the high-water mark near Peggys Cove, Nova Scotia, with no co-occurring species. *H. telephium* was collected from an urban wooded area in Halifax, Nova Scotia (+44°62'99.47", -63°58'14.11"), growing alongside *F. filiformis* and *D. spicata*. June samples of *H. telephium* were retrieved from the same location. *D. spicata* was sampled from the same areas as *S. spurium* (44°62'69.31", -63°57'63.36") and *H. telephium* (+44°62'99.47", -63°58'14.11"). *P. compressa* was sampled from the same area as *S. acre* (+44°66'88.67", -63°57'20.01") and adjacent to a parking lot (+44°64'26.47", -63°35'43.41"). *S. bicolor*

was sampled from a roadside location (+44°79'17.38", -63°14'98.23") and in a sparsely forested area (+44°69'72.06", -63°15'49.95").

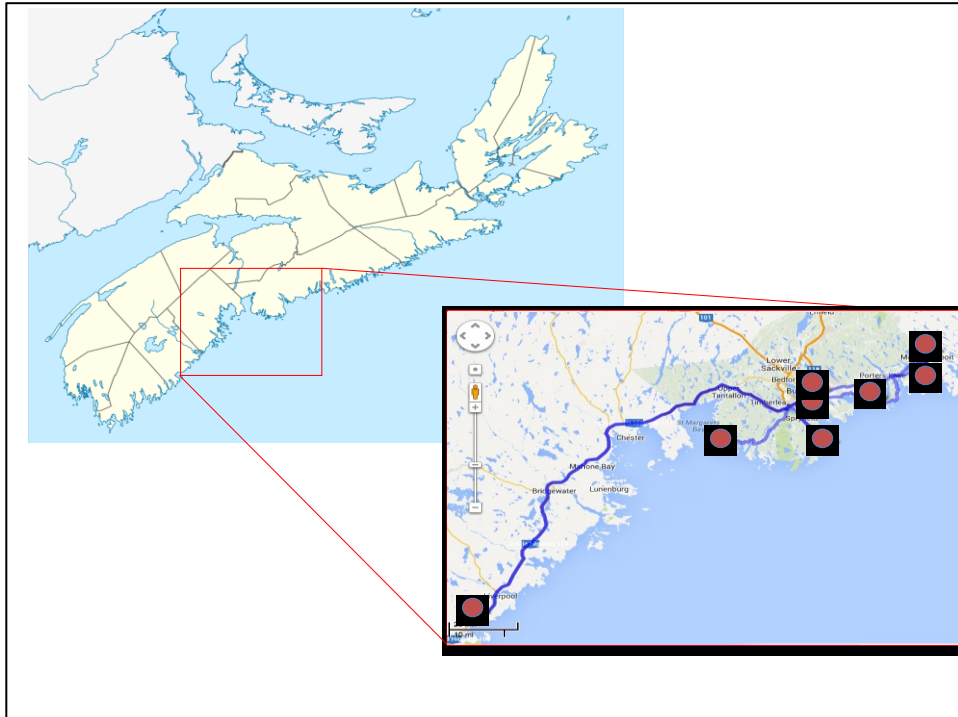


FIGURE 3.2: Map of Nova Scotia sampling locations

3.2.1.2 Green roof sampling

Mycorrhizal colonization was quantified for *S. acre*, *S. bicolor*, *P. compressa*, and *D. spicata* sampled during September, 2011 from an on-going green roof experiment conducted on the library roof of Saint Mary's University in Halifax, Nova Scotia (Lundholm et al., 2010). During the Saint Mary's green roof trial from which the samples were sourced, plants had been collected from field populations, with soil left adhering to roots systems in order to reduce transplantation stress, and propagated in modular self-contained units filled with 6 cm of growing medium (Sopraflor X, Soprema Inc., Drummondville, QC, Canada). Each module contained only a single plant species

(monoculture). After establishment, plants were not given supplementary water or nutrients, and were left to maintain themselves for four years before collection for the current study. For the green roof species, ten individuals of each species were used; two individuals were drawn from a single module, drawing from a total of five modules per species.

3.2.1.3 Bioassay sampling

The bioassay study consisted of a growth chamber bioassay experiment utilizing a commercially available growing medium (Soprema Sopraflor XTM) composed of expanded shale, sand, vegetable compost, perlite and blond peat. Four treatments of this soil were selected: 'fresh medium' which had yet to leave its sealed shipping container and three treatments which had been used in an ongoing green roof experiment (Lundholm et al., 2010). During the Lundholm et al. (2010) experiment, Soprema Sopraflor XTM had been arranged in container trays and used to propagate monocultures of field-collected plant species. For the current experiment, medium was selected which either had contained monocultures of *S. bicolor*, an obligate mycorrhizal species, or *S. acre*, a non-mycorrhizal species, or which had been left unvegetated. Additionally, controls were created by autoclaving each of the four treatments at 121°C for one hour over three separate cycles in a Tuttnauer 5596-1R-SP autoclave.

One hundred twenty five ml of each growing medium treatment was thoroughly mixed and placed in surface-sterilized freely draining 6.35 cm² trays and sown with three leek plants (*Allium porrum* L.). Leek seeds had been surface-sterilized for 45 minutes in a 0.5% sodium hypochlorite solution and rinsed in distilled water before being propagated

on sterile filter paper for 48 hours. Four trays of each treatment were arranged in blocks in a complete random block design comprising eight blocks, with treatment placement randomized within blocks to account for any climate variation present within the growth chamber. Plants were grown inside a Conviron ATC26 growth chamber for sixty days at a temperature of 22.0°C with a 16-hour photoperiod illuminated at 300 $\mu\text{Es}^{-1}\text{m}^{-1}$. Plants were watered daily with distilled water to saturation. After sixty days, plants were destructively harvested and roots were washed free of media by rinsing under tap water over a 1-mm sieve before being cut from shoots and stored in 50% ethanol.

3.2.2 Sample Preparation

All samples were prepared using the following methods. Entire plants with intact root systems were collected for assessment. Roots were severed from shoots and washed free of growing media, rinsed with tap water over a .25-cm sieve, cut into 1 cm sections and stored in 50% ethanol. Prior to assessment, roots were removed from ethanol, rinsed with distilled water and placed in 250-mL wide-bottom BernardinTM mason jars with 150 mL of 10% KOH solution. Samples were then placed in a Tuttnauer 5596-1R-SP autoclave and cleared at 121°C for 45 minutes. After clearing, roots were rinsed with distilled water to remove excess KOH and stained via a modification of the ink and vinegar technique (Vierheilig et al., 1998; Walker, 2005). Cleared roots were acidified overnight in 1% acetic acid and then placed in 150 mL of a staining solution composed of ink and acetic acid (7% acetic acid in 1:100 v/v Nobel Chinese Ink) for 12 hours. Roots were removed from the staining solution and placed in 150 mL of acidified glycerol (1:1:1 water, acetic acid and laboratory-grade glycerol) for 45 minutes. Root sections were then placed in a petri dish with enough acidified glycerol to allow them to float freely. From the petri

dish, root sections were randomly selected and arranged end to end on 75 x 25 x 1 mm microscope slides, aligned parallel to the long axis. A single drop of acidified glycerol was added to the roots and a cover slip was applied. Twelve hours of moderate pressure was added to the roots and a cover slip was applied. Twelve hours of moderate pressure was then applied to the slides in order to disperse trapped air bubbles and compress root samples for two-dimensional reference.

3.2.3 Assessment of Colonization

Samples were assessed using an OlympusTM BX43 compound microscope. Assessment was conducted using one of two protocols: the magnified intersect method (McGonigle et al., 1990) or what is being termed the ‘intense’ magnified intersect (IMI) method. The IMI method is similar to the magnified intersect method, except that at each intersect, all fungal structures are recorded. This gives an absolute value of the quantity of each AM fungal structure present at each intersect, as opposed to a presence/absence measurement. The IMI method was used only on samples collected from the green roof, and was applied to both AM fungal structures (arbuscules, vesicles, AM hyphae) and structures belonging to dark septate endophytes (DSE hyphae and microsclerotia). The MI method was used on all field and bioassay samples. As root systems of individual plants were cut into 1 cm sections, three randomly selected sections from each individual were used for a single microscope slide, with two slides being used to characterize an individual. Fifty microscopic transects were made along each 1 cm section of root, with transects being spaced at 100 μm . The colonization status of each species was therefore characterized by three thousand intersects for green roof samples (ten individuals x two subsamples x 150 intersects per subsample) and 6000 intersects for field samples (20 individuals x two subsamples x 150 intersects per subsample).

Concomitant with the assessment of colonization, morphological characteristics of AM fungal hyphae, vesicles and arbuscules were recorded in order to identify and attain a general inventory of the various Glomeromycotan genera that were present within sample roots (Abbott, 1982).

3.2.4 Data Analysis

In both the survey of green roof and field-collected plants, as well as in the growth chamber bioassay experiment, mean numbers of each AM fungal structure (vesicles, arbuscules and hyphae) were calculated for each species to allow for cross-species comparisons of all structures. Levene's test was used to gauge the homogeneity of variance, and scatter plots, box plots and histograms were used to visually inspect for normality. This was followed by analyses of variance (ANOVA) on each variable and inspection of the residuals for normality. Data on field-collected material were log-transformed as this improved the normality of the residuals. Tukey's honestly significant difference (HSD) post-hoc tests were conducted to assess significant pairwise differences ($\alpha = 0.05$) in AM fungal structure formation between species. All statistical analyses were conducted using PASW version 18.0.0 (IBM Corp.).

3.3 Results

3.3.1 Colonization of Field-Collected Samples

In field samples assessed using the MI method, no significant intra-species differences were found between sites or dates of collection for any of the analyzed species, prompting the decision to pool all species data. AM fungal colonization was observed in all species except *S. acre* (Fig. 3.3). Observed colonization status was comparable to

recorded literature values (Wang et al., 2006), except for succulent species. *S. acre* was devoid of AM fungal colonization in this study, but has been found to display sporadic AM fungal colonization by others (Harley et al., 1987). Similarly, the few studies of *H. telephium* in the literature have reported it as being a non-mycorrhizal plant (Harley et al., 1987) contrary to the findings of this study. No AM fungal colonization information for *S. spurium* could be found in the literature, but was observed to be colonized here. *R. rosea* is typically described as being non-mycorrhizal (Harley et al., 1987), but was found to be consistently colonized in this study.

Significant differences in intraradical AM fungal structure formation were viewed both between and within different host life-form groups. AM fungal colonization was significantly different between the grass species, with greater formation of all three intraradical AM fungal structures in *D. spicata* versus *P. compressa* ($p < 0.0001$; Fig 3.3). *P. compressa* showed only minimal AM fungal colonization, forming no arbuscules, very few vesicles, and minimal intraradical hyphae. Colonization was also different among succulent species, with all species showing some degree of colonization except *S. acre*, which was found completely uncolonized in all samples. There were no significant differences in the formation of AM fungal structures between *R. rosea* and *S. spurium*, while both these species formed significantly greater amounts of AM fungal structures in comparison with *H. telephium* ($p < 0.0001$; Fig. 3.3). *H. telephium*, while displaying both vesicular and hyphal colonization, was observed to be devoid of arbuscules. This may have been the result of time of sampling, location of the plants (heavily shaded beneath a forest canopy), or the ephemeral nature of arbuscules. Arbuscules were encountered most

often within *S. bicolor* and *D. spicata*, while vesicles were encountered most often within *S. bicolor* and *R. rosea*.

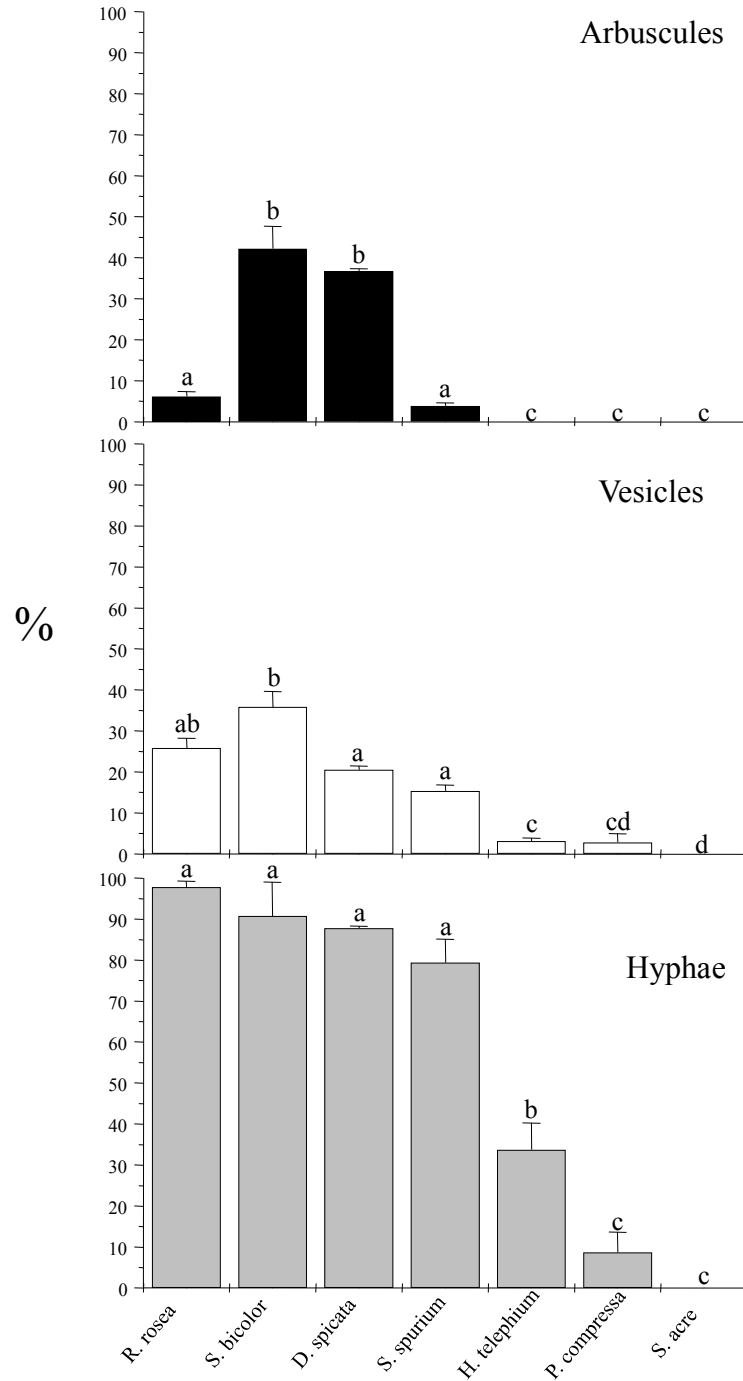


FIGURE 3.3: Percent colonization of field-collected plants

3.3.2 Colonization of Green Roof Samples

Observations obtained using the IMI method on the green roof species displayed more significant differences in AM fungal structure formation between species than had been

detected by the MI method (Fig. 3.4). Additionally, information recorded concerning colonization of roots by dark septate endophytes (DSE) revealed no distinct inter-species differences (Fig. 3.4).

In field samples analyzed with the MI method, no significant differences were observed for arbuscule or intraradical hyphae formation between *D. spicata* and *S. bicolor*. In the green roof samples, however, amounts of hyphae and arbuscules were significantly higher in *S. bicolor* than *D. spicata*, *S. acre* or *P. compressa* ($p < 0.0001$; Fig. 3.4) while the formation of all intraradical AM fungal structures were higher in *D. spicata* than *S. acre* or *P. compressa* ($p < 0.0001$; Fig. 3.4). Similar to field samples, green roof samples of *P. compressa* were sparsely colonized by AM fungi and green roof samples of *S. acre* were not colonized by AM fungi.

Formation of DSE hyphae and microsclerotia was observed in all species, although differences between species were not significant. However, there seemed to be greater, although not significant, formation of DSE hyphae and microsclerotia within *P. compressa* as compared with the other green roof species.

Given the absence of arbuscules within *P. compressa*, identification of AM fungal genera was completed only for *S. bicolor* and *D. spicata* (See Appendix A: Identification of Arbuscular Mycorrhizal Genera). *S. bicolor* was almost entirely dominated by *Glomus* spp. (including *G. tenuis*), which were identified in almost all observations. Members of the *Acaulospora* spp. constituted the remaining observations. Conversely, *D. spicata* was colonized in approximately equal proportions by *Glomus* spp. and *Acaulospora* spp.

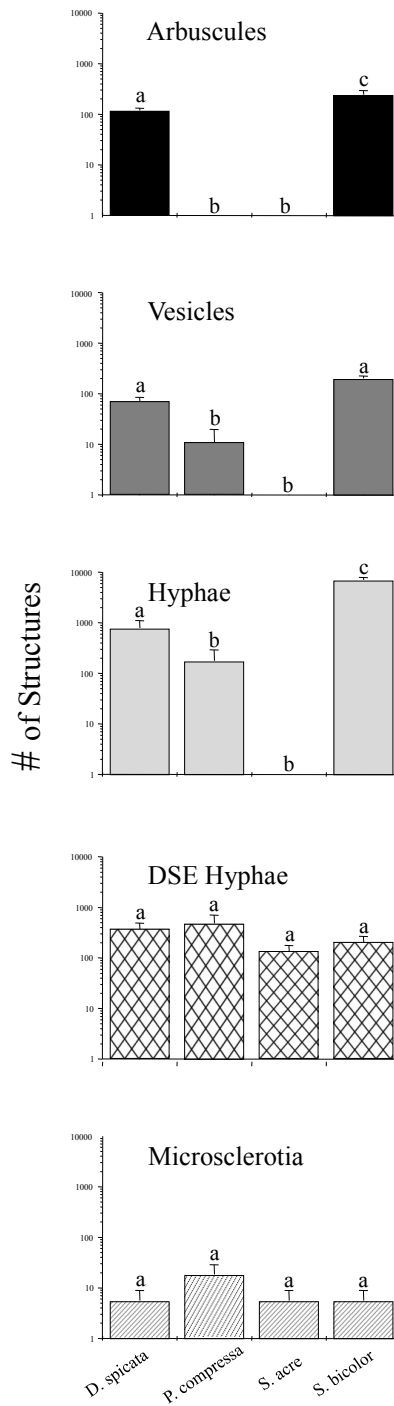


FIGURE. 3.4: Abundance of AM and DSE structures within roots of green roof plants

3.3.3 Colonization of Bioassay Samples

The formation of intraradical AM fungal structures was significantly higher ($p < 0.0001$; Fig. 3.5) in leek plants grown in growing media which had been used to propagate field-collected *S. bicolor* than in leeks from all other treatments. There were no significant differences in AM fungal colonization between the remaining three treatments. No AM fungal colonization was observed in control treatments. Arbuscules and vesicles were not observed in leeks grown in fresh growing media. AM fungal colonization was minor in all non-*S. bicolor* treatments.

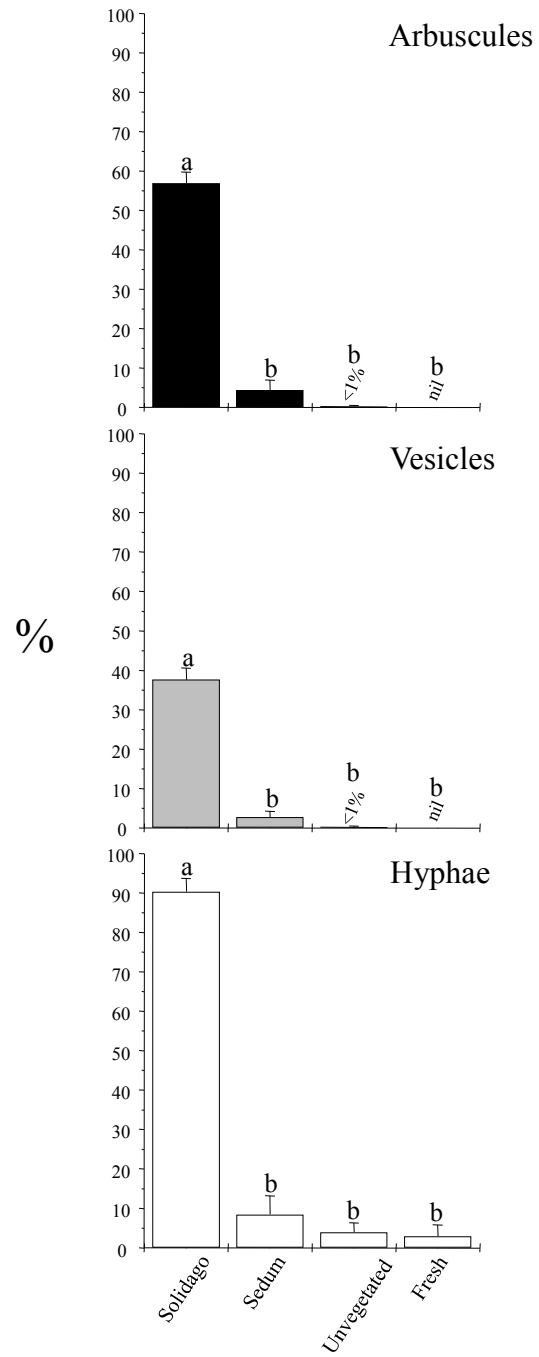


FIGURE. 3.5: Percent colonization of leeks grown in media of differing histories

3.4 Discussion

The fungal colonization of a selection of popular and proposed green roof plant species, including succulents, forbs and grasses, was assessed in both a green roof system and in the field. Literature concerning the colonization of these species is scarce, and to the best of my knowledge, this is the first study to specifically examine these symbioses under green roof conditions.

3.4.1 Colonization of Field Samples

Roots of all plants analyzed were colonized by fungi to some degree, and most formed AM symbioses with the exception of *S. acre*, which was colonized by dark septate endophytes (DSE) only. All other plant species analyzed formed classic endomycorrhizal structures, including vesicles and arbuscules, with the exception of *P. compressa* and *H. telephium*, which did not form arbuscules.

In fact, arbuscule formation was quite variable across species, with *S. bicolor* containing them at 42% of intersects and *D. spicata* containing them at 37%. In contrast, arbuscules were observed at 6% and 4% of intersects for *S. spurium* and *R. rosea* respectively. The significant differences between *S. bicolor* and *D. spicata* on the one hand, and *R. rosea* and *S. spurium* on the other, suggests that these species may have different levels of dependency upon AM fungi (Graham et al., 1991; Graham et al., 1997). Elevated formation of arbuscules indicates an active symbiosis in which bidirectional transfer of nutrients is occurring (Bago et al., 1998; Karandashov et al., 2005). Arbuscules are formed in order to transfer the nutrients absorbed by the extraradical hyphae to the host, but are also the sites of carbon transfer from the host to the fungus (Bago et al., 2000).

Being an ephemeral organ of nutrient exchange, lasting only 2-5 days before collapsing, arbuscules are indicative of recent activity both inside and outside the plant root (Toth et al., 1984; Alexander et al., 1988). The minimal formation of arbuscules within *R. rosea* and *S. spurium* may represent similarly reduced nutrient transfer. Conversely, reduced arbuscule formation has previously been observed in mature root samples, samples retrieved during periods of minimal plant growth, and samples retrieved from soils receiving chemical phosphorous additions (Brundrett, 1991). It is possible that any of these factors may have influenced arbuscule formation in this study, although there was no significant colonization variation between samples retrieved during different seasons; indeed, the sampling procedure was randomized to ensure that equal numbers of young and mature roots were sampled. Further, none of the sampling sites were located near areas likely to be receiving chemical fertilizers (e.g. golf courses, residential lawns, agricultural areas, etc.). As such, it seems likely that the two species from the Crassulaceae have minimal dependence upon AM fungi for accessing nutrients.

Vesicles, on the other hand, were observed in all field samples in which AM fungi were present, with greatest prevalence in *R. rosea*, *S. spurium*, *D. spicata*, and *S. bicolor*. Vesicle formation occurred in the grass and forb species, with the greatest numbers formed in *D. spicata* and *S. bicolor*. Vesicles were also observed in *P. compressa*, although in significantly lower amounts than in *D. spicata*. Vesicles are lipid storage structures (Olsson et al., 2005) which allow AM fungi to overwinter and maintain viability through periods of stress when they may not be receiving adequate amounts of carbon from their host. Vesicle formation within green roof plants may be necessary for

the continuing viability of the AM fungal symbioses within this environment, potentially allowing them to survive frequent periods of stress.

Nonetheless, percent colonization is not necessarily the only measure of symbiotic functionality, as the volume of soil colonized by extraradical mycelia is also important given that it accounts for the largest biomass fraction of the AM fungus, and is responsible for nutrient and water absorption. Indeed, the volume of water translocated from mycobiont to host has been more strongly linked with the volume of soil explored by extraradical hyphae as opposed to intraradical colonization. Additionally, as different soil-nutrient and climatic conditions may elicit different patterns of mycorrhizal colonization, it may be that the conditions under which the survey was conducted were not conducive to arbuscule formation in these species. Regardless, it is clear that all species analyzed, barring *S. acre*, are capable of hosting AM fungi.

3.4.2 Green Roof Samples

The results of the more intensive analysis of the green roof plant species mirrored field sample observations, with all species aside from *S. acre* forming intraradical structures and *P. compressa* failing to form arbuscules. However, while most species engaged in the symbioses, it was clear that *S. bicolor* and *D. spicata* hosted the highest levels of colonization, as evidenced by the relatively large number of arbuscules formed within their cortical cells. By calculating the quantity of intraradical structures at individual intersects, it became apparent that arbuscules and hyphae were significantly more common in *S. bicolor* than in all other plants. Vesicle formation in *S. bicolor* was also relatively high (and statistically similar to that of *D. spicata*). The formation of

intraradical structures in the green roof samples indicates that the AM fungal symbioses continue to be viable and operational within such a highly engineered substrate.

In addition to AM fungi, the presence of dark septate endophytes (DSE) was also recorded. The term DSE refers to a broad and ubiquitous group of fungi that form melanized, septate hyphae which colonize root tissue without causing a negative host response (Kernaghan et al., 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 et al., 2008), although the body of literature concerning DSE is much smaller than that of AM fungi. While the evidence is still somewhat sparse, it appears that AM fungi and DSE may compete for host resources when both colonize the same root system, although competition may be reduced through spatio-temporal stratification of colonization (Perez-Naranjo, 2009). In this study, however, no correlation was found between colonization by AM fungi and DSE. DSE colonization was observed at statistically similar levels across all plant species, irrespective of their colonization by AM fungi.

3.4.3 Bioassay of Growing Media

It was initially hypothesized that fresh growing medium would contain substantial amounts of viable AM fungal inocula and it was not assumed the media would be sterile. Indeed, when bags of growing medium were opened, they were observed to contain stem and leaf fragments belonging to various *Sedum* spp., and an actively growing unidentified moss species. However, the bioassay experiment resulted in negligible colonization of leek plants propagated in fresh media, indicating a limited supply of viable AM fungal

propagules. Statistically similar results were found for leeks propagated within growing media that had been used in the Lundholm et al. (2010) experiment, as either un-vegetated controls or to cultivate *S. acre*. Given the durability of AM fungal propagules (Smith et al., 2010), it is unlikely that many lost viability during production or transportation, suggesting that the fresh growing medium possessed minimal inoculum potential. Furthermore, it would appear that the growing medium did not accrue additional AM fungal inocula by simply remaining on the green roof, or through cultivation of *S. acre*. As the fresh growing medium had negligible colonization potential in its initial state, and this potential did not increase when the medium was left on the green roof, we can infer that natural dispersal processes failed to deliver viable propagules. It would appear that the green roof was outside the natural dispersal range of the local AM fungal populations, a situation which was probably exacerbated by the paucity of vegetated surfaces adjacent to the roof top.

Compared to fresh media, significantly higher colonization potential was observed only in media that had been used to cultivate *S. bicolor* (green roof samples of *S. bicolor* were also found to be heavily colonized by AM fungi). Given that the fresh growing medium contained minimal inoculum potential, and that this did not increase through natural dispersal mechanisms, it appears that the cultivation of *S. bicolor* was responsible for the introduction of AM fungal propagules onto the green roof.

3.4.4 Dispersal of AM Fungi into the Green Roof

In order for plants to become colonized by AM fungi, they must come into contact with viable inocula. In natural soils, uncolonized roots encounter established extraradical

hyphae, colonized root fragments, and spores, all of which serve to increase the colonization potential of their growing medium (Allen, 1991; Friese et al., 1991; Allen et al., 1992). Plants in the green roof survey, and leeks in the bioassay experiment, must have come into contact with at least one of these sources in order to become colonized.

AM fungal propagules were likely inadvertently introduced when individual *S. bicolor* plants were transplanted into the green roof. *S. bicolor* plants harvested from the green roof were derived from populations that had originally been collected from the field.

Given that *S. bicolor* readily engages in the AM symbiosis under natural conditions (as indicated by the field survey), it is likely that the original *S. bicolor* plants were colonized by AM fungi when they were harvested from the field. As these *S. bicolor* plants were eventually transplanted into the green roof, they would have introduced AM fungal inocula into the growing medium, raising its colonization potential and ensuring that succeeding generations of plants would become colonized. This same situation was likely responsible for the observed colonization of green roof samples of *D. spicata* and *P. compressa*.

If the previously described scenario is accurate, then the propagation of plants from seed, within fresh growing media, will not result in the formation of the AM fungal symbiosis.

If AM fungi are to be used within green roofs, they will have to be intentionally introduced, as natural dispersal mechanisms cannot be relied upon. Furthermore, attempts to utilize AM fungal nurse plants may prove fruitless depending on the design of the green roof. Given the modular design of the Lundholm et al. (2010) green roof, dispersal of AM fungi may be curtailed. As the inocula of AM fungi are not effectively wind dispersed (Jumpponen et al., 2012), their ability to move from module to module may be

extremely limited. Further, living AM fungal mycelia are an extremely important source of inoculum for uncolonized plant roots. Hyphae can grow at a rate of 3 mm day⁻¹, colonizing compatible root systems as they are encountered (Camel et al., 1991). Mycelia are seasonally persistent, surviving periods of both host dormancy and death, as well as episodes of low soil moisture and extreme temperatures (Parniske, 2008). However, if mycelial advance is blocked, as it would be in a modular green roof, colonization outside individual modules will be prevented. However, these problems would likely be a non-issue within a non-modular green roof.

3.4.5 Mycorrhizal Dependence and Succulents

The combination of their drought tolerance and lack of mycorrhizal dependency has led to succulents such as *S. acre* becoming over-represented within green roof plant assemblages (Getter et al., 2008). Their evolutionary history has left them well-adapted to the harsh edaphic conditions of mountainous slopes, exposed outcrops, coastal barrens and bedrock fissures (Uno et al., 1987; 't Hart, 1997; Gontcharova et al., 2009; Byalt, 2011). They display several features related to survival within the thin, coarse-textured, azonal soils, including adaptations to low soil moisture and intermittent aridity (VanWoert et al., 2005) such as: succulent tissues for water storage; thick, waxy leaf cuticles; tuberous roots or rhizomes; facultative CAM photosynthesis; and small, deeply set and stomata (Clausen, 1975; Denton, 1982; Gurevitch et al., 1986; 't Hart, 1997; Eggli, 2002; Byalt, 2011).

One of the most popular species for green roof systems is *S. acre*, a highly drought-tolerant species, which can be propagated from cuttings and grown in as little as 3 cm of

medium (Monterusso et al., 2005). While its morphological and physiological adaptations appear to have made it suitable for green roof cultivation, its relationship to soil microorganisms may be no less important. Members of the Crassulaceae generally appear to have extremely low mycorrhizal dependencies, with comparatively few species forming obligate relationships with AM fungi. This is supported by the results of the current study, as *S. acre* was observed to be uncolonized by AM fungi across multiple seasons and sites while our other Crassulaceae species exhibited minimal colonization as well. Uncolonized roots of *S. acre* were even observed with germinating AM fungal spores directly adjacent (Fig. 3.6).

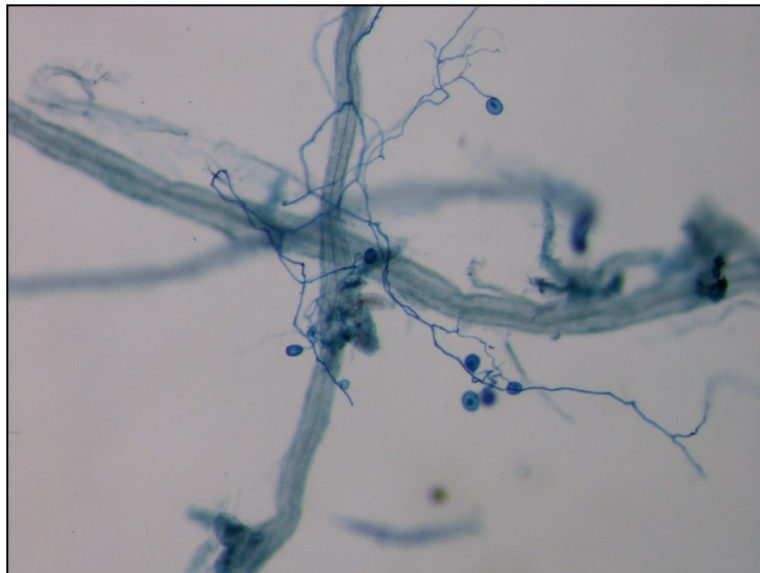


FIGURE 3.6: AM fungal spores germinating in close proximity to roots of *S. acre*

This point becomes especially interesting in the context of the green roof environment, given the results of the bioassay experiment. As the colonization potential of fresh media was extremely low, any obligate or facultative AM plants dependent upon their

mycobionts during periods of elevated stress (e.g. drought) would be at a severe disadvantage (Allen, 2007; Janos, 2007). Without the presence of their mycobionts, AM-dependent plants may be unable to access adequate amounts of water and nutrients, or compete with other organisms (Facelli et al., 2010).

Conversely, *S. acre* and other non-mycorrhizal species of Crassulaceae may not suffer these disadvantages in the AM-depauperate green-roof growing media. While the non-mycorrhizal members of the Crassulaceae are pre-adapted for survival without mycorrhizal relationships, the growth of plants with greater AM dependence may be inhibited in the green roof system, exacerbating the current over-reliance upon species of Crassulaceae. The dominance of *Sedum* spp. over plants with higher mycorrhizal dependence in green roofs is therefore likely not just a matter of their hardiness and adaptation to green roof conditions, but also due to their advantage over mycorrhizal-dependent plant species.

The above discussion is not meant to advocate the exclusion of succulents from green roof systems as green roofs designed for arid or mediterranean climates will likely still need to include succulents as major component of their vegetative assembly. However, given the potential benefits associated with plant diversity, and the inclusion of AM fungi within green roof systems, reliance upon non-mycorrhizal species such as *S. acre* may need to be reconsidered.

Alternatively, succulent species that are capable of forming AM symbioses might be utilized. The other three Crassulacean species surveyed all displayed consistent signs of AM fungal colonization. *S. spurium*, a plant already in use within green roofs, was

colonized (this is the first recorded incidence of AM colonization in this species), with both arbuscules and vesicles present. In a study of the evapotranspirative properties of several succulents, *S. spurium* was found to facilitate the greatest level of water removal from the growing medium (Berghage et al., 2007), although the amounts transpired by *S. spurium* were still an order of magnitude less than by the forb *S. canadensis* (Compton et al., 2006). Still, *S. spurium* may be a particularly useful green roof plant due to its flexible water-use strategies (Berghage et al., 2007), which allow it to conserve water while soil moisture is low and rapidly use and store water when it becomes available. Similarly, *R. rosea*, a circum-boreal member of the Crassulaceae, was observed to form AM fungal structures in the present study. This species is well adapted to the harsh edaphic conditions of bedrock fissure communities where soil texture is extremely coarse, organic content is low, and soil moisture can fluctuate rapidly (Ohba, 1975; Woodward et al., 1975).

Also, *H. telephium*, a species previously reported to be non-mycorrhizal (Wang et al., 2006), was found to consistently form vesicles (but not arbuscules) in the present study. This species may be particularly adept at surviving drought, given that it is able to store significant amounts of water within its tuberous root systems (Woodward et al., 1975). These species have been successfully used in green roofs before (Lundholm et al., 2010), and in some cases, succulents have acted as nurse plants, making water more available to less-drought-tolerant species (Butler et al., 2011). Through the utilization of succulents capable of hosting AM fungi, green roof designers can maximize the benefits of both the drought-tolerating nature of the Crassulaceae and the improved nutrient and water scavenging of mycorrhiza.

3.5 Conclusion

Considering the importance of AM fungi for their hosts, the relative sterility of growing media is an obvious barrier for green roof designers attempting to include AM-dependent plants within their vegetative layer. This is problematic given that the goals of green roof implementation revolve around the provisioning of various ecosystem services and enhancement of urban biodiversity (Oberndorfer et al., 2007). As approximately two thirds of all terrestrial plant species form the AM fungal symbiosis (Wang et al., 2006), many species will be prevented from establishing within green roof environments, seriously curtailing the potential benefits of mycorrhizal colonization.

As commercially available growing media are unlikely to contain sufficient colonization potential, and as natural dispersal methods cannot be relied upon to deliver sufficient propagules to the green roof, the integration of AM fungi into green roof systems will likely require deliberate introduction of AM fungal propagules into growing media. Methods for achieving this include the use of commercial pelletized inoculants, application of field soils with high colonization potential, integration of colonized root segments, and the propagation of nurse plants.

While commercial AM inocula have been developed, they tend to be composed almost entirely of a few *Glomus* spp., which may be a significant drawback given the distinct responses of various host species to different AM fungi (Klironomos, 2003; Jansa et al., 2008). Field soil may also be an inappropriate medium for introducing AM fungal propagules, as it may contain undesirable microorganisms (pathogens etc.) capable of

damaging green roof plants. This leaves the introduction of previously colonized plants, or plant root fragments, as the best option for introducing mycorrhizal inoculum to green roof systems.

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CHAPTER FOUR

CONCLUSION

It was the aim of this thesis to review the prospective benefits of the integration of AM fungi into green roof design and to assess the prevalence of AM fungi within a green roof system. The process of achieving these aims included three discrete tasks: 1) an evaluation and synthesis of the literature concerning both green roofs and AM fungi; 2) the quantification of the AM fungal colonization of plant roots from both the field and a green roof; and 3) an assessment of the AM fungal colonization potential of a commercially produced growing medium. The results allowed for an up-to-date summary of the relationship between various green roof plant species and AM fungi, as well as the processes allowing or preventing green roof plants from becoming colonized. These results will hopefully provide the impetus for the green roof industry to begin to include AM fungi into green roof design. I hope that this thesis will also act as a guide for future research into the maximization of green roof biodiversity and ecosystem service provisioning through the integration of mycorrhizal fungi.

The literature review provided copious examples of edaphic situations analogous to green roof conditions, in which colonization by AM fungi improved host fitness. Within a green roof system, AM fungi may be particularly useful due to their ability to enhance host drought tolerance, providing hosts with access to micropores, increasing uptake of solutes involved in osmotic adjustment, and augmenting aspects of host physiology including transpiration rate and photosynthetic capacity. Furthermore, the extensive absorptive network provided by the extraradical mycelia may help to prevent erosion and

excessive leaching of nutrients while facilitating the flow of labile and recalcitrant organic matter into growing media. Additionally, information has been compiled on various AM-induced changes to plant physiology with the potential to enhance green roof ecosystem provisioning. For example, increased transpiration rates may enhance the speed at which a growing medium can be returned to its maximum retention capacity, while greater biomass production may result in enhanced albedo. Both factors may improve stormwater mitigation and cooling functions.

Through studying the differences in AM fungal colonization between field-collected plant species, the relative levels of AM dependence of the selected plant species were determined. The measurement of AM fungal colonization of green roof plants, in conjunction with the bioassay of a commercial growing medium, allowed for the conceptualization of the dispersal mechanisms involved in AM fungal colonization of green roof plant roots. Overall, the results indicate that some of the most common green roof plants, including *Sedum acre*, *Sedum spurium*, and *Poa compressa*, have negligible dependency upon AM fungi. This characteristic likely confers no disadvantage, given that the commercial growing medium contained minimal quantities of viable AM fungal propagules, a trait that may effectively prevent the establishment of AM-dependent species. The dearth of viable AM fungal propagules within growing media may operate as a filter which increases the representation of non-AM host species at the expense of more AM-dependent plants. Essentially, this study has elucidated the potential impacts that AM fungi (or the lack thereof) may have on the diversity of green roof plant assemblages.

For the approximately 200,000 AM-dependent plant species, the absence of AM fungi may present a significant obstacle to their integration within green roof plant assemblages. Without interaction with AM fungal symbionts, AM-dependent plant species may experience reduced access to sufficient soil nutrients and water during critical periods of drought, when water is scarce and nutrients have reduced mobility within the soil solution (Gobat, Aragno, & Matthey, 2004). Many plants in arid environments rely on their AM fungal symbionts to enhance their water uptake from soil regions that are inaccessible to the plant's root system, such as micropores, and bridge the zones of nutrient depletion which form around plant roots (Allen, 2009). Considering the conditions of low soil moisture and nutrient availability inherent within green roofs, attempting to propagate AM-dependent species within green roof growing media may prove highly ineffective. Conversely, species with low AM dependence, such as the *Crassulaceae*, will experience no such obstacles.

Ultimately, the value of integrating AM fungi into green roof design may be goal-dependent, as green roofs can be constructed with a relatively small set of plant species which do not require an AM fungal mycobiont. If enhanced plant species diversity is not an objective, then AM fungi may not be required. However, it is unlikely that the ecosystem service provisioning of green roofs can be maximized without increasing the species diversity of utilized plant assemblages. It would seem that research on the suitability of plant species for use in green roofs has been hampered by inattention to soil microbiology in general, and AM fungi in particular. Assessing the ability of AM-dependent plants to survive in any given environment, without reference to the presence or absence of their AM-partner, may prevent the formulation of reliable conclusions.

Future green roof research must therefore account for the root ecology of subject plants, and determine their dependence upon, and responsiveness to, colonization by AM fungi. This research cannot depend upon previous studies, as these have tended to utilize edaphic conditions similar to those found under agricultural settings, which are highly dissimilar to green roof growing media. Instead, green roof conditions must be replicated exactly in order to attain relevant results, meaning that the various growing types of media as well as moisture conditions typical of green roofs must be replicated experimentally. Furthermore, the great diversity of host-mycobiont interactions must be considered, as different AM fungi will affect various hosts in a multiplicity of ways. Microbial synergies may come into play when multiple species of AM fungi colonize root systems simultaneously. Acknowledging this may produce greater gains than simply relying on the small number of *Glomus* spp. often used within commercial inoculants.

Furthermore, green roof studies must be conducted over longer time periods, as both plants and fungi are capable of effectively modifying their soil environments. The changes that they initiate within green roof growing media may lead to alterations in plant diversity and abundance, so that species that dominate within fresh media may decline as conditions change. Finally, the integration of AM fungi into green roof design must be analyzed on the basis of potential costs and benefits to the consumer. Given that green roofs are produced as commodities, their constituent components, including AM fungi, must add sufficient value as to outweigh their costs. Factors which may gauge whether AM fungi are cost-effective might include reduced fertilizer and irrigation requirements, lower plant mortality rates leading to less plant replacement, and decreased erosion rates, preventing excess loss of growing media. If future research can quantify

these benefits, then the potential economic benefits may prove to be the strongest impetus for the integration of AM fungi into green roofs.

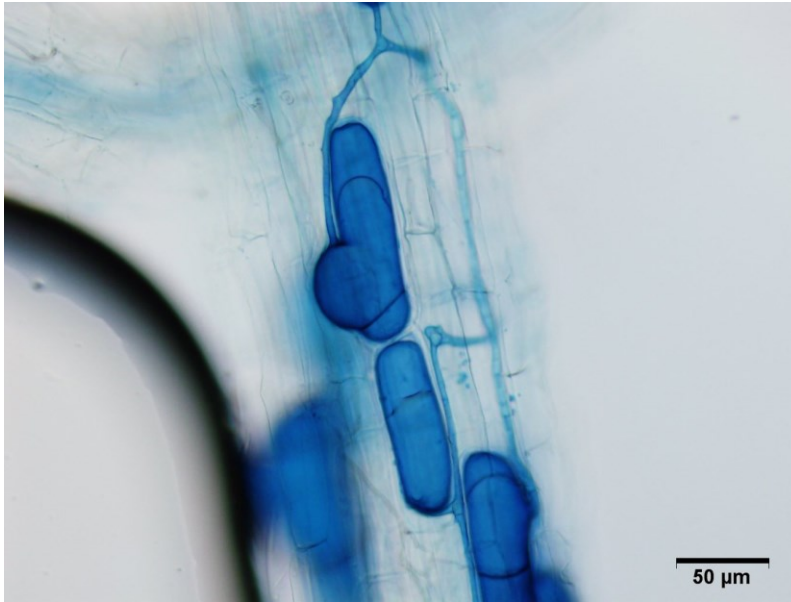
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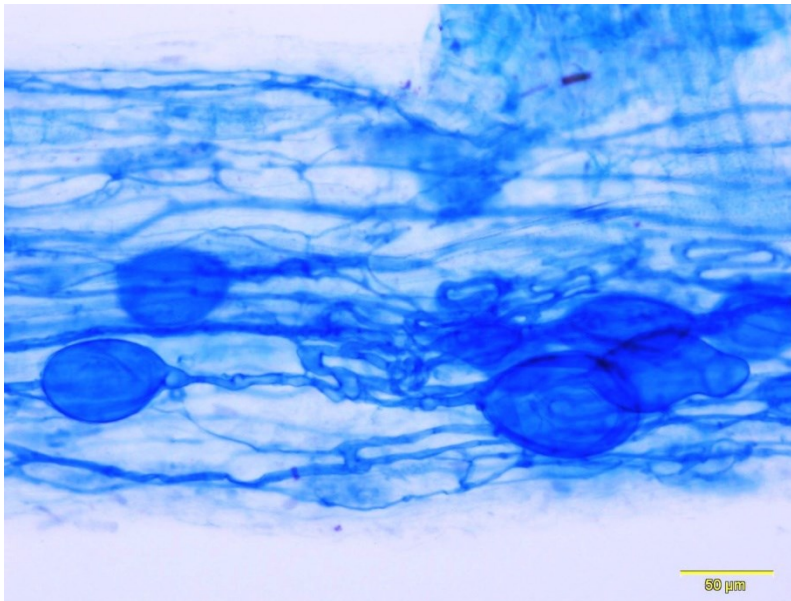
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APPENDIX A: Identification of Arbuscular Mycorrhizal Genera



Irregularly lobed vesicles of an *Acaulospora* spp. in *Danthonia spicata*.



Consistently H-branching hyphae of *Glomus* spp. in *Solidago bicolor*.

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