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Senior Seminar
Fall 2008

Mycorrhizae in the Arboretum and the Urban Environment

Mycorrhizae are tiny fungi that form symbiotic relationships with the roots of host plants. Early stages of the evolution of mycorrhizal symbiosis are traced back to the Devonian and Silurian eras of early geologic time. The fossil record suggests that associations between fungi and primitive land plants served as an important part of the plants' colonization of the land, which began during these periods (Mukerji et. al 1). Seven types of mycorrhizae are currently recognized: endomycorrhizae, ectomycorrhizae, ectendomycorrhizae, ericoid, orchid, arbutoid, and monotropoid mycorrhizae. The first two are the most common types, with many of the others being specially adapted to individual orders or families of plants. Mycorrhizal infestation results in a mutually beneficial "common life" shared between the fungus and the host plant (Wilcox 225). Mycorrhizae benefit plants by increasing nutrient and water uptake and protecting against soil borne pathogens. In exchange, the host plant provides a source of carbon for the fungus. Mycorrhizae are crucially important parts of nearly all of the earth's ecosystems due to the benefits they provide to plants. A strong fungal association increases the host plant's resistance to stress and tolerance of poor conditions. The fungal symbiosis and its formation is known to vary depending on the nature of the immediate environment. A variety of soil factors influence the formation and survival of mycorrhizae, including pH, compaction, soil nutrient content, soil moisture, and temperature.

The importance of mycorrhizae in natural ecosystems has been well documented. Since mycorrhizal associations enable host plants to thrive under all manner of stress conditions, it could logically be assumed that they are especially necessary in disturbed and high-stress environments such as those found in urban areas. At the same time, many of the stresses that affect plants in these environments are also known to influence the

formation and survival of mycorrhizae. These stressors have the potential to impact populations of fungi in an area subject to them. This leads to the question of what populations of mycorrhizae in highly stressed areas are like, especially as compared to sites with fewer obvious stresses.

This question leads to the experimental part of this research. The purpose of the study was to conduct a comparative study of populations of mycorrhizae in two different disturbed environments: the Landscape Arboretum at Ambler Campus and the urban tree pits of West Philadelphia. Though both areas have been subject to some level of disturbance in the recent past, their current conditions are visibly quite different. Trees in the arboretum are surrounded by large areas of open space, something lacking in the urban environment. Urban trees are subject to stresses such as high compaction and poor soils, whereas the soil of the arboretum is specially managed and amended for optimum plant performance. Several trees were chosen from each environment and the conditions at their sites were examined. Root samples were collected, studied, and the vesicular-arbuscular fungi present were counted and compared.

Part I: Types of Mycorrhizae

Endomycorrhizae, many of which are classified as vesicular-arbuscular mycorrhizae based on their anatomical structures, are the most common type of mycorrhizal fungi. Vesicular-arbuscular fungi make up the majority of endomycorrhizae. They are characterized by intracellular penetration of plant roots by aseptate hyphae (Wilcox 230). Their fruiting bodies are rarely visible, and often are not present at all

(Illinois Mycological Society). Hyphae of vesicular-arbuscular mycorrhizae enter the cells in the cortical regions of plant roots and colonize the space between the cell wall and the plasma membrane. In this area, the fungi form distinctive branched structures called arbuscles. These arbuscles become tightly wrapped by the plant cell's plasma membrane. Exchange of nutrients between the mycorrhizal fungus and the plant takes place at this interface. Intracellular arbuscles are short-lived structures that, depending on variety, "may grow, mature, and degenerate all within about 15 days, and at the same time as new ones are formed in other cells" (Smith & Read 393). The other distinctive feature of this kind of fungi is the presence of vesicles. Vesicles are swollen fungal end cells that form either between cells or in the cell walls of the host plant's roots (Illinois Mycological Society). Endomycorrhizae do not usually colonize the vascular and meristematic tissues of plant roots (Harrison 360).

Ectomycorrhizae are found in 140 genera of seed plants and play a vital role in many of the world's ecosystems (Mukerji et. al. 29). Most of them are classified as basidiomycetes, with a minority belonging to the phylum Ascomycota (Wilcox 226). They colonize the exterior surface of the root and form a distinctive sheath-like structure around it. The fungus then forms a structure called a hartig net, which consists of a network of hyphae. The hartig net enters intercellular spaces on the surface of the root, penetrating to the depth of a few cells (Illinois Mycological Society). Hyphae of the hartig net "undergo branching thus expanding the surface area for nutrient exchange" (Mukerji et. al. 136). Ectomycorrhizae are found where plants have adapted to soils that are lacking in nutrients. These fungi can be broadly receptive or highly specific in terms

of their associations with plants (Wilcox 226-227). Unlike Endomycorrhizae, ectomycorrhizae often produce conspicuous epigeous fruiting bodies. Some mycorrhizae feature characteristics of both end- and ectomycorrhizae, such as a fungal mantle as well as intracellular penetration in the same fungus. These fungi are classified as ectendomycorrhizae.

Other more minor subgroups of mycorrhizal fungi include ericoid mycorrhizae, orchid mycorrhizae, arbutoid mycorrhizae, and monotropoid mycorrhizae. Many of these fungi form associations with specific orders or families. Ericoid mycorrhizae are found in association with plants in the Ericaceae, Empetraceae, and Epacridaceae families. These plants often occur in extreme conditions, such as acidic soil. Instead of adding surface area to the roots and increasing nutrient absorption, these fungi “play an important role in releasing enzymes and other exudates into the substrate to make recalcitrant substance available to the plant” (Mukerji et. al. 30). Orchid mycorrhizae form associations with the roots and protocorm of orchid species, influencing seedling establishment and providing carbon to the protocorm, which lacks chlorophyll (Mukerji et. al. 31). Arbutoid and monotropoid mycorrhizae colonize the roots of plants in the order Ericales. Monotropoid mycorrhizae associate with achlorophyllous plants in the subfamily Monotropoidae. The fungi provide a source of carbon for the monotropes through a common association with green plants (Mukerji et. al. 32).

Part II: Functions of Mycorrhizae

One of the most notable and critical functions of mycorrhizae is their role in plant nutrient uptake. Fungal hyphae increase the surface area of the plant, increasing the amount of nutrients that can be taken in and translocated. This increase in surface area due to association with mycorrhizae can be very dramatic: “the effective surface area of a tree’s roots, for example, can be increased a fantastic 700 to 1000 times by the association” (Lowenfels & Lewis 61). Nutrients, both inorganic and organic, are absorbed by the fungal vegetative mycelium and then transferred “to the plant along one or more symbiotic interfaces where the two symbionts are in close contact” (Smith & Read 379). Carbon from the plant is also transferred to the fungus at these interfaces, of which VAM fungi are capable of forming at least two. These include the intracellular arbuscular interface and hyphal interfaces, which can be intra- or intercellular. The arbuscular interfaces are thought to be more transitory, with the hyphal interfaces being the more robust of the two (Smith & Read 380). Phosphorus is the nutrient most associated with mycorrhizae. This essential macronutrient is part of plant cell biochemical compounds such as sugar phosphates (intermediates of respiration and photosynthesis reactions), nucleic acids, nucleotides used in energy metabolism, coenzymes, phospholipids, and plays a key part in reactions involving ATP (Taiz & Zieger 75, 80). Potassium, zinc, magnesium, iron, and calcium uptake are also increased in the presence of strong mycorrhizal associations (Lowenfels & Lewis 61). Because of this ability to increase nutrient uptake, mycorrhizae play a crucial role in a number of the world’s ecosystems. This is especially true of those with sub-optimal conditions for plant growth and development. Nitrogen and phosphorus concentrations in the foliage of plants

grown in low-fertility sites can be equal to or exceed those found in sites with high soil nutrient content (Entry et. al. 128). In effect, mycorrhizae allow plants to succeed to a much larger extent than they would without fungal associations.

In addition to increased nutrient uptake, mycorrhizae also benefit plants by increasing the amount of moisture they are able to absorb from the soil. Mycorrhizal plants have been found to be more resistant to drought stress in many cases. In addition to increasing the surface area of roots, the fungi are thought to improve a plant's water relations by "increasing root hydraulic conductivity, increasing transpiration rate and lowering stomatal resistance" (Mukerji et. al. 62). This increase in moisture uptake provides host plants with increased resistance to drought. Plants under drought stress are also benefited by increased nutrient uptake resulting from an infestation of mycorrhizae, since nutrient availability in soil is decreased as the soil dries out (Smith & Read 159). Other functions of mycorrhizae include deterring and controlling soil pathogens and decreasing soil toxicity (Wilcox 225).

Part III: Soil Factors That Influence Mycorrhizae

Nutrient content of soils effects the formation of mycorrhizal relationships. Phosphate, in particular, is a notable variable to consider when examining the presence of mycorrhizal fungi at a site. This nutrient is a partial regulator of vesicular-arbuscular mycorrhizal symbiosis. Fungal colonization of plant roots has been found to have an inverse correlation with the plant's phosphate status (Harrison 362). In other words, high levels of phosphorus in soil result in decreased development of mycorrhizae, while low

levels of the nutrient suggest that larger populations of fungi will be present. The proportion of plant root lengths that have been colonized by mycorrhizae has been found to increase with decreasing nutrient availability (Entry et. al. 128). This limiting effect is not limited to VAM fungi; the formation of ectomycorrhizae is known to be “hampered in the presence of high P:N ratio” (Mukerji et. al. 137). While high nutrient content in a soil can limit the formation of mycorrhizae, it does not necessarily serve to arrest the fungal infestation (Slankis 438). The presence of smaller populations of fungi in soils with plentiful nutrient content suggests that fungal populations are regulated by the degree of requirement for the benefits of the symbiosis in a particular ecosystem. Predictably, a severe deficiency of inorganic nutrients such as nitrogen or phosphorus bears no ill consequence for the formation of mycorrhizae (Slankis 438).

Populations of mycorrhizae are also influenced by the pH of soil. Vesicular-arbuscular mycorrhizae vary in their responses to pH. Their response depends on the particular species of fungi: some types do well in acidic soils, others exhibit a better response in soils with a higher pH (Entry et. al. 127). Ectomycorrhizae are usually found in acidic conditions, especially in moist soils with a high content of organic matter (Entry et. al. 127). When tested, most species of ectomycorrhizae exhibit optimum growth at a pH of 4-6, and some thrived even as low as 2.7-3 (Slankis 441-442). Different strains of one species have different optimum pH ranges, which suggests that the fungi may be capable of adapting to different soil conditions. Some species show a wide range of pH tolerance, such as *Cenococcum grandiforme* which is capable of thriving in a pH range of

2.4-7 (Slankis 443). Despite this ability to function in different pH levels, soil alkalinity is known to limit the formation of mycorrhizae (Mukerji et. al (137).

Compaction is a soil factor with a strong effect on mycorrhizal fungi. It is also a major plant stressor in its own right. Compacted soils are frequently found in urban and agricultural areas where the soil has been subjected to pressure from heavy machinery or human foot traffic. Fungal structures are very fragile. Compaction can crush mycorrhizae or even drown them by creating an anaerobic environment in which they cannot function (Lowenfels & Lewis). Increased soil compaction has been found to cause a dramatic reduction in the formation of mycorrhizae (Entry et al). This damage to the fungal population places increased stress on the host plant by reducing or eliminating the benefits that a good mycorrhizal symbiosis provides.

Soil moisture is another environmental condition known to have an impact on the formation and function of mycorrhizae. Ideal moisture conditions for mycorrhizae vary by species. Many fungi, especially ectomycorrhizae, display greater abundance and increased growth under moist conditions. Other varieties are adapted to excess moisture and even flooded conditions (Slankis 444-445). Some arbuscular endomycorrhizae form associations with wetland and aquatic species (Entry et. al 125). Populations of vesicular-arbuscular mycorrhizae increase under conditions of drought, as demonstrated by an increased percentage of colonized root tissue in plants grown in a moisture deficit (Entry et. al. 125). Among wetland plants, fungal colonization has been found to increase during drier seasons (Entry et. al 125). In a comparative study of different levels of soil moisture, “higher rates of arbuscular mycorrhizal colonization were found in moist soil

compared with rates found in very dry or flooded soils” (Entry et. al 125). Colonization of roots by vesicular arbuscular mycorrhizae is at its maximum in conditions of soil moisture that supports plant growth (Mukerji et. al. 63).

The effects of excess soil moisture are more negative. Flooded conditions lead to a deficit of oxygen, which limits development of mycorrhizae as well as the roots of their host plants. If prolonged, these anaerobic conditions can lead to profound “changes in root physiology, including phosphate leakages, decreasing permeability to water and nutrients, arresting growth, and eventually killing roots” (Slankis 445). Is species adapted to waterlogged soils, fungal survival is dependent on the host plant’s ability to supply oxygen to the fungus, which depends on the physiological state of the roots (Slankis 445). Germination of mycorrhizal spores is inhibited by low concentrations of oxygen (Entry et. al. 126), which leads to a reduction in the population of mycorrhizae in flooded areas.

Temperature is a known factor in influencing mycorrhizal development. Soil temperature and soil moisture together have a strong effect on colonization of plant roots by mycorrhizal fungi. Optimal soil temperatures for the formation of most kinds of vesicular arbuscular mycorrhizae fall close to 30° C. The fungi usually form in a temperature range of between 18 and 40° C, but the influence of temperature on fungal development and symbiosis depends greatly on the specific combination of fungus and host plant, as well as the host plant’s developmental stage (Entry et. al. 126).

Part IV: Experimental Research

A total number of 11 trees representing three different species were selected for comparison in this experiment. The species initially chosen for research were the southern magnolia (*Magnolia grandiflora*), river birch (*Betula nigra*), and red oak (*Quercus rubra*). One specimen of scarlet oak (*Quercus coccinea*), a close relative of the red oak, was included in the final set of oak trees. Four trees of each species were chosen except for the southern magnolia, which is represented by three trees due to lack of availability. The experimental sets of trees each contain two specimens located in the Temple Ambler arboretum and two from the urban environment of West Philadelphia. Each tree was assigned a short number and letter code name for the purposes of organization and quick identification. A table of the trees, their locations and code names is included below (fig. 1). Tree names consist of initials of the genus and species names followed by a number. The pairs of trees were chosen for similarities in size and condition. None of them were visibly infected by pathogens at the time of selection. The trees were also chosen based on characteristics of the site. Arboretum trees were preferred to come from areas with open, unpaved soil. Most of the urban trees are located in small tree pits in sidewalks, surrounded by pavement and minimal areas of open soil. All of them exhibited a degree of compaction visible to the naked eye.

Tree Name	Species	Location
MG1	Southern Magnolia (<i>Magnolia grandiflora</i>)	Ambler Arboretum
MG2	Southern Magnolia (<i>Magnolia grandiflora</i>)	West Philadelphia
MG3	Southern Magnolia (<i>Magnolia grandiflora</i>)	West Philadelphia
BN1	River Birch (<i>Betula nigra</i>)	Ambler Arboretum
BN2	River Birch (<i>Betula nigra</i>)	Ambler Arboretum
BN3	River Birch (<i>Betula nigra</i>)	West Philadelphia

BN4	River Birch (<i>Betula nigra</i>)	West Philadelphia
QR1	Red Oak (<i>Quercus rubra</i>)	Ambler Arboretum
QR2	Red Oak (<i>Quercus rubra</i>)	Ambler Arboretum
QR3	Red Oak (<i>Quercus rubra</i>)	West Philadelphia
QC	Scarlet Oak (<i>Q. coccinea</i>)	West Philadelphia

Figure 1-Tree names, species, and locations

The first part of the experimental process was to send soil samples from the test sites to Pennsylvania State University for analysis. These tests examined soil pH and nutrient content for the test sites. Phosphorus is the primary nutrient to be addressed in this report due to its relationship to mycorrhizal formation and function. Conditions were found to vary a great deal from site to site, even between those in close proximity to each other. Urban tree samples were located in small, compacted spaces, that are visibly dry for much of the year. They were found to exhibit varying degrees of nutrient content. Sampling sites in the arboretum, in contrast, featured wide expanses of open soil in the vicinity of the trees. Like the urban sites, these areas displayed variation in nutrient content and pH. Levels of phosphorus varied; in general, the urban sites displayed higher phosphorus content than those in the arboretum, most of which fell below optimum (see fig. 2). The range of pH values at test sites is 5.7-7.2 (see attached graphs-figs. 3 &4).

Tree Name	Environment	Phosphorus Level
MG1	Arboretum	Below Optimum
MG2	Urban Philadelphia	Below Optimum
MG3	Urban Philadelphia	Optimum
BN1	Arboretum	Below Optimum
BN2	Arboretum	Below Optimum
BN3	Urban Philadelphia	Optimum
BN4	Urban Philadelphia	Optimum
QR1	Arboretum	Below Optimum
QR2	Arboretum	Optimum
QR3	Urban Philadelphia	Above Optimum
QC	Urban Philadelphia	Above Optimum

Figure 2-Phosphorus levels at Test Sites

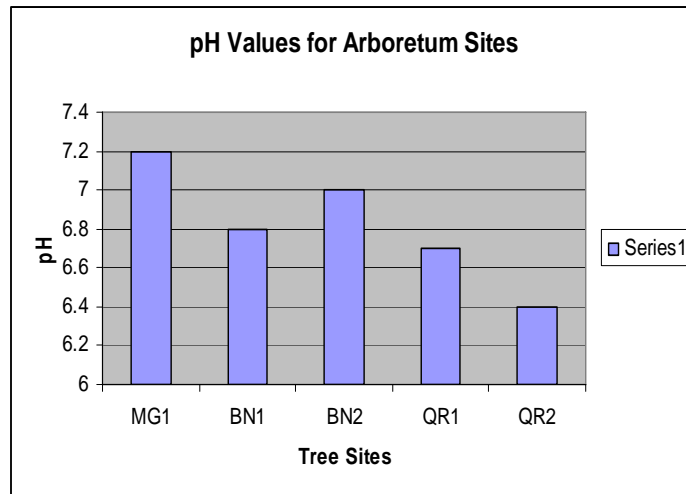


Figure 3-Soil pH in the Arboretum

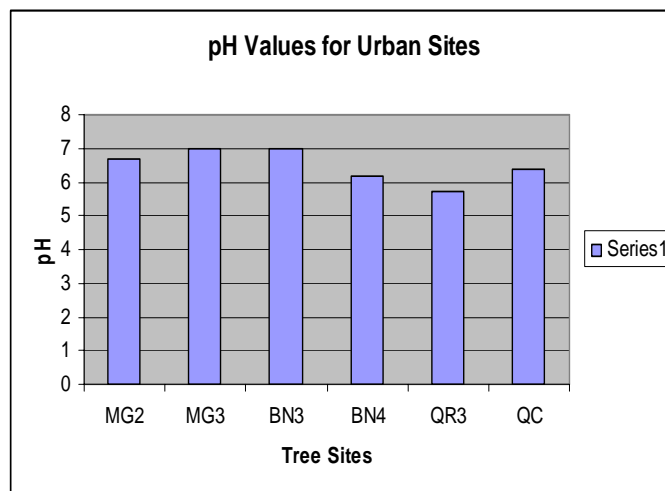


Figure 4-Soil pH at Urban Test Sites

Compaction at the sites was later tested with a device borrowed from the department. As with pH, the sites displayed a great amount of variation from one to the next. Heavily compacted conditions were found in both the arboretum and the urban sites. Compaction testing did not go as planned due to difficulty with the testing device. When inserted into soil, the compaction tester is intended to give a reading of the amount of pressure necessary to break through any compacted layers. This pressure reading is intended to be listed along with the depth of the tester’s shaft that could be sunken into

the soil. At a number of the test sites, the device did not provide a reading on how much pressure was being placed on the soil. For this reason, measurements of compaction in this report will be based on the depth of subsidence the soil displayed, as measured by how much of length of the testing device was capable of entering the soil. Though not the most accurate method for testing compaction, this partial data will provide a rough measure of the amount of compaction present at the test sites. Compaction levels are visualized in graphs below (figs. 5 & 6). A table of all of the trees sampled in this experiment and the results of all tests is included at the end of this paper (fig 22).

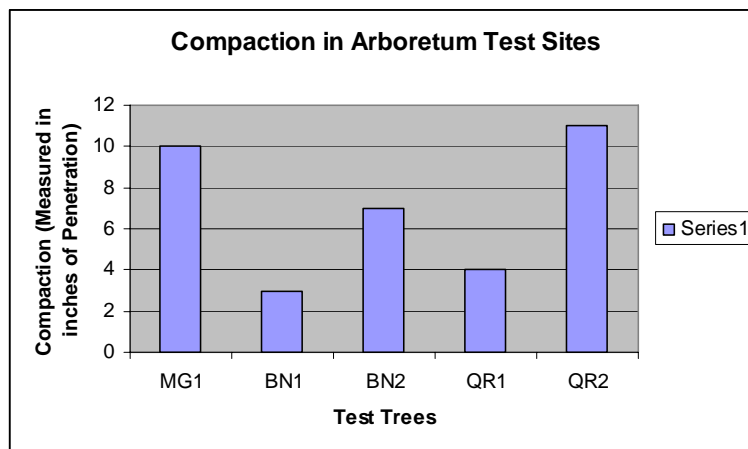


Figure 5-Soil Compaction in the Landscape Arboretum

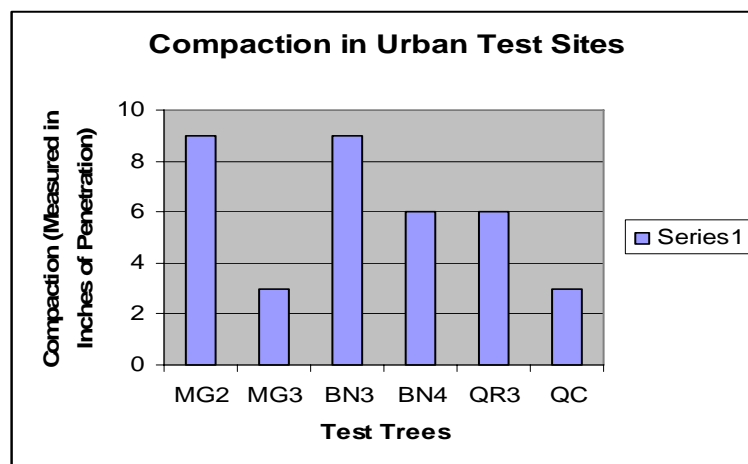


Figure 6-Soil Compaction at Urban Test Sites

The laboratory part of the experiment was based on an experimental protocol for viewing endomycorrhizae from the Association for Biology Laboratory Education (ABLE). The basic process was to clear the pigments from root samples collected at the testing sites and stain them in such a way as to make the structures of mycorrhizae visible under examination with a microscope. To accomplish these ends, root samples were rinsed, placed in a solution of 10% potassium hydroxide, and incubated in a water bath of 80° C. The incubation times required varied by species of tree being tested and the protocol was adapted to each variety of root in order to achieve the best results, each alteration being recorded for future use. In addition to varying the incubation time by species, the potassium hydroxide solution was changed at various intervals during the incubation period. This was done in order to observe the results of the process more clearly and to produce an improved record of the incubation time needed by each species.

The first set of roots tested were those of the Southern magnolia. They were incubated for a 30 minute period followed by a change of the potassium hydroxide solution and a second incubation period, also having a duration of 30 minutes. River birch was the second species to be tested, and took a longer period of time to clear. The first incubation lasted for 20 minutes, at which point the potassium hydroxide solution was nearly black in color due to the clearing of root pigments. It was discarded and replaced with fresh solution, then allowed to incubate for 50 minutes. This was followed by successive incubations of 55 and 30 minute durations, each with a fresh change of potassium hydroxide solution. Roots of the red oak were the last to be tested, and also required the longest incubation time. The first period in the water bath lasted for 30

minutes, as did the second. After this, the roots were incubated for one hour, followed by a fourth change of potassium hydroxide solution and a final incubation time of 30 minutes.

The beakers containing the roots and potassium hydroxide solution were removed from the water bath and a small amount of 30% hydrogen peroxide was added and allowed to act on the roots for 10 minutes. The bulk of root pigment clearing occurred during this part of the process. One drop of peroxide was listed as the correct amount according to the ABLE instructions, but this was varied by species after the importance of this step was observed. The magnolia roots were treated with one drop, the birch roots with several, and the oak roots with almost one milliliter. After completion of the 10 minute period, the roots were removed and rinsed prior to being placed in a solution of 10% hydrochloric acid for five minutes. At this point, the root tissue was visibly cleared of most pigments and the roots were a very pale yellow in color. They were then placed in a solution of aniline blue in 85% lactic acid and returned to the 80° C water bath for a 30 minute incubation period. All of these steps were performed as directed, and not varied by species. At the end of the process, the roots were removed from this solution and stored in 85% lactic acid until they were mounted on slides for examination.

In order to examine the fungal associations found in the tree roots, one root from each test site was mounted on a microscope slide. The roots were not sectioned prior to being mounted. In the case of the magnolia roots in particular, this proved to be impossible due to degradation of the root lignin during the clearing process. Each root sample was removed from the 85% lactic acid it had been stored in, rinsed in fresh lactic

acid, and wet-mounted in distilled water on a microscope slide. The slides were then labeled with the code name of the tree the roots were collected from and examined at different levels of magnification. Mycorrhizae were visible as blue masses inside the root cortical tissue due to the staining process. Fungal structures were visible when the slides were viewed on higher levels of magnification. Cell walls and vascular tissue of the roots are visible in most of the slides as well. Sets of photographs of each sample were taken to record images of their fungal associations and allow for further analysis outside the laboratory. These sets include photographs on magnifications of 4x and 10x.

Analysis of the fungal populations was conducted by overlaying the photographs with a grid consisting of 300 squares. Photographs taken on magnifications of 10x were chosen for analysis due to the greater area of root tissue and greater level of detail visible in them. Two photographs were used from each test site. These were chosen based on the clarity, focus, visibility of fungal tissue, and overall quality of the photograph. The fungal content of each square of the grid was recorded as total, partial, minimal, or absent depending on the percentage of fungal tissue visible. Total coverage was defined as a percentage of fungi near 100%. High coverage meant that a square was approximately 75-99% covered, and high medium coverage meant that 50-75% of the square in question was covered with stained fungal tissue. Low medium coverage meant that 25-50% of the slide was covered, low coverage was defined as up to 25%, and absent was defined as a total lack of visible fungal tissue. Each category was assigned a numerical value-5 for total coverage, 4 for high coverage, 3 for high medium, 2 for low medium, 1 for low coverage, and 0 for absent.

The sums of the numbers per photograph were then counted and added together in order to obtain numerical population data for each test site. The amount of coverage was divided by the number of squares on the grid that displayed root tissue to determine an average amount of mycorrhizae per grid cell for each slide. In cases where the root sample did not occupy the entire photograph, as seen with the tiny fibrous roots of the river birch, the average was obtained by counting the number of squares that were occupied by root tissue and using that as the dividing number. When the averages from the representative photographs were combined, an average score of mycorrhizae coverage per grid square was calculated. This provided the population data for each test site.

The visibility of fungi in photographs taken of the root samples is varied. Roots from the southern magnolia and river birch trees responded well to the experimental protocol. Mycorrhizae were clearly visible when they were examined under the microscope, and traces of the original roots pigments were scarce. Some parts of the birch roots were darker than the ideal, but most of them were still capable of yielding data when examined. In order to achieve better results in the future, the amount of hydrogen peroxide added to the roots should be increased. The red oak samples, in contrast, did not clear adequately to be examined for data due to high concentrations of tannins and other compounds present in the roots. One protocol for clearing roots to view mycorrhizae states that field-grown or older roots are often more difficult to clear for this reason. The same protocol also suggests using an autoclave in lieu of the water bath for incubation purposes (Burton). They were too dark for fungi to be visible under the microscope, with

the exception of the tiniest fragments of some root hairs. These alone would not provide adequate information to compare populations of mycorrhizae at the sites, so there is no data from the set of red oak trees in this report. One photograph used for data collection from each test site is included in this paper to provide a representation of the sort of images collected (figs. 7, 8, 9, 10, 11, 12, 13). Photographs from the red oak trees sampled are included for posterity (figs. 14, 15, 16, 17); data was not collected from them for reasons discussed above.

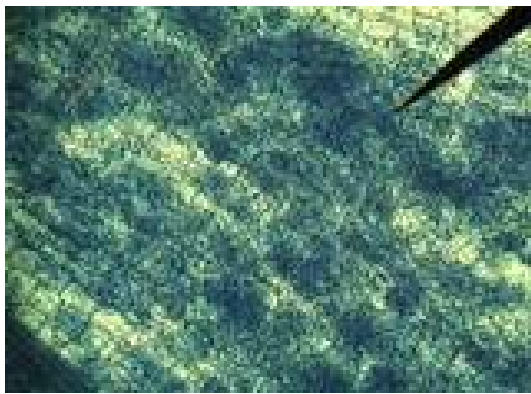


Figure 7-Arboretum Magnolia Root

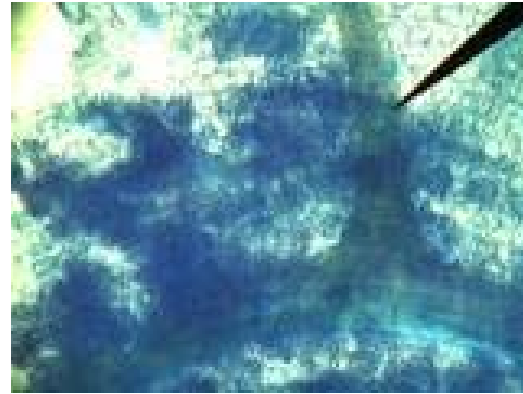


Figure 8-Urban Magnolia Root



Figure 9-Urban Magnolia Root

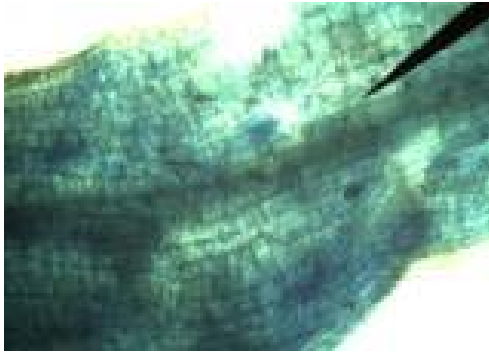


Figure 10-Arboretum Birch Root

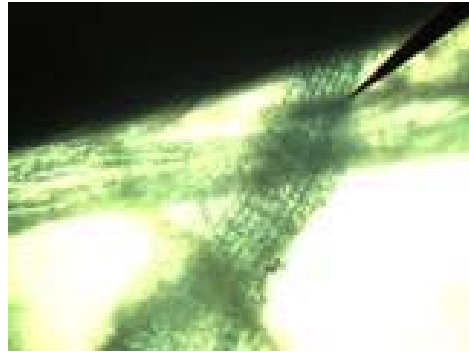


Figure 11-Arboretum Birch Root

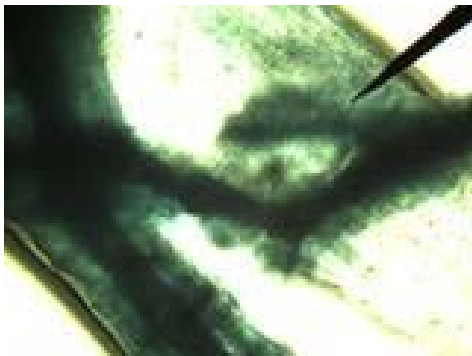


Figure 12-Urban Birch Root



Figure 13-Urban Birch Root

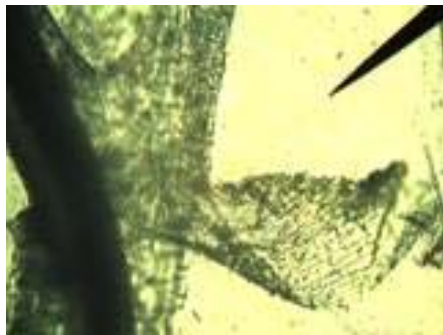


Figure 14-Arboretum Oak Root



Figure 15-Arboretum Oak Root



Figure 16-Urban Oak Root



Figure 17-Urban Oak Root

At preliminary examination, the slides of the southern magnolias appeared to display very similar populations of mycorrhizae from one test site to the next, with a slight increase in fungi present at the urban sites. Careful counting both confirmed and contrasted with this observation. The average mycorrhizae content of grid cells in the tree from the Ambler arboretum was 3.9. This represents coverage of close to 75% per square. Percentages are rounded to the nearest multiple of five for ease of graphing. The two averages from the photographs were 4.2 and 3.6. The first urban test tree had an average of 4.4, close to 90%. The average scores for both photographs from this site were 4.4. The second urban magnolia's average was 4.5, also close to 90% fungal coverage per square, taken from averages of 4.4 and 4.5. Both the average numerical scores and percentage values are represented in the graphs below (figs. 18, 19). Despite appearing very similar at first, there is a definite difference in the population of mycorrhizae between the southern magnolia roots from the two environments sampled in this experiment. Both of the trees located in urban Philadelphia displayed an average fungal population of close to 15% larger than the sample from the arboretum.

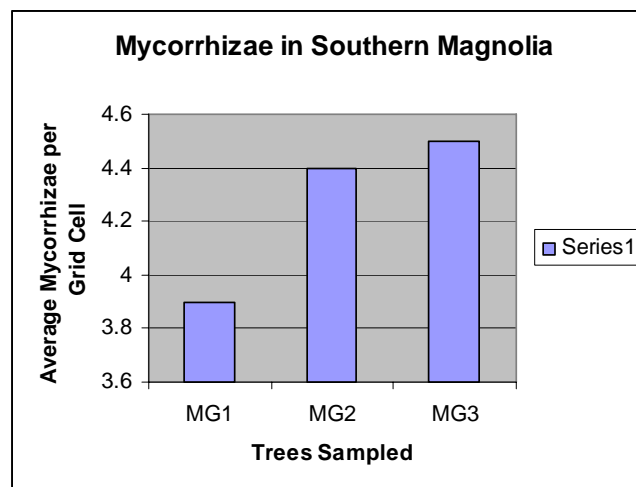


Figure 18-Mycorrhizae in Southern Magnolia

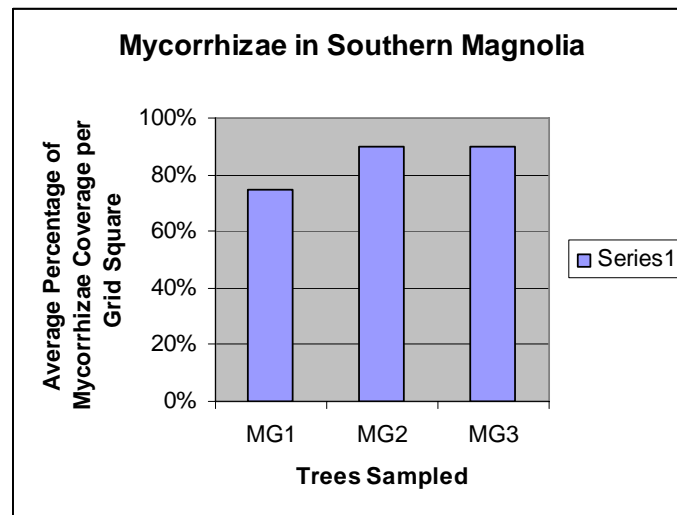


Figure 19: Mycorrhizae in Southern Magnolia

Mycorrhizae in river birches, the second set of trees, followed a similar pattern. The first arboretum tree had an average score of 3.7 per grid cell, close to 75%. This percentage came from averages of 4.2 and 3.1 from the two photos of this sample. The second tree from the arboretum also had an average of 3.7 or 75%. This same number was found to be the average of both of the photos from this tree. Average populations of fungi in the urban trees were slightly higher. The average score for the first urban birch was 4.0, averaged from 4.1 and 3.9. This is a population of close to 80% fungal coverage per square of the grid. The second urban tree had a slightly higher average score per square: 4.2, or approximately 85%. Individual scores of the two photographs were 4.3 and 4.0. There was a 5-10% difference in population of mycorrhizae between the urban trees and those located in the arboretum, with the urban trees hosting the larger number of fungi (see figs. 20, 21). The difference between populations was smaller in the river birches as compared to the magnolias that were sampled, but still visible and quantifiable.

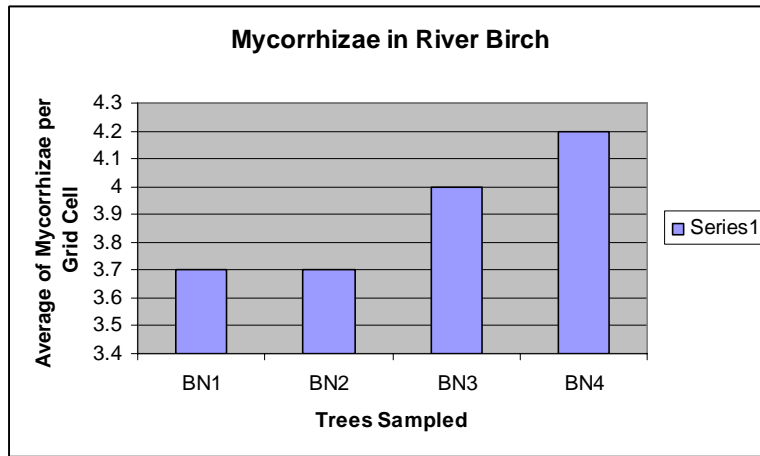


Figure 20: Mycorrhizae in River Birch

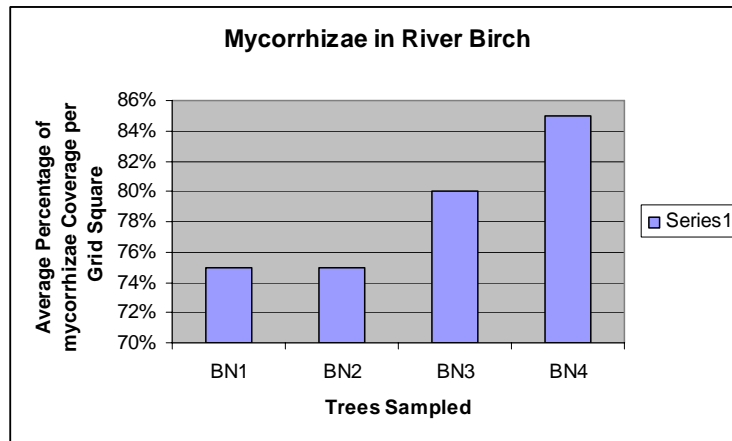


Figure 21: Mycorrhizae in River Birch

There are a number of possible reasons for the greater number of mycorrhizae in the urban southern magnolias. It is important to consider that this set of trees was hindered by an uneven number of test sites between the two locations due to availability of plants of this type, which could have potentially skewed the test results. The variance in pH and nutrient content between the sites was not large enough to account for the difference in population. One of the urban sites was heavily compacted, but this did not appear to have a major inhibiting effect on the fungi. Heavy fertilization with compost or other organic soil amendments are capable of limiting mycorrhizal development by

raising soil nutrient content (Lowenfels interview). This may be done in the arboretum, but there was no conclusive proof. Both urban sites, despite differing levels of compaction, were hosts to very similar populations of mycorrhizae. One idea as to the cause of the difference in population is the fact that mycorrhizae, being crucial to a plant's survival under stress conditions, could likely be found in greater numbers in areas where more of them are required by plants in order to thrive.

As with the southern magnolias, the river birches from urban sites proved to have larger populations of mycorrhizae. The difference in population between sites was smaller in this case, but still present and worth noting. Soil pH values at the test sites were not drastic, but the urban tree with the lowest pH (6.2) was also the one with the most fungi. One of the arboretum sites was heavily compacted, but its mycorrhizal association was nearly identical to the other arboretum tree which exhibited less compaction at its test site. Soil phosphorus levels were below optimum at both arboretum sites and above optimum at the urban sites. This would suggest that the populations of fungi should be larger in the arboretum, but this was not the case. The trees from the arboretum were surrounded by larger amounts of open space, which is known to be beneficial to the trees, but not especially influential to populations of mycorrhizae (Lowenfels interview). Soil organisms are capable of reducing mycorrhizae populations by producing unfavorable conditions for the fungi or consuming them (Lowenfels interview). This is another factor with a potential role in the arboretum's populations of mycorrhizae. In another similarity shared with the southern magnolias, all of the river birches sampled for this experiment were visibly healthy. The higher populations of fungi

at the urban sites could be a response to increased need for them in the presence of combined stresses. The large populations of mycorrhizae certainly appear to provide good results. Current information on the evolution of the fungus-green plant symbiosis suggests that plants with strong fungal association have a selective advantage and increased ability to survive conditions of all sorts (Mukerji et. al. 1). Given that the trees chosen for this research were visibly healthy despite being located in the stressful urban environment, the strong mycorrhizal associations that they exhibit are very likely to be a major factor in their success.

Mycorrhizal fungi are a crucial part of the soil food web. Their many functions- increasing nutrient and water absorption, protecting against pathogens, and increasing stress tolerance- provide invaluable benefits to green plants in every ecosystem. This is especially true of areas with poor soil quality or major plant stressors. Some plants will not germinate or reach their mature state successfully without a good mycorrhizal association (Lowenfels interview). The results of this research, showing the presence of slightly larger populations of fungi in stress-filled urban environments, demonstrate the importance of mycorrhizae in enabling plants to thrive under less than ideal conditions. Despite the presence of factors that are known to be limiting to mycorrhizae (compaction, high levels of phosphorus in the soil, etc-see fig. 22), all test sites displayed strong symbiotic relationships between plant and fungus. This could be indicative of a fungal ability to overcome these influential factors in areas where a strong association would be especially beneficial to one or both symbionts. Mycorrhizal associations from greater numbers of test sites will be compared in future research in an attempt to determine if the

pattern of increased populations of mycorrhizae in urban areas found in this pilot

experiment holds true when studied using more statistically significant numbers of trees.

Tree	Species	Environment	Soil pH	Phosphorus Level	Compaction (depth of tester penetration)	Average Mycorrhizae
MG1	Southern Magnolia	Arboretum	7.2	Below Optimum	10"	Approximately 75%
MG2	Southern Magnolia	Urban Philadelphia	6.7	Below Optimum	9"	Approximately 90%
MG3	Southern Magnolia	Urban Philadelphia	7	Optimum	3"	Approximately 90%
BN1	River Birch	Arboretum	6.8	Below Optimum	3"	Approximately 75%
BN2	River Birch	Arboretum	7	Below Optimum	7"	Approximately 75%
BN3	River Birch	Urban Philadelphia	7	Optimum	9"	Approximately 80%
BN4	River Birch	Urban Philadelphia	6.2	Optimum	6"	Approximately 85%
QR1	Red Oak	Arboretum	6.7	Below Optimum	4"	N/A
QR2	Red Oak	Arboretum	6.4	Optimum	11"	N/A
QR3	Red Oak	Urban Philadelphia	5.7	Above Optimum	6"	N/A
QC	Scarlet Oak	Urban Philadelphia	6.4	Above Optimum	3"	N/A

Figure 22-Chart of Trees, Soil Conditions, and Populations of Mycorrhizae

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