Ecological Importance of Ectomycorrhizal Linkages in the Ozark Mountains and the Fernow Experimental Forests

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Ecological Importance of Ectomycorrhizal Linkages in the Ozark Mountains and the Fernow Experimental Forest
Ecological Importance of Ectomycorrhizal Linkages in the Ozark Mountains and the Fernow Experimental Forest

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Biology

by

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Abstract

Underground stem-to-stem linkages involving ectomycorrhizal (ECM) fungi are probably important in forest ecosystems, since these linkages could assist in the survival of established trees as well as increasing the growth and development of seedlings and saplings. This study compared forest communities of the Ozark Mountains of northwest Arkansas and the Fernow Experimental Forest in West Virginia by examining species richness, diversity, relative abundance, and the potential for stems to exhibit spatial distribution and clustering patterns that reflected the existence of linkages by ECM fungi. Data on forest communities in the Ozarks were obtained from eight plot locations in Devil's Den State Park, four plots in Pea Ridge National Military Park, and three plots in the Buffalo National River Park. Data also were collected from ten plots within the Fernow Experimental Forest. The two regions were chosen for their similar topography and the overall dominance of trees, including Quercus (oaks) and Fagus (beech), that had the potential to form ECM linkages.

Euclidean distance calculations revealed that spatial relationships existed among ECM trees, seedlings, and saplings in which seedling and sapling displayed decreasing stem height with increasing distance from a tree. Furthermore, when the clustering algorithm MCLUST was applied to the ECM species in the two regions, stems tended to form clusters within 4 m of a tree or near each other. Although species richness and relative abundances of particular trees in the forest communities in the two regions were not similar, both were dominated by ECM trees, albeit belonging to different species. White oak dominated the forests in the Ozarks, whereas red oak and beech were the primary ECM trees in the Fernow Experimental Forest. ECM associations undoubtedly involved numerous fungal taxa but appeared to be dominated by the members of the genera Amanita and Russula, based on sequence data obtained from root tips and
frUITING BODYs.

In summary, this study demonstrated the occurrence of patterns of spatial distribution among ECM-forming trees, seedlings, and saplings in which the presence of the trees appeared to provide a symbiotic 'host effect' that assisted in the survival and development of smaller stems.
Acknowledgments

First and foremost, I would like to thank my adviser, Dr. Steven Stephenson. I would like to thank him for his support, guidance, knowledge, and his ability to make the field of forest ecology an exciting and attainable subject. Having Steven as an adviser made my research a unique and challenging aspect of forestry that I feel any student would be proud to take on.

I would like to thank Dr. Tom Schuler for his support during the Fernow Experimental forest survey. Thank you, Tom, for setting up room-and-board, for taking time out to show my assistant and me around the forest, and for your continued support with equipment, supplies, and the opportunity to present to your colleagues and you.

A special thanks goes out to Francis Onduso and Leah Markum for all of the work they put into helping me survey the plots within the Ozarks (Francis) and the Ozark and Fernow Experimental Forest (Leah). I wouldn't have been able to do it without you, thank you. And I would like to thank Laura Walker and Dr. Matt Smith for their help and guidance with the DNA analysis.

I wouldn't be here today if it wasn't for the support of all my teachers, assistants, and committee members, so I would also like to thank Dr. Johnny Gentry, Dr. Timothy Kral, Dr. Gary Huxel, and Cheri LaRue for believing in me and taking the time to help me reach this milestone in my life.

I would also like to extend a huge thank you to Chad Richards for believing in me and seeing my talents and making me proud of them. Your support, help, guidance, personal skills, and love is something I am forever grateful for, thank you.

Finally, it is with the greatest honor that I thank my mother, Sandra Bursick. It is with her continued belief, understanding, support, cheer, and undying love that I have found strength
and comfort through the hardest times. She raised me to be an independent, hardworking, compassionate student and I owe her the greatest of thanks for being such an astounding model. Thank you to my father, Raymond Bursick, for teaching me at a young age how important life is and to never take what you have in front of you for granted. I would also like to thank my sisters, Tausha and Raeann, for their love and kindness. Thank you to Rae, Isaiah, Kia, Jack, and my brother-in-law, Mike Roseborough, for being a wonderful part of my life and reminding me to stay amazed, young, and challenged.
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Chapter 1. Introduction

1.1 Historical Review of Forests within Eastern North America

In the early 1600s, forests in the United States occupied approximately 46% of the landscape, some 422 million hectares (1044 million acres). This area declined by more than 10% by 1992, with forests covering 32% and 298 million hectares (737 million acres) (MacCleerly 1992). The forests of the United States showed a slight increase to 33% cover in 2002 (Smith 2002), with essentially the same value (33.2%) reported in 2012 (World Bank 2012). A large portion of the forest land in the United States is found in the Appalachian Mountains of eastern North America (Figure 1.1), which occupies an area approximately 482 km (300 miles) wide and 2414 km (1500 miles) in length. Figure 1.1 indicates the full extent of eastern North America and the dominant forest types.

Although the map in Figure 1.1 indicates current forest types, it took hundreds of years of development before they got to where they are today. The forests across the United States have been sculpted and changed since the Native Americans lived off the rich and diverse natural resources these forests provided. Native Americans depended on the land for shelter, food, hunting, and agriculture. They used the land to meet the needs of the people, which in many cases resulted in cutting down trees over small areas to produce fields for crops and plant seeds.

The management of the areas was constant and required frequent burns in order to eliminate competition, reduce weed infestation, and increase the minerals in the soil. These practices were then passed down to European settlers when they first moved into the region. As populations grew, so did the need for land, crops, and homes. People depended on the forest for their survival, which meant clearing out forested regions to build homes and railways and increase agricultural development. The drop in the area of land covered by forests from the
Figure 1.1. Forest regions recognized by Braun (1950) in eastern North America (adapted from Dyer 2006).
early 1600s to the 1900s was due to this development and set the stage for the types of forests we see today.

A. History of Northwest Arkansas and the Appalachian Mountain Forests

The forests of northwest Arkansas and the Appalachian Mountains provide excellent examples of how human and environmental influences on a forest can alter its overall structure and composition. Northwest Arkansas used to have a number of prairies and open regions, some of which were probably due to clear cutting and the use of fire for controlling the growth of the underbrush. As people moved into new regions, the forest expanded into the open areas, creating dense areas of new growth. In the Appalachians, settlers also employed burning for agricultural uses (MacCleery 1992), and clear cutting was done on a dangerously large scale, which nearly eliminated all of the old-growth forests within that region. The land also was used for harvesting fruits and nuts, raising cattle, and hunting for large game animals, which further disturbed the area as grazing and browsing continued to put stress on the surrounding trees. In the end, the dominant trees within the forests were those that could withstand regular fire, undergo early succession from forest edge into prairies, and survive variable soil conditions.

Most of the early accounts of forest types and ranges of particular trees were recorded in land deeds, in county border reports, or by logging agencies. These reports were limited in number and did not describe the density or distribution of trees in the forests. As people became aware of the negative consequences to the environment deforestation caused, it became clear that something needed to be done to regulate this type of practice. One of the earliest studies of eastern North America looked into the differentiation of deciduous forests and how the dominance of certain species was a direct cause of environmental changes and determined in large part by the magnitude of the disturbance (Braun 1950). Braun (1950) traveled throughout
eastern North America and ultimately recognized nine large regions that she separated on the basis of climate and dominant trees. These were the mixed mesophytic, western mesophytic, oak-hickory, oak-chestnut, oak-pine, southeastern evergreen forest, beech-maple, maple-basswood, and hemlock-white pine forest regions. Her accounts of the areas she examined in her first study are, to date, the most extensive records of the soil composition, topography, and forest structure available. A review of the same regions (Figure 1.2) by Dyer (2006) was carried out using forest inventory data and analysis of sampled plots. When compared to Braun's map (Figure 1.1), there are noticeable overlaps in forest types, with variation only a result of Dyer (2006) using a different method for classifying forest types. Overall, eastern North America has had similar forest composition, with the dominant trees being the same between 1950 to 2006. However, these studies, although extensive and invaluable, do not address the issues surrounding the decline in oak regeneration within these regions.

B. The Oak Decline: Factors Associated with Declining Oak Regeneration

Timber harvests were commonplace during early European settlement and became a problem to national forests as human populations and timber demand increased. As a result, the oaks (genus *Quercus*), the dominant quality hardwood across America, were being clearcut at an alarming rate. Fortunately, early concerns began to surface that supplies of oak would not be able to meet the demand, and researchers started to question timber harvesting methods and their impact on the forest. Dating back as early as the 1800s, Clark (1992) describes the first reported concern for oak regeneration in the United States as the first government supported study of live oak *Quercus virginiana*, a tree commonly used in shipbuilding. This first study, although focusing on oak products and not the health of the forest, was the beginning of the important role society has in the management of forests. In the coming years, many researchers
Figure 1.2. The forest regions recognized by Braun (1950) as delimited by Dyer (2006).
carried out studies (Tool 1960, Den Uyl 1961, Holt and Fisher 1979, Oak et al. 1988, Spetich 2004) and produced books (Leffelman and Hawley 1925, Hawley 1946, Smith 1962) on the subject of declining oak regeneration, but little headway was made in reversing this trend. One of the leading problems behind developing successful forest management practices was a lack of understanding what factors led to the decline of oak regeneration in the first place. Moreover, with forests being an open system, it was not known whether purely human factors caused the problem or if there were other environmental factors to be taken into consideration.

Although disturbance factors like deforestation undoubtedly impacted oak regeneration, a certain amount of disturbance has been attributed to the growth and survival of oaks (Spetich 2004), but the level of disturbance must be controlled. Spetich (2004) suggests that competition between oaks and surrounding vegetation, coupled with an understanding of plant growth rates, success of resource acquisition, and ability to grow in unfavorable conditions (poor lighting and soil richness) should be taken into consideration before beginning any oak regeneration management practice. Furthermore, it has been suggested that studies should also extend beyond 20 years in order to take into consideration the regeneration rate of oaks (Brose et al. 2008).

Along with human disturbances, other environmental factors such as animals feeding on seedlings, climate change, diseases, infestations, predation of acorns by insects and animals, and decreased fire frequency have been linked to the decline in oak regeneration (Lorimer 2008). Climate change is a possible precursor to other disturbances such as disease, infestation, and predation as demonstrated in the rise in red oak borer in the Ozarks in association with the droughts that have taken place in the last 50 years (Tool 1960, Basset et al. 1982, Law and Gott 1987, Spetich 2004). The long droughts that took place in the Ozark Mountains in 1959,

C. Practices Used to Assist Oak Regeneration

Restoring an ecosystem to its natural conditions must involve management practices that include natural disturbances, but also limit events that are unnatural or extreme. One of the leading management practices used to enhance oak regeneration is the application of prescribed burns to reduce disease and insect populations, competition for resources, and enhance soil richness. In west central Virginia, Brose and Lear (2004), during a seasonal cycle between 1994 to 1998, conducted a study of prescribed fires on oak dominated stands to address the change in composition and structure of oaks after differing levels (low, medium, high) of prescribed burns were applied. Their study revealed an increased morality for young stems (root collars less then 0.64 cm) in all fire regimes, which indicated prescribed fires should be reserved for sites with mature stems undergoing advanced reproduction. Brose (2004) extended the concern of the previous study, stating that although prescribed fire has been shown to assist in oak regeneration, it is important to also consider stand development so that oak regeneration is not lost and reproduction of current stands is not inhibited.

The presence of prescribed fires and carefully planned management practices are important tools in addressing the decline in oak regeneration, but they are not the only resources available. As early as 1955, researchers were looking into the potential of using mycorrhizal fungi to improve the development of oaks due to the mutually beneficial symbiosis that takes place between oaks and ectomycorrhizal fungi (Imshenetskii 1955). The role of ectomycorrhizal fungi proves beneficial to the host plant by providing access to resources it would not normally have, which includes assisting developing seedlings in their early and
vulnerable stage of life (Garrett et al. 1979). The growth of plants, such as oaks, with ectomycorrhizal associations has been shown to be greater than those without (Fisher and Cox 1978). They increase access to nutrients and water (Abbot and Robinson 1984), and the increased access to phosphorus and nitrogen results in increased plant production (Koide 2002). Given these results, the application of mycorrhizal fungi to struggling ecosystems where oak regeneration is low could assist in the survival and reproduction of established trees as well as increasing the growth and development of younger stems like seedlings and saplings.

1.2 Forests Studied in Eastern North America

The two study areas used during this project, the Fernow Experimental Forest and the Ozark Mountains, are upland forest regions that occupy the central Appalachians and the Boston Mountains in northwest Arkansas, respectively (Stephenson et al. 2007). The two areas were chosen for their similar topography, the presence of oak-dominated forests, and the overall dominance of trees with potential ectomycorrhizal linkages.

The first part of the study was carried out in the upper northwest region of the Ozark Mountains (Figure 1.3), which is dominated by hardwoods, with *Quercus* (oak) and *Carya* (hickory) being the most dominant taxa present (Spetich 2002). The study sites were within Devil’s Den State Park, and Pea Ridge National Military Park, and Buffalo National River Park. Devil's Den State Park (35°46'28N, 94°14'30'W) covers a total area of 1,011 hectares with elevations reaching 301 m and characterized by a rocky terrain of sedimentary layers (National Park Service — Devil's Den 2012).

Pea Ridge National Military Park (36°27'16 N, 94°02’03' W) covers a total area of 1740 hectares, reaches a maximum elevation of 392 m, and is located in a preserved area of land dedicated to protecting the site of the American Civil War Battle of Pea Ridge (National Park
Figure 1.3. Map of Arkansas (Strausberg and Hough 1997) with star symbol indicating the three study sites, Pea Ridge National Military Park (top star to the right of highway 71), Devil’s Den State Park (left of the Ozark National Forest boundary), and Buffalo River National Park (middle of map near the right).
The Buffalo National River stretches over 241 kilometers in length, has limestone and sandstone bluffs over 152 meters high, and is noted for its varied topography: caves, springs, waterfalls, and sink holes (National Park Service — Buffalo 2012). The region of interest during this study (Buffalo National River Park — 36°04.273 N, 93°09.464 W) occupies a small portion of the river’s length but provides a good indication of species diversity.

The three localities studied make up only one portion of the vast 486,000 hectares of the Ozark National Forest but do fall within the Ozark Mountains, which is where most of the National Forest land is found (State Parks 2012). The Ozark Mountains, also called the Ozark Plateau, are the product of millions of years of geologic evolution since the continents first split from a super-continent some 750 million years ago (Clark 2008). The continued fragmentation (540 million years ago) of the continental crust split the land into pieces, infusing plates with seawater and resulted in the eventual creation of the different oceans around the continents (Clark 2008). The ocean basin spread sediments across the continents, molding large erosion-resistant bedrock, and eventually, after the ocean subsided, left deposits of rocks, fine sands, shells, and grains (Clark 2008). The rocks within the Ozark highlands are, as a result, horizontal, sedimentary, and limey (Read 1952). Northwest Arkansas experiences hot summers and cool winters with temperatures, on average, reaching as high as 32º C in the hottest part of summer, July and August, and as low as 7º C in the coldest part of winter, January (Arkansas wiki 2013).

The second part of the study was carried out in the 1,861.6 hectare Fernow Experimental Forest (Figure 1.4), located within the Monongahela National Forest near the town of Parsons in West Virginia (United States Department of Agriculture [USDA] 2012). The Fernow is
dominated by second- and third-growth Appalachian hardwoods, with *Quercus* (oak) and *Fagus* (beech) among the most dominant taxa present (Stephenson et al. 1994). Most of the landscape occupied by forests is composed of hard-fractured sandstone, softer shales, and limestones, with elevations ranging from 533 to 1,112 m (USDA 2012), and most slopes are between 10 and 60 percent (Stephenson et al. 1994). The forest climate is fairly cool, with an annual mean temperature of about 9°C, and an annual precipitation of about 147 centimeters (USDA 2012).

The two regions considered in this study have a generally similar topography, although the Fernow Experimental Forest tends to have steeper slopes. The Fernow Experimental Forest is characterized by soil types dominated by loams and silt that originated from “acid shales and sandstones on the western half of the Forest and from sandstones, shales and limestone on the” eastern half (Muzika et al. 1999). Average soil pH over the entire Forest is about 4.5 (Helvey and Kochenderfer 1991). However, Stephenson (unpublished data) recorded a mean pH of 4.1 for >100 soil samples collected in the Fernow Experimental Forest.

In northwest Arkansas, the soils are derived largely from sandstones and shales (Ware et al. 1992). Those soils that occur at higher elevations are more acid (Ware et al. 1992) and consist mostly of well drained areas of stony and sandy loams. The lower elevations are dominated by intertwined shale and sandstone located under a layer of Pennsylvanian sandstone (Stephenson et al. 2007). In northwest Arkansas, soil pH is higher than on the Fernow Experimental Forest. Stephenson (unpublished data) recorded a mean pH of 5.9 for >100 soil samples collected in the Pea Ridge National Military Park.

The next chapters will introduce mycorrhizal linkages (Chapter 2) and examine the composition and relative abundance of seedlings, saplings, and trees within the Ozarks and the Fernow Experimental Forest in central Appalachians (Chapter 2), their spatial distribution from
samples within these two forest (Chapter 2), and the fungi associated with them (Chapter 3). These chapters will provide a comparative study of the two regions with the intent of addressing oak dominance, but also emphasize how they may be assisted by the presence of their symbiotic partners, ectomycorrhizal fungi.
Figure 1.4: The surrounding border, in yellow, of the Fernow Experimental Forest in West Virginia. (USDA 2009)
Chapter 2. Mycorrhizal Linkages

2.1 Introduction

A German biologist by the name of A. B. Frank first coined the term “mycorrhizae,” which translates as “fungus-root” (Frank 1885). He hypothesized that mycorrhizae represent a pervasive mutualistic symbiosis in which fungus and host nutritionally rely on each other; that the fungus extracts nutrients from both mineral soil and humus and translocates them to the tree host; and that the tree, in turn, nourishes the fungus” (Trappe 2005).

Today, it is known that mycorrhizal associations occur in 80 to 90 percent of all vascular plants and that fossil evidence of the symbiosis between plant and fungi occurs within an Early Devonian plant, Aglaphyton major, dating back 400 million years ago (Raven et al. 2005).

The symbiotic relationship between plants and fungi involves two main types of mycorrhizal associations that are distinguished by the structures that form the physical relationship between the two. The two types of associations are endomycorrhizal and ectomycorrhizal. In both types of mycorrhizae, a network-like structure (mycelium) responsible for the symbiotic connection between the tree and fungus grows underground. The mycelium resembles a root system, as it extends from the fruiting body of a fungus to the tree roots (Figure 2.1), but it functions quite differently.

The typical morphological features of an Russula fungus (or mushroom) is illustrated in Figure 2.1. The fruiting body (consisting of cap, gills, and stalk) is above ground and the individual filaments (known as hyphae) that make up the mycelium network are below ground. Russulas are just one group of fungi belonging to a larger phylum known as the Basidiomycota (~22,300 species), but they are representative of the "typical" mushroom the average person might see (Raven et al. 2005). Additional groups of fungi are described in section 2.3.
Figure 2.1. *Russula lutea* fruiting body attached to tree roots through an underground mycelium from which the fungus is derived.
Fungal hyphae can be quite complex and contain several features that allow the filaments to travel through the humus and topsoil with relative ease. The hyphae are divided by small partitions called septa, each with central pore (perforations) that allow for a continuous flow of cytoplasm from cell to cell (Raven et al. 2005). The hyphae grow rapidly, as much as a kilometer in 24 hours, and contain cell walls that are composed of chitin, which make the fungi resistant to microbial degradation (Raven et al. 2005). The microscopic size, speed of growth, cell walls of chitin, and self-fusion of hyphae creates a highly successful web-like network that allows the fungi to reach nutrients within the soil that would otherwise be out of reach.

The network of hyphae, responsible for nutrient uptake, is one of the most important features for survival. However, the chitin within the cell walls of the hyphae prevent them from being able to consume organisms directly, a constraint they overcome by secreting (near tips of hyphae) enzymes onto a food source that then breaks up the material into a more manageable size for absorption (Raven et al. 2005). Because mushrooms cannot photosynthesize, they rely on the mycelium to spread throughout the soil in search of a symbiotic host to obtain the additional energy they need. This symbiotic relationship is achieved differently, depending on the type of fungi present, and that is what separates endo- from ectomycorrhizal fungi. The prefixes “endo” and “ecto” have a Greek origin and translate directly as “inside” and “outside”, respectively, which is indicative of how each type of fungi extracts nutrients from a particular substrate, either inside the root cell or outside it. The nature of endomycorrhizal associations will be briefly reviewed in the next section, but the primary research focus will be on ectomycorrhizal fungi.

2.2 Ectomycorrhizal Fungi

The ectomycorrhizal fungi are a small group, with only about three percent of plants
forming symbiotic associations (Stephenson 2010). Unlike the hyphae of endomycorrhizal fungi, those of ectomycorrhizal fungi do not penetrate the cortex cells within the plant roots. Ectomycorrhizal fungi create an association with a host by forming a network, known as a Hartig net, along the outside of the cortical region. The fungal mycelium is visible outside of the root tips as a thick matted layer, known as the sheath or mantle, with hair-like extensions which are visible to the naked eye.

One important characteristic of the mycelium is the placement of individual hyphae along the feeder roots of the plants. This placement is not an accident and is thought to be an attracted response to the root tip exuding substances as it grows (Stephenson 2010). The hyphae, in turn, can be seen aiding in the production of thicker, more branched roots, due to nutrient transfer; the increased diameter of the feeder root tips is clearly present. The mat of hyphae is typically about 20 to 30% of the total volume of the mantle, with the mantle being differentiated into two layers (Stephenson 2010).

The structure of the hyphae is different for each layer. The outer layer is made up of tightly packed thick-walled hyphae with few spaces, and the inner layer is composed of hyphae that make a thin-walled spacious layer (Stephenson 2010). The inner layer is the point of transfer between the fungus and the plant. Hyphae begin the symbiotic association by penetrating the inner layer of the root and then growing among the cells in the outermost part of the cortex, creating the Hartig net shown in Figure 2.2 (Stephenson 2010). The outer layer acts to increase surface area by extending outward into the surrounding soil.

Although ectomycorrhizal fungi form associations with tree root tips differently than endomycorrhizal fungi, they both provide the necessary link so that the tree and fungus can benefit from a nutrient transfer. These benefits will be examined in section 2.4, but first the
Figure 2.2. The Hartig net shown within the cortex of a tree root (adapted from Stephenson 2010b).
next section will cover the types of fungi responsible for mycorrhizal (both ectomycorrhizal and endomycorrhizal) associations.

2.3 Mycorrhizal Fungi

The phylum Basidiomycota was mentioned previously as one of four major phyla of fungi. The other three fungal phyla are Chytridiomycota, Zygomycota, and Ascomycota. The two smaller groups are the Zygomycota (~1060 species) and Chytridiomycota (~790 species), with the largest number of species belonging to the Ascomycota (~32,300 species) and Basidiomycota (~22,300 species) phyla (Raven et al. 2005). With combined species numbers in excess of 50k (arguably much larger), it would be impossible to describe each of them and their possible associations; therefore, a select few will be addressed, providing a view of the typical fungi responsible for mycorrhizal associations.

Cytrids, being a small group and predominately aquatic, do not have mycorrhizal associations with plants. Instead, they tend to be varied in structure and habitat. Some cytrids can be found living in water, while others can be found in cow dung or even existing as parasites in algae, protozoa, and other parts of plants or saprophytes (Raven et al. 2005). Their role in the plant kingdom is more of a nuisance than a symbiotic partner.

The majority of ascomycetes are fungi considered as mold and produce many of the serious plant diseases such as brown rot, chestnut blight, powdery mildews, and Dutch elm disease (Raven et al. 2005). However, the ascomycetes also include several members that form mycorrhizal associations with plants.

One of the largest groups of ascomycete fungi are the Dothideomycetes, which has over 19,000 species, are primarily pathogens and saprobes, with the exception of *Cenococcum geophilum*, which forms ectomycorrhizal associations (Dothidomycetes 2012). The ectomycorrhizal association of *Cenococcum geophilum* is demonstrated in Figure 2.3 where the
hyphae of this fungus are shown surrounding the root tips of a black oak tree.

The Pezizomycetes form another large class of fungi within the Ascomycota, with several ectomycorrhizal fungi present. The Pezizomycetes have one order, the Pezizales, which have approximately 1680 species, several of which are commonly known (morels and truffles) and can be parasitic, saprobic, and, more importantly, mycorrhizal (Kirk et. al. 2008). Several fungi that undergo mycorrhizal association belong to this order, such as Pinirhiza humarioides, P. daqingensis, P. geoporoides, and P. tricophaeoides (Wei et al. 2010). Of the more common forms, the morels are also found with mycorrhizae associations. Although once thought to be mostly saprophytic, two morels (Figure 2.4), Morel rotunda and Morel esculenta (Dahlstrom et al. 2000), have been found in vito to form ectomycorrhizal associations between Norway spruce (Picea abies).

The most common mycorrhizal associations found macroscopically and with trees belong to phyla Basidiomycota. The common name for the different groups of mushrooms that belong to the Basidiomycetes are jelly fungi, boletes, amanitas, stinkhorns, chantrelles, earth stars, puffballs, and bracket fungi (Basidiomycota 2012).

For the basidiomycetes, the typical type of mycorrhizal association is ectomycorrhizal, which have hyphae that grow on and around the outside of a host plant’s root tips (Figure 2.5). As the introduction to ectomycorrhizal fungi suggested, the visible structures that indicate the presence of a mycorrhizal association are the absence of root hairs, broadened feeder root tips, and discoloration along those tips (Figure 2.5).

Figure 2.5 illustrates the diversity of ectomycorrhizae that exists between species of plants and the types of fungi that undergo mycorrhizal associations with them. The mycorrhizal trees will be considered further in section 2.4, but first it is worthwhile to investigate the fungal
Figure 2.3 *Cenococcum geophilum* surrounding the root tips of a black oak tree.
Figure 2.4. Fruiting structure of three morel mushrooms (image used with permission of Emily Johnson).
Figure 2.5. The morphological variety in tree root tips, the subsequent mycorrhizal associations with highly varied structures, colors, and attachments. Top left image shows black oak root tips infected with a black colored mycorrhiza, bottom left are blue-white metallic colored mycorrhiza formed on red oak root tips, and the top and bottom right images indicate black colored mycorrhiza and a fruiting body from the *Russla lutela* fungi found on white oak root tips, respectively.
fruiting bodies responsible for producing the mycelium.

The fungal fruiting body is generally separated by how and what produces spores. Some groups produce spores on the surface of gills, others have spores within pockets of a protected cap, and still others produce spores on an exposed surface of the fruiting body. Spore production is also a feature of the fruiting body that is used to distinguish similar species from each other. These spores produce a variety of spore prints that can be used to determine species by color or by looking at spore ornamentation (smooth, rough, warded, etc.) under a microscope. Beyond spore production, fungi also have a variety of diagnostic features, such as the cap, stalk, "teeth", annulus, vulva, gills, pores, or the lack of any or all of these features.

The fungi illustrated in Figure 2.6 also include several species of *Aminita*, *Boletus*, and *Russula* that have mycorrhizal associations, as depicted (for *Russula*) by the hyphae attached to tree root tips in Figure 2.5. Morphologically, the various species are similar, but upon closer inspection, one can see the vastly different features pointed out previously. This now takes us to the next section which will look at the types of trees that undergo symbiotic relationships with fungi.
Figure 2.6. Fruiting bodies of several different types of fungi.
2.4 Mycorrhizal Trees

Trees that form mycorrhizal associations are separated into two groups, endomycorrhizal and ectomycorrhizal. Endomycorrhizal associations occur with maple, elm, ash, magnolia, hickory, cypress, cherry, juniper and many others, whereas ectomycorrhizal trees are oak, birch, beech, chestnut, pine, spruce, hemlock, and fir (Stephenson 2010b).

The separation of mycorrhizal trees into different associations (endomycorrhizal or ectomycorrhizal) might have something to do with the types of byproducts produced by fungal hyphae. As mentioned earlier, the mycelium produces an enzyme that is excreted into the soil or surrounding food source in order to break food down to the molecular level for absorption. Because this “digestive” process takes place outside the fungal host, the enzymes mix with the surrounding soil and add to the soil whatever byproduct is produced during the breakdown of the larger organism. This, in turn, creates a much different soil environment for tree roots than would otherwise be the case without the fungus present. Arbuscular (endomycorrhizal) fungi are known to produce a glycoprotein called glomalin, which is thought to be a major source of carbon in the soil (Hijri and Sanders 2005). This glycoprotein could have a greater importance to trees such as maple, ash, and hickory, which have endomycorrhizal associations. Of course the production of carbon in the soil is not unique to endomycorrhizal fungi, but it could be a drawing source.

Several ectomycorrhizal fungi are known to produce lignolytic and proteolytic enzymes, which allow them to dissolve organic matter, and these could be enzymes that also mutually benefit ectomycorrhizal trees (Lucas and Casper 2008).

The exact role of endo- and ectomycorrhizal fungi on trees is not entirely understood, and there continues to be considerable debate on how these associations take place, why they are tree
specific, and what exact mutual benefit each receives because of the association. This last point takes us to section 2.5, *The Importance of Mycorrhizal Associations*, which will address some of the ongoing research into the importance of mycorrhizal associations between plants (specifically trees) and fungi.

### 2.5 The Importance of Mycorrhizal Associations

Many studies have shown that mycorrhizal associations between fungi and trees are so important that the lives of the two organisms depend on it. This is believed to be a result of the reduction of environmental stresses on plants due to the presence of the fungal partner (Stephenson 2010). The advantage to having a fungal association is an increased surface area of roots (increased access to nutrients) provided by the fungal association, which can also assist other surrounding plants. Although studies have shown that proximity to linked plants is beneficial to surrounding plants, there is still much debate on how close a plant would need to be in order to take advantage of the symbiotic relationship. This is further fueled by studies that have found plants, regardless of type, can share a connection between one fungal host (Perry et al. 1989, Read et al. 1985, and Read 1988).

These underground connections help with the transfer of carbon and nutrients from mycorrhizal fungi to nearby plants. Amaranthus and Perry (1994) and Perry et al. (1989) also found that negative interactions caused by competition between species was reduced by the presence of mycorrhizal fungi. This was thought to be a result of the fungal hyphae providing increased nutrient availability, access to different soil depths, and active transport during periods of environmental stress (heat, water loss, etc.).

In addition to the benefits of access to increased soil and other sources of nutrients for plant roots by hyphae, there is a symbiotic relationship that exists between the tree and fungus.
Because the fungus cannot photosynthesize, it needs to obtain sugar and other nutrients that provides the energy it needs from an outside source. The fungal hyphae naturally attach themselves to the ends of growing root tips that readily allow for a linkage to be established. This linkage then allows the photosynthesizing tree to "share" the energy it acquires through the hyphal connection. The fungus has the benefit of having a direct link to glucose, and the tree has the benefit of increased surface area of its roots, which gives it access to water and other nutrients it would not otherwise have.

The next benefit of having a symbiotic relationship relates the different nutrients that are both produced in and absorbed by the mycelium in the soil. Not only does the mycelium break down organic matter and absorb that into the hyphae, which are attached to roots, but it also absorbs “N, P, K, Ca, S, Cu, and Zn from the soil and translocate[s] there to the associated plants” (Habte 2000). The plants with mycorrhizal associations also benefit from an increased rate of absorption, as they have their own root system and that of the hyphae working for them.

The biggest benefit, however, is the increased depth of soil the plant roots can reach due to the fungal hyphae. Generally, the rate of growth for plant roots is slow, placing a limit on the depth they can reach, which makes for a small area for them to undergo nutrient absorption. This area, known as the root zone, can quickly become depleted through competition, erosion, drought, or other natural stresses, which makes survival problematic (Habte 2000). However, if a plant has a mycorrhizal association, its roots are no longer limited to the nutrient-poor root zone and can get access to other sources. This gives these plants a major advantage over those who do not have the association.

The remaining chapters will address the regions of interest (Chapter 3), the forest composition and communities in the regions (Chapter 4), and the potential spatial distribution
(Chapter 5) and clustering (Chapter 6) caused by linkages between the regions ECM fungi (Chapter 7), trees, seedlings, and saplings.

Chapter 3. Regions of Study

3.1 Introduction to Sampling Methods

As mentioned previously in the introductory chapter, the first part of the study reported therein was carried out in the upper northwest region of the Ozark Mountains, specifically within the Buffalo National River Park, Devil's Den State Park, and the Pea Ridge National Military Park (Figure 3.1). The Buffalo National River stretches over 241 km in length; however, the region of interest during this study (36° 04.273 N, 93° 09.464 W) occupies a small portion of the river’s length but provides a good indication of species diversity.

Devil's Den State Park (35° 46'28"N 94° 14'30"W) covers a total area of 1,011 ha with elevations reaching 301 m and characterized by a rocky terrain of sedimentary layers (Devil's Den State Park 2009). Pea Ridge National Military Park (36° 27'16"N 94° 02'03"W) covers a total area of 1740 ha, reaches an maximum elevation of 392 m, and is located in a preserved area of land dedicated to protecting the site of the American Civil War Battle of Pea Ridge (National Park Service — Pea Ridge 2012). A 10 x 10 m plot size was used to collect data on the forest communities within the Ozarks. The plots were placed in localities throughout the general study area where the forests present contained a mixture of both ectomycorrhizal (ECM) and non-ECM trees. Study sites were selected from GIS-based forest vegetation maps. Plots were placed in areas of relatively uniform topography, well away from forest edges and with no and with no evidence of recent major disturbance (e.g., treefall gaps). Each plot was delimited by a large fiberglass measuring tape and two smaller fiberglass cross tapes. Units (meters and centimeters), which were obtained using a handheld distance measuring device, were marked on
Figure 3.1. Map of the state of Arkansas (Foti 2011), with the Ozark Mountains displayed in deep green and the three study locations, Pea Ridge (top yellow star), Devil's Den (yellow star below and to the left of Pea Ridge), and the Buffalo National River Park (yellow star to the right of Devil's Den) indicated.
the tapes to map the exact (within ± 1 centimeter) locations of all trees (stems ≥10 cm DBH), small trees (≥2.5 cm but <10 cm DBH), saplings of tree species (<2.5 cm but ≥1 m tall) and what were (for the purpose of the present study) considered to be “established” seedlings of tree species (≥10 cm but <1 m tall). The addition of two fiberglass cross tapes allowed for easy separation of the large 10 x 10 m plot into more manageable 5 x 5 m subplots for stem counts. The large (50 m) tape was intertwined through the stakes set at each of the four corners that marked the outside border of the plot. Accuracy in the placement of plots was assisted by an electronic laser-guided (ELG) measuring device, which used a receiver and digital measuring pad to determine exact distances within the plot. The ELG device calculates distance by using an ultrasonic wave to transmit light between two devices and uses the time it takes to bounce this wave between them to calculate distance.

Once the plot border and area were established, the next step involved species identification for all stems within the plot, which was done by first recording their placement in the plot, making an identification, and then determining height or diameter at breast height (DBH). Stem placement along the plot was determined with the help of an assistant who, using the 50 m tape for reference, would record the approximate placement of a stem along the tape line. The within plot measurement was taken using the ELG device by placing the ELG receiver above a stem within the plot and having the assistant hold the measuring pad at the 50 m tape plot line and recording the distance, in meters, within the plot. Finally, a meter stick was used to obtain the height in centimeters or, in the case of a tree, a diameter tape was used to record the diameter at breast height (DBH). Each stem’s species was identified (to the extent possible), and its DBH (trees and small trees) or height (saplings and seedlings) was recorded on a field data sheet designed for the project.
There were a total of fifteen plots placed within the Ozarks. This included eight within Devil's Den State Park, four within Pea Ridge National Military Park, and three within the Buffalo National River Park. Each location was chosen to reduce disturbance effects, but because they were located within parks, there were some features (tree gap control, controlled burns, and hiking paths) that were unavoidable. In particular, Pea Ridge National Military Park was visited through two different seasons, which resulted in one of the plots (summer 2011) not having a recent burn while the other three (summer 2012) had undergone recent controlled burns. Because of this unplanned disturbance, additional burned sites were also observed in the Fernow Experimental Forest such that a comparison between the two regions could be made.

The second part of this study was carried out in the 1,862 hectare Fernow Experimental Forest, located in the Monongahela National Forest near the town of Parsons in West Virginia (United States Department of Agriculture [USDA] 2012). During the 1994 field season, Stephenson et al. (1994) established 105 permanent plots within the Fernow Experimental Forest. Data on the composition of forest communities obtained in the present study were collected from several of these permanent plots. An effort was made to encompass as wide a range of forest types as possible, including those with beech present along with oaks; oaks are the dominant taxa present in this region of eastern North America (Stephenson et al. 1994). Collecting data involved setting up 5 x 5 m plots at seven different localities (Stephenson study sites 6a, 6b, 10, 13, 21, 25, and 66) in areas along Fork Mountain, Camp Hollow, Stone Lick and Wilson Hollow roads and along Canoe Run and within the Otter Creek Wilderness (Figure 3.2). The high stem density in the Fernow Experimental Forest required using a smaller plot size so that a better sample reflecting the overall composition and structure could be obtained. Three additional plots were located off John B. Hollow Road, which correlated with hot-burn (repeats of 4 to 5
years) study sites.

For consistency and reduction of disturbance effects, each site was positioned such that the plot placement was along the contour of the slope, away from road edges, and within a slope range of 10 to 30 percent. Using a 50 m tape, plot stakes, and an electronic measuring device, plot arrangement, location of trees, seedlings, saplings, their height or DBH, and species name was recorded the same as northwest Arkansas with the exception of using a 5 m square plot.
Figure 3.2. Map of the Fernow Experimental Forest (USDA-Fernow 2011). Note: the yellow line delimits the forest and blue lines indicate forest roads. The approximate locations of study sites are indicated with stars.
3.2 Difference in Seedling and Sapping Coverage for Northwest Arkansas and the Fernow Experimental Forest

A. Introduction

The dominant tree species in northwest Arkansas and west central West Virginia have been members of the genus *Quercus* (oak) from as early as there are records until today. One of the earliest reports on the forests of the Ozarks was made by an English naturalist, Thomas Nuttall, in 1819 (USDA 2010). He described the area as dominated by bottomland hardwoods, bald cypress, large areas of grass prairies, pine woodland, and a dominance of gum, hickory, and oak trees in the uplands (USDA 2010). During this era, forests covered nearly 95 percent of the land, a figure that fell to approximately 70 percent in the late 1920s after railway production and civilization movements began (USDA 2010). Current trends, as of 2010, put Arkansan forests at 56 percent forested, which comprises 33.3 million acres and is estimated to be 18.8 million acres of oak and other hard woods with 41 percent softwoods. The northwest region of Arkansas is a complex mosaic of pastures and forests characterized by mixed dominance of pine/oak, various hardwoods, and some hardwood/cedar communities (see USDA 2010 Figure 3.2). The overall region has continued to show a dominance of white oak, southern and northern red oak, and hickory, as was noted in 1819. However, despite the continued dominance of oak, in recent years there have been several studies indicating a decline in oak regeneration, but little is known of the cause.

Just like the Ozarks, in the early 1950s and late 1960s, much of the Fernow Experimental Forest was dominated by hardwoods with some red spruce and hemlock (Core 1966), with many of the species being logged and removed for civilian purposes. Early surveys at that same time, between 1951-1959, showed a dominance of oaks, especially northern red oak and chestnut oak, where later, in 1987-2009, the dominance appeared to have shifted to red and sugar maple
In 1999, a study of 105 plots within the Fernow Experimental Forest was carried out to quantify the composition in the overstory and understory, and this study found that the dominant overstory species was northern red oak, with sugar maple, beech, and red maple dominating the understory (Muzika et al. 1999). Again, this region shows a decline of oak regeneration by a reduced dominance in the understory.

The next section will compare the structure and composition of the seedling and sapling strata of selected study areas in the Ozark Mountains and the Fernow Experimental Forest within the central Appalachian Mountains in order to determine the relative diversity and abundance of the species present and to assess the level of oak dominance in these forests in an effort to quantify the extent of the oak decline.

B. Structure and Composition of the Seedling and Sapling Strata in Northwest Arkansas

As already noted, acquiring the stem counts and abundance of species throughout northwest Arkansas was carried out in a number of individual plots within Devil's Den State Park, Pea Ridge National Military Park, and the Buffalo National River Park. Pooled data from all plots within this region are provided in Table 3.1, which provides an overall view of species distribution. Each of the individual study areas will be considered in greater detail below, which will provide a per-region analysis of the distribution of species for the selected areas within the Ozarks.

The first location, the Ozark Mountains region of northwest Arkansas (Table 3.1) is characterized by a wide range of species. Relative abundance (Table 3.1) was calculated for all plots within northwest Arkansas by adding the counts obtained for each species separately and then combining these values to obtain a final species count. The final species count was then divided by each species and multiplied by 100 to derive its percent relative abundance.
Furthermore, values of total abundance, by size class (Table 3.2), were compiled to distinguish seedling and sapling abundance by height within the region. The height ranges in Table 3.2 were used to broaden the range of values observed and to allow a clear separation of seedlings, saplings, and trees within the plot.

The composition of species and their relative abundance for eight sites selected within Devil's Den State Park are presented in Table 3.3. Relative abundance was calculated using the same methods as used for the entire northwest Arkansas region (i.e., adding the counts obtained for each species separately, combining these values to obtain a final species count, and then dividing the final species count by each species and multiplying by 100). A total abundance by size class (Table 3.4) was also constructed to broaden the range of stems observed and to allow a clear separation of seedlings, saplings, and trees within Devil's Den State Park.

The relative abundance (Table 3.5) and total abundance by size class (Table 3.6) were also constructed for Pea Ridge to emphasize the species present and to broaden the range of stems observed, thereby allowing for a clear separation of seedlings, saplings, and trees. Finally, the relative abundance (Table 3.7) and total abundance by size class (Table 3.8) tables were produced for the Buffalo National River Park.
Table 3.1. Total stem counts and relative abundance (%) for plots sampled in the Ozark Mountains of northwest Arkansas. Nomenclature follows USDA (2013).

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Taxon</th>
<th>Stem counts</th>
<th>Relative abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maple</td>
<td><em>Acer spp.</em></td>
<td>680</td>
<td>19.2</td>
</tr>
<tr>
<td>White oak</td>
<td><em>Quercus alba</em></td>
<td>481</td>
<td>13.6</td>
</tr>
<tr>
<td>Ash</td>
<td><em>Fraxinus spp.</em></td>
<td>358</td>
<td>10.1</td>
</tr>
<tr>
<td>Black oak</td>
<td><em>Quercus velutina</em></td>
<td>343</td>
<td>9.7</td>
</tr>
<tr>
<td>Hickory</td>
<td><em>Carya spp.</em></td>
<td>301</td>
<td>8.5</td>
</tr>
<tr>
<td>Black cherry</td>
<td><em>Prunus serotina</em></td>
<td>250</td>
<td>7.1</td>
</tr>
<tr>
<td>Elm</td>
<td><em>Ulmus spp.</em></td>
<td>209</td>
<td>5.9</td>
</tr>
<tr>
<td>Northern red oak</td>
<td><em>Quercus rubra</em></td>
<td>199</td>
<td>5.6</td>
</tr>
<tr>
<td>Post oak</td>
<td><em>Quercus stellata</em></td>
<td>141</td>
<td>4.0</td>
</tr>
<tr>
<td>Serviceberry</td>
<td><em>Amelanchier arborea</em></td>
<td>93</td>
<td>2.6</td>
</tr>
<tr>
<td>Blackjack oak</td>
<td><em>Quercus marinlandica</em></td>
<td>74</td>
<td>2.1</td>
</tr>
<tr>
<td>Black gum</td>
<td><em>Nyssa sylvatica</em></td>
<td>73</td>
<td>2.1</td>
</tr>
<tr>
<td>Flowering dogwood</td>
<td><em>Cornus florida</em></td>
<td>66</td>
<td>1.9</td>
</tr>
</tbody>
</table>
Table 3.1. Continued.

<table>
<thead>
<tr>
<th>Tree Type</th>
<th>Scientific Name</th>
<th>Frequency</th>
<th>Relative Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eastern redbud</td>
<td><em>Cercis canadensis</em></td>
<td>61</td>
<td>1.7</td>
</tr>
<tr>
<td>Eastern red cedar</td>
<td><em>Juniperus virginiana</em></td>
<td>61</td>
<td>1.7</td>
</tr>
<tr>
<td>Hackberry</td>
<td><em>Celtis occidentalis</em></td>
<td>57</td>
<td>1.6</td>
</tr>
<tr>
<td>Southern red oak</td>
<td><em>Quercus falcata</em></td>
<td>46</td>
<td>1.3</td>
</tr>
<tr>
<td>Persimmon</td>
<td><em>Diospyros virginiana</em></td>
<td>29</td>
<td>0.8</td>
</tr>
<tr>
<td>Chinkapin oak</td>
<td><em>Quercus muehlenbergii</em></td>
<td>8</td>
<td>0.2</td>
</tr>
<tr>
<td>Sassafras</td>
<td><em>Sassafras albidum</em></td>
<td>7</td>
<td>0.2</td>
</tr>
<tr>
<td>Sweetgum</td>
<td><em>Liquidambar styraciflua</em></td>
<td>2</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>3540</td>
<td>100</td>
</tr>
</tbody>
</table>

*Acer* spp. includes *Acer rubrum* and *Acer saccharum*; *Carya* spp. includes *Carya cordiformis*, *C. ovata*, and *C. tomentosa*; *Ulmus* spp. includes *U. alata* and *U. americana*; *Fraxinus* spp. includes *F. americana* and *pennsylvanica* (but mostly the former).
Table 3.2. Total stem counts by size class for plots sampled in the Ozark Mountains of northwest Arkansas.

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Stem size class (cm)</th>
<th>Trees</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 to 6</td>
<td>6 to 12</td>
<td>12 to 20</td>
</tr>
<tr>
<td>Ash</td>
<td>5</td>
<td>23</td>
<td>54</td>
</tr>
<tr>
<td>White oak</td>
<td>2</td>
<td>21</td>
<td>30</td>
</tr>
<tr>
<td>Eastern redbud</td>
<td>8</td>
<td>28</td>
<td>21</td>
</tr>
<tr>
<td>Hackberry</td>
<td>1</td>
<td>15</td>
<td>26</td>
</tr>
<tr>
<td>Black oak</td>
<td>3</td>
<td>20</td>
<td>11</td>
</tr>
<tr>
<td>Southern red oak</td>
<td>2</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td>Hickory</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Elm</td>
<td>6</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Eastern red cedar</td>
<td>0</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>Flowering dogwood</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Black cherry</td>
<td>0</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Northern red oak</td>
<td>0</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Chinkapin oak</td>
<td>0</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Persimmon</td>
<td>0</td>
<td>0</td>
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</tr>
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Table 3.2. Continued.

<table>
<thead>
<tr>
<th></th>
<th>0</th>
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<th>0</th>
<th>0</th>
<th>2</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serviceberry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sassafras</strong></td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><strong>Blackjack oak</strong></td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>28</td>
<td>145</td>
<td>199</td>
<td>171</td>
<td>20</td>
<td>52</td>
<td>615</td>
</tr>
</tbody>
</table>

*Acer* spp. includes *Acer rubrum* and *Acer saccharum*; *Carya* spp. includes *Carya cordiformis*, *C. ovata*, and *C. tomentosa*; *Ulmus* spp. includes *U. alata* and *U. americana*; *Fraxinus* spp. includes *F. americana* and *pennsylvanica* (but mostly the former).
Table 3.3. Stem counts and relative abundance for plots sampled in Devil's Den State Park in northwest Arkansas.

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Stem counts</th>
<th>Relative abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maple</td>
<td>708</td>
<td>29.7</td>
</tr>
<tr>
<td>White oak</td>
<td>313</td>
<td>13.2</td>
</tr>
<tr>
<td>Black oak</td>
<td>278</td>
<td>11.7</td>
</tr>
<tr>
<td>Black cherry</td>
<td>238</td>
<td>10.0</td>
</tr>
<tr>
<td>Ash</td>
<td>220</td>
<td>9.2</td>
</tr>
<tr>
<td>Northern red oak</td>
<td>185</td>
<td>7.8</td>
</tr>
<tr>
<td>Hickory</td>
<td>145</td>
<td>6.1</td>
</tr>
<tr>
<td>Black gum</td>
<td>73</td>
<td>3.1</td>
</tr>
<tr>
<td>Serviceberry</td>
<td>72</td>
<td>3.0</td>
</tr>
<tr>
<td>Elm</td>
<td>44</td>
<td>0.1</td>
</tr>
<tr>
<td>Flowering dogwood</td>
<td>39</td>
<td>1.6</td>
</tr>
<tr>
<td>Persimmon</td>
<td>38</td>
<td>1.6</td>
</tr>
<tr>
<td>Southern red oak</td>
<td>8</td>
<td>0.3</td>
</tr>
<tr>
<td>Sassafras</td>
<td>6</td>
<td>0.3</td>
</tr>
</tbody>
</table>
Table 3.3. Continued.

<table>
<thead>
<tr>
<th>Tree Type</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eastern red cedar</td>
<td>5</td>
<td>0.2</td>
</tr>
<tr>
<td>Post oak</td>
<td>3</td>
<td>0.1</td>
</tr>
<tr>
<td>Hackberry</td>
<td>3</td>
<td>0.1</td>
</tr>
<tr>
<td>Sweetgum</td>
<td>2</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2380</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

*Maple (Acer spp.) includes Acer rubrum and Acer saccharum, Hickory (Carya spp.) includes Carya cordiformis, C. ovata, and C. tomentosa. Elm (Ulmus spp.) includes U. alata and U. americana. Ash (Fraxinus spp.) includes F. americana and pennisylvanica (but mostly the former).
Table 3.4. Total stem counts by size class for plots sampled in Devil's Den State Park in northwest Arkansas.

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Stem size class (cm)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>Trees</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 to 6</td>
<td>6 to 12</td>
<td>12 to 20</td>
<td>20 to 60</td>
<td>60 to 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maple</td>
<td>170</td>
<td>412</td>
<td>99</td>
<td>25</td>
<td>2</td>
<td>0</td>
<td>708</td>
</tr>
<tr>
<td>White oak</td>
<td>23</td>
<td>110</td>
<td>76</td>
<td>30</td>
<td>1</td>
<td>73</td>
<td>313</td>
</tr>
<tr>
<td>Black oak</td>
<td>18</td>
<td>85</td>
<td>99</td>
<td>59</td>
<td>3</td>
<td>14</td>
<td>278</td>
</tr>
<tr>
<td>Black cherry</td>
<td>57</td>
<td>149</td>
<td>16</td>
<td>3</td>
<td>1</td>
<td>12</td>
<td>238</td>
</tr>
<tr>
<td>Ash</td>
<td>44</td>
<td>111</td>
<td>26</td>
<td>38</td>
<td>1</td>
<td>3</td>
<td>220</td>
</tr>
<tr>
<td>Northern red oak</td>
<td>1</td>
<td>59</td>
<td>59</td>
<td>35</td>
<td>1</td>
<td>30</td>
<td>185</td>
</tr>
<tr>
<td>Hickory</td>
<td>4</td>
<td>57</td>
<td>51</td>
<td>8</td>
<td>1</td>
<td>24</td>
<td>145</td>
</tr>
<tr>
<td>Black gum</td>
<td>2</td>
<td>30</td>
<td>32</td>
<td>2</td>
<td>1</td>
<td>6</td>
<td>73</td>
</tr>
<tr>
<td>Serviceberry</td>
<td>21</td>
<td>32</td>
<td>8</td>
<td>2</td>
<td>0</td>
<td>9</td>
<td>72</td>
</tr>
<tr>
<td>Elm</td>
<td>9</td>
<td>12</td>
<td>10</td>
<td>11</td>
<td>0</td>
<td>2</td>
<td>44</td>
</tr>
<tr>
<td>Flowering dogwood</td>
<td>10</td>
<td>11</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>15</td>
<td>39</td>
</tr>
<tr>
<td>Persimmon</td>
<td>5</td>
<td>33</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>38</td>
</tr>
<tr>
<td>Southern red oak</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Sassafras</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
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</table>
Table 3.4. Continued.

<table>
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<th>Tree Type</th>
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<th>2</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eastern red cedar</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Post oak</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Hackberry</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Sweetgum</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>368</td>
<td>1107</td>
<td>484</td>
<td>219</td>
<td>11</td>
<td>191</td>
<td>2380</td>
</tr>
</tbody>
</table>

*Maple (Acer spp.) includes *Acer rubrum* and *Acer saccharum*, Hickory (Carya spp.) includes *Carya cordiformis*, *C. ovata*, and *C. tomentosa*. Elm (Ulmus spp.) includes *U. alata* and *U. americana*. Ash (Fraxinus spp.) includes *F. americana* and *pennsylvanica* (but mostly the former).
Table 3.5. Stem counts and relative abundance (%) for plots within Pea Ridge National Military Park in northwest Arkansas.

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Stem counts</th>
<th>Relative abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northern red oak</td>
<td>141</td>
<td>24.2</td>
</tr>
<tr>
<td>Elm</td>
<td>130</td>
<td>22.3</td>
</tr>
<tr>
<td>Hickory</td>
<td>119</td>
<td>20.4</td>
</tr>
<tr>
<td>Blackjack oak</td>
<td>74</td>
<td>12.7</td>
</tr>
<tr>
<td>White oak</td>
<td>37</td>
<td>6.3</td>
</tr>
<tr>
<td>Black oak</td>
<td>24</td>
<td>4.1</td>
</tr>
<tr>
<td>Eastern red cedar</td>
<td>21</td>
<td>3.6</td>
</tr>
<tr>
<td>Serviceberry</td>
<td>19</td>
<td>3.3</td>
</tr>
<tr>
<td>Flowering dogwood</td>
<td>15</td>
<td>2.6</td>
</tr>
<tr>
<td>Ash</td>
<td>2</td>
<td>0.3</td>
</tr>
<tr>
<td>Black cherry</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Hackberry</td>
<td>1</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Table 3.5. Continued.

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>583</td>
<td>100</td>
</tr>
</tbody>
</table>

*Maple (Acer spp.) includes Acer rubrum and Acer saccharum, Hickory (Carya spp.) includes Carya cordiformis, C. ovata, and C. tomentosa. Elm (Ulmus spp.) includes U. alata and U. americana. Ash (Fraxinus spp.) includes F. americana and pennisylvanica (but mostly the former).
Table 3.6. Total stem counts by size class for plots sampled in Pea Ridge National Military Park in northwest Arkansas.

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Stem size class in (cm)</th>
<th>Trees</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 to 6</td>
<td>6 to 12</td>
<td>12 to 20</td>
</tr>
<tr>
<td>Northern red oak</td>
<td>75</td>
<td>30</td>
<td>9</td>
</tr>
<tr>
<td>Elm</td>
<td>88</td>
<td>31</td>
<td>6</td>
</tr>
<tr>
<td>Hickory</td>
<td>26</td>
<td>28</td>
<td>35</td>
</tr>
<tr>
<td>Blackjack oak</td>
<td>13</td>
<td>30</td>
<td>11</td>
</tr>
<tr>
<td>White oak</td>
<td>5</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>Black oak</td>
<td>5</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Eastern red cedar</td>
<td>5</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Serviceberry</td>
<td>8</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Flowering dogwood</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Ash</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Black cherry</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Hackberry</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>225</td>
<td>139</td>
<td>79</td>
</tr>
</tbody>
</table>

*Species names are the same as those listed in Tables 3.1-3.5.*
Table 3.7. Stem counts and relative abundance for plots sampled in Buffalo National River in northwest Arkansas.

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Stem counts</th>
<th>Relative abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash</td>
<td>137</td>
<td>22.3</td>
</tr>
<tr>
<td>White oak</td>
<td>131</td>
<td>21.3</td>
</tr>
<tr>
<td>Eastern redbud</td>
<td>61</td>
<td>9.9</td>
</tr>
<tr>
<td>Hackberry</td>
<td>53</td>
<td>8.6</td>
</tr>
<tr>
<td>Black oak</td>
<td>41</td>
<td>6.7</td>
</tr>
<tr>
<td>Southern red oak</td>
<td>38</td>
<td>6.2</td>
</tr>
<tr>
<td>Hickory</td>
<td>37</td>
<td>6.0</td>
</tr>
<tr>
<td>Elm</td>
<td>35</td>
<td>5.7</td>
</tr>
<tr>
<td>Eastern red cedar</td>
<td>35</td>
<td>5.7</td>
</tr>
<tr>
<td>Flowering dogwood</td>
<td>12</td>
<td>2.0</td>
</tr>
<tr>
<td>Black cherry</td>
<td>11</td>
<td>1.8</td>
</tr>
<tr>
<td>Northern red oak</td>
<td>11</td>
<td>1.8</td>
</tr>
</tbody>
</table>
Table 3.7. Continued.

<table>
<thead>
<tr>
<th>Species</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinkapin oak</td>
<td>8</td>
<td>1.3</td>
</tr>
<tr>
<td>Persimmon</td>
<td>2</td>
<td>0.3</td>
</tr>
<tr>
<td>Serviceberry</td>
<td>2</td>
<td>0.3</td>
</tr>
<tr>
<td>Sassafras</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>615</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

*Maple (Acer spp.) includes Acer rubrum and Acer saccharum, Hickory (Carya spp.) includes Carya cordiformis, C. ovata, and C. tomentosa. Elm (Ulmus spp.) includes U. alata and U. americana. Ash (Fraxinus spp.) includes F. americana and pennsylvanica (but mostly the former).*
Table 3.8. Total stem counts by size class for plots within Buffalo National River Park in northwest Arkansas.

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Stem size class (cm)</th>
<th></th>
<th></th>
<th></th>
<th>Trees</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 to 6</td>
<td>6 to 12</td>
<td>12 to 20</td>
<td>20 to 60</td>
<td>60 to 100</td>
<td></td>
</tr>
<tr>
<td>Ash</td>
<td>5</td>
<td>23</td>
<td>54</td>
<td>48</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>White oak</td>
<td>2</td>
<td>21</td>
<td>30</td>
<td>62</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Eastern redbud</td>
<td>8</td>
<td>28</td>
<td>21</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hackberry</td>
<td>1</td>
<td>15</td>
<td>26</td>
<td>10</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Black oak</td>
<td>3</td>
<td>20</td>
<td>11</td>
<td>6</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Southern red oak</td>
<td>2</td>
<td>17</td>
<td>11</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Hickory</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>15</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Elm</td>
<td>6</td>
<td>3</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Eastern red cedar</td>
<td>0</td>
<td>4</td>
<td>11</td>
<td>8</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>Flowering dogwood</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>7</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Black cherry</td>
<td>0</td>
<td>7</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Northern red oak</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chinkapin oak</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Persimmon</td>
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<td>0</td>
<td>0</td>
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<td>0</td>
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</tr>
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</table>
Table 3.8. Continued.

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<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serviceberry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sassafras</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Blackjack oak</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>28</td>
<td>145</td>
<td>199</td>
<td>171</td>
<td>20</td>
<td>52</td>
<td>615</td>
</tr>
</tbody>
</table>

*Maple (Acer spp.) includes Acer rubrum and Acer saccharum, Hickory (Carya spp.) includes Carya cordiformis, C. ovata, and C. tomentosa. Elm (Ulmus spp.) includes U. alata and U. americana. Ash (Fraxinus spp.) includes F. americana and pennsylvanica (but mostly the former).
C. Analysis of Relative Abundance and Size Class Distributions for Northwest Arkansas

Looking at the abundance (Table 3.2) and relative abundance (Table 3.1) data for all plots within northwest Arkansas, stems from 6 to 12 cm are the most abundant, with the most abundant taxon being maple (680). However, because the majority of maples are below 12 cm, it is doubtful many will reach the sapling or tree stage, as indicated by the reduced counts shown in this region, with only 99 in the 12 to 20 size class, 25 in the 20 to 60 size class, and a total of 2 red maples between 60 and 100 cm. An interesting reverse trend is seen for oaks, whose total population is low below 6 cm, only 143 between 2 and 6 cm, but increases in numbers, to 387, as heights from 6 to 12 cm are reached, and stem counts remain high with 321 from 12 to 20 cm, 253 from 20 to 60 cm, 23 in the 60 to 100 size class, and 157 trees. The relative abundance for maple is 19.2% for 680 stems, whereas the corresponding figure for oak is 36.2% for 1284 stems. Given their distributions by size class, it would appear that despite the abundance of red maple in northwest Arkansas, the dominant trees are likely to remain oaks due to the large number of stems above 20 cm. Beyond the dominance of maple and oak, hickory, with 265 stems and 13.6% relative abundance, is worth mentioning. Hickory has a more even distribution among the lower size classes, where the numbers fall below 40 stems between 2 to 6 cm and 20 to 60 cm, but stem counts are 85 and 88 for 6 to 12 cm and 12 to 20 cm, respectively.

Given these data, it would be a safe assumption that the dominant species within the Ozarks will remain members of the genus *Quercus*, with codominance belonging to hickory. Furthermore, although maple does show a noteworthy dominance (19.2% relative abundance), it would seem that with the reduced number of larger stems, this would not mean an overall established dominance for the larger size classes or trees.

The species with the highest relative abundance for Devil’s Den State Park (Table 3.3)
were red maple (28.5%), white oak (13.2%), black oak (11.7%), black cherry (10.0%), and red oak (6.9%). These data are comparable to the overall species abundance for the entire northwest Arkansas region, which is to be expected since this is where a majority of plots were sampled.

Looking at the abundance (Table 3.4) and relative abundance (Table 3.3), the species from 6 to 12 cm are the most numerous, with the most abundant taxa being maple (409 stems) followed by black cherry (149 stems), white oak (110 stems), black oak (85 stems), and red oak and hickory tied with 54 stems each. Interestingly, the smallest size class (2 to 6 cm) is also dominated by red maple (170 stems), whereas black cherry, white oak, and black oak occur in much smaller quantities: black cherry (57), white oak (23), black oak (18), and red oak (1). In the 12 to 20 size class, the red maple and black cherry stem counts drop off to 89 and 16 stems, respectively, whereas black oak stem counts rise to 99 stems, and an appreciable number are present for white oak (76), red oak (57), and hickory (54). The 20 to 60 cm size class is dominated by black oak (59 stems), white oak (30 stems), and red oak (23 stems), whereas black cherry (3 stems), red maple (8 stems), and hickory (8 stems) are characterized by a decreased dominance.

For the 60 to 100 size class, few of the main species dominated, but there were three stems present for black oak and two for red maple. Of the dominant species listed, white oak had the greatest number of trees (73) and was followed by red oak (29), hickory (24), and black oak (14).

Given the results from the entire northwest Arkansas region and those obtained from Devil's Den State Park, the previous assumption, that the dominant species within the Ozarks belong to the genus Quercus holds true, as is the case for the codominance of hickory. The stems
with the greatest relative abundance (Table 3.5) for all of the field sites in Pea Ridge National Military Park were post oak (23.7%), elm (22.0%), hickory (20.2%), and blackjack oak (12.7%). Of these species, post oak and blackjack oak had the largest number of trees present with 11 each (Table 3.6). The total stem counts by size class, given in Table 3.6, show a clear dominant size class (2 to 6 cm) that had a total of 225 stems, of which 88 were elm, 75 were red oak, 25 were hickory, and 10 were blackjack oak. Each of these species had similar stem counts within the 6 to 12 cm size class, with elm having 31 stems, post oak having 30, hickory having 28, and blackjack oak having 20. The only notable stem count in the 12 to 20 cm size class was hickory with 35 stems, whereas the others (post oak, elm, and red oak) all fell below 10 stems. In the upper size (i.e., those with class ranges between 20 to 100 cm), the stem counts were greatest for blackjack oak (29 stems), hickory (29 stems), post oak (13 stems), and elm (3 stems).

Given the results from Pea Ridge National Military Park, the dominance of oak remains apparent along with the consistent presence of the codominant hickory. Unlike Devil's Den State Park, Pea Ridge Natational Military Park did not have an overwhelming presence of maple stems. In fact, there were zero stems recoded for all size classes.

Relative abundance (Table 3.7) for the Buffalo National River Park sites was distributed among the greatest number of species for the three regions, but the overall pattern is the same as for the others regions, where oaks are the dominant trees present. Ash (mostly white ash) has a high stem count (136) with a relative abundance of 22.1%, which makes it the most dominant in this region, but white oak is nearly equal with 131 stems and 21.3% relative abundance. The stem counts drop off considerably for other species, with the next counts at 61 stems and 9.9% relative abundance for hackberry and 53 stems and 8.6% relative abundance for eastern redbud.

The dominant size class for the combined Buffalo National River study sites (Table 3.8)
falls between 12 to 20 cm, with 199 stems counted in that class, which were composed mostly of white ash (54 stems), white oak (30 stems), hackberry (26 stems), and eastern redbud (21 stems). The least dominant size class, unlike the previous areas discussed thus far, is the 2 to 6 cm size class. The next highest dominant size class (20 to 60 cm) has 171 stems and is dominated by white oak (62 stems) and white ash (48 stems).

The 6 to 12 cm size class has a total of 145 stems, which consists of eastern redbud (28 stems), white ash (22 stems), white oak (21 stems), southern red oak (17 stems), and hackberry (15 stems). Of the species mentioned, white oak has the greatest number of stems in the 60 to 100 cm size class (7 stems) and trees (9). However, for the entire Buffalo National River study site (Table 8), the species represented by the most trees are eastern red cedar with 14 trees and winged elm and mockernut hickory, each with 10 trees.

Given the results from the relative abundance (Table 3.7) and stem counts by size class (Table 3.8) for the Buffalo National River, the dominant species are white ash and white oak with stem height counts greatest between 12 and 60 cm. Although hickory was not as dominant in this region as the other northwest Arkansas plots, there was a greater diversity in the number of different hickory (bitternut, mockernut, and shagbark) and a large number of trees, 11 in total.

Based on the results from the Buffalo National River Park, Pea Ridge National Military Park, and Devil's Den State Park field locations, the dominant tree species within northwest Arkansas are all oaks, with hickory as the main codominant. For the genus *Quercus*, those with the largest stem counts (Table 3.2) and relative abundance (Table 1) are white oak and black oak, 481 stems and 13.6% and 319 stems and 9.7%, respectively. Maples, when grouped together, had 707 total stems and a relative abundance of 20.6% within the northwest Arkansas study area. Given that the majority of maple stem counts, 582 of the 707 stems, fell within smaller size
classes (2 to 12 cm), the tendency here was to find a large number of small stems produced, but with many of these displaying little evidence of ever reaching establishment beyond 60 to 100 cm or as trees.

D. Structure and Composition of the Seedling and Sapling Strata in the Fernow Experimental Forest

The Fernow Experimental Forest has a forest composition similar to that of the Ozark Mountains, and thus, is an appropriate place for comparison due to the fact that the dominant tree species belong to the genera *Quercus*, *Fagus* and *Betula*. Study sites from which data were collected within the Fernow Experimental Forest included as wide a range of forest types as possible, including those with beech present along with oak. Acquiring the stem counts and abundance of species throughout the Fernow Experimental Forest was carried out in a total of ten sites, three of which correlated with hot-burns (repeats of 4-5 years).

Relative abundance (shown in Table 3.9) was calculated for all plots within the Fernow Experimental Forest by adding the counts obtained for each species separately and then combining these values to obtain a final species count. The final species count was divided by the total for each species and multiplied by 100 to calculate its percent relative abundance. Furthermore, the total abundance by size class (Table 3.10) was also constructed for this region to distinguish seedling and sapling abundance by height within the area. The height ranges listed in Table 3.10 are the same as those used in northwest Arkansas (Table 3.2), which were used to broaden the range of values observed, to allow a clear separation of seedlings, saplings, and trees within the plot, and to assist in comparing the two regions.
Table 3.9. Total stem counts and relative abundance (%) for plots sampled in the Fernow Experimental Forest, West Virginia.

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Taxon</th>
<th>Stem counts</th>
<th>Relative abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maple</td>
<td><em>Acer</em> <em>spp.</em></td>
<td>1507</td>
<td>47.7</td>
</tr>
<tr>
<td>Northern red oak</td>
<td><em>Quercus rubra</em></td>
<td>646</td>
<td>20.4</td>
</tr>
<tr>
<td>American beech</td>
<td><em>Fagus grandifolia</em></td>
<td>290</td>
<td>9.2</td>
</tr>
<tr>
<td>Black gum</td>
<td><em>Nyssa sylvatica</em></td>
<td>188</td>
<td>6.0</td>
</tr>
<tr>
<td>Chestnut oak</td>
<td><em>Quercus prinus</em></td>
<td>183</td>
<td>5.8</td>
</tr>
<tr>
<td>White ash</td>
<td><em>Fraxinus americana.</em></td>
<td>155</td>
<td>4.9</td>
</tr>
<tr>
<td>Tulip poplar</td>
<td><em>Leriodendron tulipifera</em></td>
<td>113</td>
<td>3.6</td>
</tr>
<tr>
<td>Sassafras</td>
<td><em>Sassafras albidum</em></td>
<td>48</td>
<td>1.5</td>
</tr>
<tr>
<td>Magnolia</td>
<td><em>Magnolia</em> <em>spp.</em></td>
<td>25</td>
<td>0.4</td>
</tr>
<tr>
<td>Black cherry</td>
<td><em>Prunus serotina</em></td>
<td>4</td>
<td>0.1</td>
</tr>
<tr>
<td>Hickory</td>
<td><em>Carya</em> <em>spp.</em></td>
<td>4</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>3159</td>
<td>100.0</td>
</tr>
</tbody>
</table>

*Species names are the same as those listed in Tables 3.1-3.5 in northwest Arkansas.*
Table 3.10. Total stem counts by size class for plots within the Fernow Experimental Forest, West Virginia.

<table>
<thead>
<tr>
<th>Common Name</th>
<th>Stem size class in (cm)</th>
<th>2 to 6</th>
<th>6 to 12</th>
<th>12 to 20</th>
<th>20 to 60</th>
<th>Trees</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maple</td>
<td></td>
<td>869</td>
<td>497</td>
<td>112</td>
<td>18</td>
<td>11</td>
<td>1507</td>
</tr>
<tr>
<td>Northern red oak</td>
<td></td>
<td>189</td>
<td>187</td>
<td>202</td>
<td>61</td>
<td>7</td>
<td>646</td>
</tr>
<tr>
<td>American beech</td>
<td></td>
<td>160</td>
<td>75</td>
<td>35</td>
<td>16</td>
<td>4</td>
<td>290</td>
</tr>
<tr>
<td>Black gum</td>
<td></td>
<td>125</td>
<td>56</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>188</td>
</tr>
<tr>
<td>Chestnut oak</td>
<td></td>
<td>11</td>
<td>60</td>
<td>78</td>
<td>31</td>
<td>3</td>
<td>183</td>
</tr>
<tr>
<td>White ash</td>
<td></td>
<td>39</td>
<td>81</td>
<td>21</td>
<td>14</td>
<td>0</td>
<td>155</td>
</tr>
<tr>
<td>Tulip poplar</td>
<td></td>
<td>61</td>
<td>48</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>113</td>
</tr>
<tr>
<td>Sassafras</td>
<td></td>
<td>17</td>
<td>20</td>
<td>6</td>
<td>5</td>
<td>0</td>
<td>48</td>
</tr>
<tr>
<td>Magnolia</td>
<td></td>
<td>2</td>
<td>13</td>
<td>3</td>
<td>7</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Black cherry</td>
<td></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Hickory</td>
<td></td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>1476</td>
<td>1035</td>
<td>468</td>
<td>152</td>
<td>28</td>
<td>3159</td>
</tr>
</tbody>
</table>

*Acer spp. includes *Acer rubrum* and *Acer saccharum*; *Carya* spp. includes *Carya cordiformis*, *C. glabra*, *C. ovata*, and *C. tomentosa*, *Magnolia* spp. includes *Magnolia accuminata* and *Magnolia fraseri*. 
E. Analysis of Relative Abundance and Size Class Distributions for the Fernow Experimental Forest

Looking at the value for abundance (Table 3.9) and relative abundance (Table 3.10), the species with the highest relative abundance are maple (47.7%), red oak (20.4%), and American beech (9.2%), with the dominant size class (2 to 6 cm) having 1476 stems counted for that class. Maple has the greatest stem count (1507), of which 1366 stems were less than a height of 12 cm. Red oak has the next highest relative abundance at 20.4% (Table 3.9) and is characterized by a fairly even distribution among the size classes from 2 to 20 cm (Table 3.10), with 189 stems for size class 2 to 6 cm, 187 stems between 6 and 12 cm, and 202 stems between 12 and 20 cm. American beech has the greatest number of stems (Table 3.10) between 2 to 6 cm, with 160 total stems, and drops off as height increases, with 75 stems between 6 and 12 cm and 35 stems between 12 and 20 cm.

The 20 to 60 cm size class (Table 3.10) is dominated by red oaks (61 stems), chestnut oak (31 stems), maple (18 stems), and American beech (16 stems). The trees within the area are dominated by maple (11 total), red oak (7), and American beech (4), with 3 trees represented by chestnut oak and hickory.

F. Comparison of Relative Abundance and Size Class Distributions for Northwest Arkansas and the Fernow Experimental Forest

Comparing the data from the Fernow Experimental Forest (Tables 3.9 and 3.10) with those of the northwest Arkansas region (Tables 3.1 and 3.2), the dominant taxa common to the two regions are oak and maple. The dominant oak species (Table 3.1) within northwest Arkansas are white oak, with a relative abundance of 13.6%, and black oak, with a relative abundance of 9.7%. In the Fernow Experimental Forest (Table 3.9), the dominant oak species is red oak with a relative dominance of 20.4%. The dominant maple species in northwest
Arkansas (Table 3.1) is red maple (19.7% relative abundance), and for the Fernow Experimental Forest, the dominant species was almost surely sugar maple (represented by numerous germinal seedlings, many too small to be identified), with a relative abundance of 47.7% (Table 3.9). Both areas show oak and maple as the primary dominants, which indicate that they are best described as oak-maple dominated forests. However, both areas show that for the dominant size classes between 2 to 12 cm, maples are the most abundant, with a stem count of 1366 (Table 3.10) corresponding to a majority of the relative abundance (43.2%) for the Fernow Experimental Forest and 579 stems (Table 3.2) corresponding to a majority (16.4% relative abundance) in the Ozark Mountains of northwest Arkansas. However, for larger size classes (12 to 100 cm), dominance shifts to oaks (10.4% relative abundance), with 369 stems (Table 3.2) for that size class in the Ozarks and 11.8% relative abundance and with 372 stems (Table 3.10) in the Fernow Experimental Forest. For the same size class (12 to 100 cm), maples have a relative abundance of 3.5% with 124 stems (Table 3.2) in the Ozarks and 4.1% relative abundance with 130 stems (Table 3.10) in the Fernow Experimental Forest.

Given these data, the chance of an oak-maple dominated forest becoming widespread seems improbable. Instead, the dominance is clearly oak, whether it is black oak or white oak in the Ozarks or red oak in the Fernow Experimental Forest. The codominant species in the Ozarks are hickories, although their relative abundance is rather low at 7.5% (Table 3.1). However, hickories are represented by larger stem counts between 12 to 100 cm, making it a better choice than maple of becoming dominant.

The Fernow Experimental Forest currently has a clear dominance of red oak (20.4% relative abundance) and maple (47.7% relative abundance) (Table 3.9). However, assessing the possible future successional trend with respect to codominance in this region is not an easy task.
Maple stem counts make up most of the smaller size classes below 12 cm, but maple also has a large proportion of stems between 12 and 60 cm, with a total of 130 stems (Table 3.10). Likewise, American beech stem counts make up most of the smaller size classes below 12 cm, but this species has 51 stems between 12 and 60 cm. Consequently, oaks and beech have been the dominating trees in the past, but there could be, at least in the general study area, a trend toward a maple-dominated forest, as well.

Chapter 4 will build upon the mentioned species found within the sites in northwest Arkansas and the Fernow Experimental Forest by addressing the forest interior, the distribution of species therein, and the community and species richness.

Chapter 4. Forest Interior, Communities, and Species

4.1 Introduction

The decline in oak regeneration has stressed the importance of studies on the establishment of seedlings and saplings in the forest interior (Spetich 2002, Collins and Carson 2004, Comita 2007). The forests of northwest Arkansas and the Fernow Experimental Forest regularly undergo management practices that researchers hope will aid in the reproductive success of young oak saplings and seedlings. To address the success of these practices, the distribution of species in the interior of the forests for both of these regions was assessed. Using the results obtained from the methods and sampling section outlined in Chapter 3, the following section, Stem Coverage and Distribution, will provide graphical representations of the distribution patterns of sapling, seedling, and tree species within study areas in the two regions.

4.2 Stem Coverage and Distribution

A. Northwest Arkansas

In the summer of 2011, ten plot locations were established in three field sites—Devil's Den National Park, Pea Ridge National Military Park (revisited in 2012), and the Buffalo National
River Park—within the mountains of northwest Arkansas during the months of May through August. The first of the eight plots within Devil's Den State Park was completed on May 19, 2011. This first plot, located at 35° 46.35 N, 094° 14.63 W, had an elevation of 160 m, a slope of 10 degrees, faced five degrees from north, and was sampled during May 2011 (Figure 4.1). Plot 1 contained several ectomycorrhizal (Figure 4.1 and Figure 4.2) and endomycorrhizal trees (Figure 4.2). Both ectomycorrhizal red oak and white oak trees had several seedlings and saplings surrounding them (Figure 4.3). Although there were no black oak trees present, there were several black oak seedlings within the plot (Figures 4.2 and 4.3). The most abundant tree within the plot was hickory, but the most abundant seedlings and saplings belonged to maple (Figure 4.1). The abundance by height, Figure 4.3, indicates there were more stems between the heights of 6 to 12 cm within this plot than any other size class, although stems in the combined size classes above 12 cm were more abundant than in the 6 to 12 cm size class.

The second plot, located at 35° 46.331 N, 094° 14.743 W, had an elevation of 162 m, a slope of 10 degrees, faced ninety degrees from north, and was sampled during May 2011 (Figure 4.2). The distribution of seedlings, saplings, and trees in plot 2 (Devil's Den State Park) had several endomycorrhizal and ectomycorrhizal tree species within the plot (Figure 4.4). The dominant trees were hickory trees, followed by red oak.

The dominant seedling and sapling species were maple (Figure 4.4), red oak, and black oak (Figures 4.4 and 4.5). The size class distribution of stem heights within plot 2 was predominantly within 6 to 12 cm (over 100) and 12 to 20 cm (over 80) size classes, as indicated in Figure 4.6. The third plot, located at 35° 46.407 N, 094° 14.443 W, had an elevation of 159 m, a slope of 19 degrees, faced forty-five degrees from north, and was sampled during June 2011 (Figure 4.7). The dominant trees within the plot were hickory, followed by red oak and red.
cedar. The plot had several endomycorrhizal seedlings and saplings (mostly hickory and ash, Figure 4.7), but was dominated by ectomycorrhizal white oak, black oak, and red oak seedlings and saplings (Figure 4.8). The size class distribution (Figure 4.9) of the seedlings and saplings within plot 3 had a large number of stems in all of the size classes from 6 to 60 cm with only a few stems with heights from 2 to 6 cm (just over 10) and 60 to 100 cm (below 10 stems).

The fourth plot, located at 35° 46.117 N, 094° 14.68 W, had an elevation of 168 m, a slope of 12 degrees, faced forty-five degrees from north, and was sampled during June 2011 (Figure 4.10). The diversity of species within plot 4 (Figure 4.10) are much lower than the first three plots, with only a few representative species, most of which are ectomycorrhizal (Figure 4.11). The dominant trees within plot 4 were white oak, followed by red oak and black cherry, and the dominant seedlings and saplings were red oak, black oak, white oak, and black cherry, respectively. The distribution of abundance by size class is nearly a bell-shaped curve centered at stem heights in the 12 to 20 cm size class (near 25 stems), which is followed by nearly equal number of stems (~ 20) in both the 6 to 12 cm and 20 to 60 cm size classes (Figure 4.12). A substantial decline in stem heights is apparent for the 2 to 6 cm (<5) and 60 to 100 (<5) size classes (Figure 4.12).

The fifth plot, located at 35° 47.051 N, 094° 15.195 W, had an elevation of 127 m, a slope of 9 degrees, faced one hundred and thirty degrees south-east, and was sampled during June 2011 (Figure 4.13). The dominant trees within plot 5 were red oak, followed by white oak, post oak, and flowering dogwood (Figure 4.13). There were large numbers of black cherry, hickory, black oak, and ash seedlings and saplings within the plot (Figure 4.13). Fewer ectomycorrhizal seedlings and saplings were found in plot 5 (Figure 4.14) than previous plots. However, of the stems present, the majority belonged to black oak followed by a few red oak and white oak
seedlings and saplings (Figure 4.14). The largest size class distribution for plot 5 belonged to the 6 to 12 cm size class, with nearly 80 stems in that class (Figure 4.15). The other size classes had considerably fewer stems, with just over 20 stems in the 2 to 6 cm size class, under less than 20 stems in the 12 to 20 cm size class, and none from 20 to 100 cm (Figure 4.15).

The sixth plot, located at 35\(^\circ\) 47.034 N, 094\(^\circ\) 15.238 W, had an elevation of 124 m, a slope of 10 degrees, faced one hundred and thirty degrees south-east, and was sampled during June 2011 (Figure 4.16). The diversity of species within plot 6 was fairly large, with several trees (white oak, black oak, flowering dogwood, and elm) and seedlings and saplings (oaks, ash, black cherry, flowering dogwood, hickory, persimmon, maple, service berry, and elm) found in the plot (Figure 4.16). Flowering dogwood was represented by the largest number of trees, followed by a smaller number of white oak and elm trees (Figure 4.16). The most abundant seedlings and saplings within plot 6 were black cherry, ash, maple, and hickory, respectively (Figure 4.16). For the ectomycorrhizal trees, white oak was the dominant species followed by black oak. These two species were also the dominant ectomycorrhizal species for seedlings and saplings (Figure 4.17).

The abundance of trees, seedlings, and saplings by size class (Figure 4.18) shows that the largest number of stems (over 160) were found in the 6 to 12 cm size class followed by the 2 to 6 cm (~ 120 stems), 12 to 20 cm (< 40), and the 20 to 60 cm (only a few) size classes. The seventh plot, located at 35\(^\circ\) 47.614 N, 094\(^\circ\) 15.458 W, had an elevation of 525 m, a slope of 21 degrees, faced sixty degrees northwest, and was sampled during June 2011 (Figure 4.19). Plot 7 had the largest number of trees, seedlings, and saplings in all of the plots within Devil's Den State Park (Figure 4.19). The dominant trees in plot 7 were white oak, with 7 trees in the plot (Figures 4.19 and 4.20). The most abundant seedlings and saplings were maple, followed by white oak.
The ectomycorrhizal stems in plot 7 were white oak, red oak, and black oak with white oak having the largest number of trees, seedlings, and saplings (Figure 4.20). The size class distributions within plot 7 were predominantly stems with heights from 6 to 12 cm (nearly 350), then 12 to 20 cm (~100), 2 to 6 cm (> 50), and 20 to 60 cm (< 50) (Figure 4.21).

The eighth plot, located at 35° 47.599 N, 094° 15.379 W, had an elevation of 555 m, a slope of 20 degrees, faced fifty degrees northeast, and was sampled during June 2011 (Figure 4.22). There were several trees in plot 8, with the most dominant species being white oak, and black gum was a close second (Figure 4.22). Red oak, hickory, and red cedar were also found in the plot, with one tree found of each species (Figure 4.22). The most abundant seedlings and saplings belonged to black gum, followed by black oak, despite the fact that there were no black oak trees present in the plot (Figure 4.22). For the ectomycorrhizal species, the dominant tree was white oak, while the dominant seedlings and saplings were black oak (Figure 4.23). Of the five seedling and sapling stem height size classes, the 6 to 12 cm size class had the largest number of stems (near 120) (Figure 4.24). The abundance of stems dropped by nearly half (just above 60) in the 12 to 20 cm size class, which was followed by an even smaller number of stems with heights in the 20 to 60 cm size class (~20), the 2 to 6 cm (< 20), and dropping considerably for the 60 to 100 cm (< 5) size class (Figure 4.24).

The Pea Ridge National Military Park had four plot locations, one completed during the summer of 2011 and the remaining three completed during the summer of 2012. The first plot, located at 36° 27.566 N, 094° 01.379 W, in Pea Ridge National Military Park had a slope of 10 degrees, faced ninety degrees south-east, and was sampled during June 2012 (Figure 4.25). The dominant trees in Pea Ridge National Military Park plot 1 were elm and post oak (Figure 4.25). Post oak, hickory, elm, and blackjack oak were the dominant seedlings and saplings in plot 1,
respectively (Figure 4.25). There were only two species of ectomycorrhizal trees, seedlings, and saplings (post oak and blackjack oak) in plot 1, with post oak dominating each group (Figure 4.26). The abundance of seedlings and saplings in Pea Ridge National Military Park plot 1 was much lower (approximately about 100 total stems, Figure 4.27) than those seen in Devil's Den State Park (over 300 in just one size class in plot 7, Figure 4.21). The Pea Ridge National Military Park plot 1 had the largest number of stems in the 6 to 12 size class (>40) followed by a nearly equal number of stems in the 12 to 20 cm and 20 to 60 cm size classes (~20), with even less found in the 2 to 6 cm (<20), and 60 to 100 cm (<5) size classes (Figure 4.27).

The second plot, located at 36º 27.619 N, 094º 02.715 W, had a slope of 12 degrees, faced seventy degrees northwest, and was sampled during June 2011 (Figure 4.28). There were only two species with trees in the plot—white oak and black oak—with white oak represented by two trees and black oak, one (Figure 4.28). The diversity of species was rather low in this plot, with the dominance belonging to oaks followed by hickory (Figure 4.28). For the ectomycorrhizal stems, white oaks were the dominant trees, seedlings, and saplings, seconded by black oaks (Figure 4.29). The largest number of stems were in the 6 to 12 cm size class (>25), followed by size classes 2 to 6cm, 12 to 20 cm, and 20 to 60 cm, all with approximately 15 stems each, leaving the 60 to 100 cm size class with the smallest number of stems (~5) (Figure 4.30).

The third plot, located at 36º 27.623 N, 094º 02.720 W, in Pea Ridge National Military Park had an elevation of 465 m, slope of 13 degrees, faced ninety degrees southeast, and was sampled during June 2012 (Figure 4.31). The dominant trees within plot 3 were post oak (5 trees), with the remaining trees belonging to hickory and blackjack oak, which had one each (Figure 4.31). The dominant seedlings and saplings were also from post oak, with an equally large number of hickories, followed by blackjack oak (Figure 4.31). There were only post oak
and blackjack oak as ectomycorrhizal trees, seedlings, and saplings in plot 3, with post oak being the most dominant in all groups (Figure 4.32). The largest number of stems were in the 20 to 60 cm size class (near 70), with a steady decline in number of stems from 12 to 20 cm (~40), 6 to 12 cm (~30), 2 to 6 cm (<20), and 60 to 100 cm (<10), Figure 4.33.

The fourth plot, located at 36° 27.418 N, 094° 01.218 W, in Pea Ridge National Military Park had an elevation of 490 m, a slope of 3 degrees, faced eighty-three degrees northeast, and was sampled during June 2012 (Figure 4.34). The blackjack oak trees were the most dominant (5 trees) in plot 4, followed by post oak (3 trees), as indicated in Figure 4.34. Despite not having any elm trees in the plot, the dominant seedlings and saplings were elm, with a large number of small post oak stems also present (Figure 4.34). The ectomycorrhizal stems in plot 4 were post oak and blackjack oak, with blackjack oak dominating the trees and post oak dominating the seedlings and saplings (Figure 4.35). The size class with the largest number of stems was 2 to 6 cm (~180), followed by the 6 to 12 cm (~40) class, and very few stems were found from 12 to 100 cm (Figure 4.36).

The Buffalo National River had three plot locations, all of which were sampled during June of 2011. The first plot, located at 36° 04.214 N, 093° 09.554 W, in the Buffalo National River, had an elevation of 317 m, a slope of 18.5 degrees, and faced ninety degrees west (Figure 4.37). The diversity of species were relatively high, with four different oak species present, along with ash, black cherry, flowering dogwood, hickory, persimmon, sassafras, elm, red cedar, and eastern redbud. The red cedar trees were the most dominant (8 trees), followed by a large number of elms (6 trees) and white oaks (5 trees) (Figure 4.37). The most dominant seedlings and saplings were white oak followed by black oak and southern red oak (Figures 4.37 and 4.38). There was a large diversity in oak species, with white oak, southern red oak, and black oak trees all in the
plot, and white oak was the most dominant (Figure 4.38). Seedlings and saplings were equally diverse, with white oak, southern red oak, black oak, and chinquapin oak present, and white oak was, again, the most dominant (Figure 4.38). The stem counts by size class distribution (Figure 4.39) had the largest number of stems in the 20 to 60 cm size class (just over 100). The 6 to 12 cm and 12 to 20 cm size classes had equal number of stems (just under 100), with a large drop in stem counts for the 2 to 6 cm (just over 20) and 60 to 100 (<20) size classes (Figure 4.39).

The second plot, located at 36° 04.273 N, 093° 09.464 W, had an elevation of 339 m, a slope of 10 degrees, and faced thirty degrees west (Figure 4.40). The dominant trees within plot 2 were hickory (7 trees), followed by white oak (2 trees) and ash (2 trees) (Figure 4.40). Ectomycorrhizal stems of white oak, southern red oak, and chinquapin oak were all in plot 2, with white oak being the dominant species of trees, seedlings, and saplings (Figure 4.41). The abundance of seedlings and saplings in plot 2 was the greatest (near 120) in the 12 to 20 cm size class (Figure 4.42). The next two size classes, 6 to 12 cm (~60 to 80 stems) and 20 to 60 cm (~60), had about half as many stems, with the least number of stems in the 60 to 100 cm and 2 to 6 cm (<20) size classes (Figure 4.42).

The third plot, located at 36° 04.286 N, 093° 09.401 W, in the Buffalo National River, had an elevation of 335 m, a slope of 12 degrees, and faced forty degrees east (Figure 4.43). The dominant tree species in plot 3 was hickory (7 trees), followed by red cedar (5 trees) (Figure 4.43). The most abundant seedlings and saplings were ash and elm, respectively (Figure 4.43). For the ectomycorrhizal stems, red oak was the most abundant of the seedlings and saplings (Figure 4.44). The largest number of stems were found in the 20 to 60 cm size class (near 50), followed by 6 to 12 cm size class (near 30), and the 60 to 100 cm (< 10) and 2 to 6 cm (<5) size classes had the lowest number of stems (Figure 4.45).
Figure 4.1. Distribution of stems within plot 1 at Devil’s Den State Park. Trees are designated with large legend symbols (e.g., large open triangles represent white oak trees), whereas saplings and seedlings of the same species have been combined and are designated by a small legend symbol (e.g., small open circles represent white oak sapling and seedlings).
Figure 4.2. Distribution of ectomycorrhizal stems within plot 1 at Devil’s Den State Park. Trees are designated with large legend symbols, whereas saplings and seedlings of the same species have been combined and are designated by a small legend symbol.
Figure 4.3. Abundance of tree seedlings and saplings by 10 cm increments within plot 1 at Devil’s Den State Park.
Figure 4.4. Distribution of stems within plot 2 at Devil’s Den State Park. Trees, saplings, and seedlings have the same designations as plot 1.
Figure 4.5. Distribution of ectomycorrhizal stems within plot 2 at Devil’s Den State Park. Trees, saplings, and seedlings have the same designations as plot 1.
Figure 4.6. Abundance of tree seedlings and saplings by 10 cm increments within plot 2 at Devil’s Den State Park.
Figure 4.7. Distribution of stems within plot 3 at Devil’s Den State Park. Trees, saplings, and seedlings have the same designations as plot 1.
Figure 4.8. Distribution of ectomycorrhizal stems within plot 3 at Devil’s Den State Park. Trees, saplings, and seedlings have the same designations as plot 1.
Figure 4.9. Abundance of tree seedlings and saplings by 10 cm increments within plot 3 at Devil’s Den State Park.
Figure 4.10. Distribution of stems within plot 4 at Devil’s Den State Park. Trees, saplings, and seedlings have the same designations as plot 1.
Figure 4.11. Distribution of ectomycorrhizal stems within plot 4 at Devils Den State Park. Trees, saplings, and seedlings have the same designations as plot 1.
Figure 4.12. Abundance of tree seedlings and saplings by 10 cm increments within plot 4 at Devil’s Den State Park.
Figure 4.13. Distribution of stems within plot 5 at Devil’s Den State Park. Trees, saplings, and seedlings have the same designations as plot 1.
Figure 4.14. Distribution of ectomycorrhizal stems within plot 5 at Devil’s Den State Park. Trees, saplings, and seedlings have the same designations as plot 1.
Figure 4.15. Abundance of tree seedlings and saplings by 10 cm increments within plot 5 at Devil’s Den State Park.
Figure 4.16. Distribution of stems within plot 6 at Devil’s Den State Park. Trees, saplings, and seedlings have the same designations as plot 1.
Figure 4.17. Distribution of ectomycorrhizal stems within plot 6 at Devil’s Den State Park. Trees, saplings, and seedlings have the same designations as plot 1.
Figure 4.18. Abundance of tree seedlings and saplings by 10 cm increments within plot 6 at Devil’s Den State Park.
Figure 4.19. Distribution of stems within plot 7 at Devil’s Den State Park. Trees, saplings, and seedlings have the same designations as plot 1.
Figure 4.20. Distribution of ectomycorrhizal stems within plot 7 at Devil’s Den State Park. Trees, saplings, and seedlings have the same designations as plot 1.
Figure 4.21. Abundance of tree seedlings and saplings by 10 cm increments within plot 7 at Devil’s Den State Park.
Figure 4.22. Distribution of stems within plot 8 at Devil’s Den State Park. Trees, saplings, and seedlings have the same designations as plot 1.
Figure 4.23. Distribution of ectomycorrhizal stems within plot 8 at Devil’s Den State Park. Trees, saplings, and seedlings have the same designations as plot 1.
Figure 4.24. Abundance of tree seedlings and saplings by 10 cm increments within plot 8 at Devil’s Den State Park.
Figure 4.25. Distribution of stems within plot 1 at Pea Ridge Military Park. Trees, saplings, and seedlings have the same designations as plot 1 within Devil’s Den State Park.
Figure 4.26. Distribution of ectomycorrhizal stems within plot 1 at Pea Ridge Military Park. Trees, saplings, and seedlings have the same designations as plot 1 within Devil’s Den State Park.
Figure 4.27. Abundance of tree seedlings and saplings by 10 cm increments within plot 1 at Pea Ridge Military Park.
Figure 4.28. Distribution of stems within plot 2 at Pea Ridge Military Park. Trees, saplings, and seedlings have the same designations as plot 1 within Devil’s Den State Park.
Figure 4.29. Distribution of ectomycorrhizal stems within plot 2 at Pea Ridge Military Park. Trees, saplings, and seedlings have the same designations as plot 1 within Devil’s Den State Park.
Figure 4.30. Abundance of tree seedlings and saplings by 10 cm increments within plot 2 at Pea Ridge Military Park.
Figure 4.31. Distribution of stems within plot 3 at Pea Ridge Military Park. Trees, saplings, and seedlings have the same designations as plot 1 within Devil’s Den State Park.
Figure 4.32. Distribution of ectomycorrhizal stems within plot 3 at Pea Ridge Military Park. Trees, saplings, and seedlings have the same designations as plot 1 within Devil’s Den State Park.
Figure 4.33. Abundance of tree seedlings and saplings by 10 cm increments within plot 3 at Pea Ridge Military Park.
Figure 4.34. Distribution of stems within plot 4 at Pea Ridge Military Park. Trees, saplings, and seedlings have the same designations as plot 1 within Devil’s Den State Park.
Figure 4.35. Distribution of ectomycorrhizal stems within plot 4 at Pea Ridge Military Park. Trees, saplings, and seedlings have the same designations as plot 1 within Devil’s Den State Park.
Figure 4.36. Abundance of tree seedlings and saplings by 10 cm increments within plot 4 at Pea Ridge Military Park.
Figure 4.37. Distribution of stems within plot 1 at the Buffalo National River Park. Trees, saplings, and seedlings have the same designations as plot 1 within Devil’s Den State Park.
Figure 4.38. Distribution of ectomycorrhizal stems within plot 1 at the Buffalo National River. Trees, saplings, and seedlings have the same designations as plot 1 within Devil’s Den State Park.
Figure 4.39. Abundance of tree seedlings and saplings by 10 cm increments within plot 1 at the Buffalo National River.
Figure 4.40. Distribution of stems within plot 2 at the Buffalo National River. Trees, saplings, and seedlings have the same designations as plot 1 within Devil’s Den State Park.
Figure 4.41. Distribution of ectomycorrhizal stems within plot 2 at the Buffalo National River. Trees, saplings, and seedlings have the same designations as plot 1 within Devil’s Den State Park.
Figure 4.42. Abundance of tree seedlings and saplings by 10 cm increments within plot 1 at the Buffalo National River.
Figure 4.43. Distribution of stems within plot 3 at the Buffalo National River. Trees, saplings, and seedlings have the same designations as plot 1 within Devil’s Den State Park.
Figure 4.44. Distribution of ectomycorrhizal stems within plot 3 at the Buffalo National River. Trees, saplings, and seedlings have the same designations as plot 1 within Devil’s Den State Park.
Figure 4.45. Abundance of tree seedlings and saplings by 10 cm increments within plot 3 at the Buffalo National River.
B. The Fernow Experimental Forest

In the summer of 2012, ten 5 x 5 m plots were established in the Fernow Experimental Forest near Parsons, West Virginia, during the month of June. Seven plots were in unburned locations that were essentially within established Stephenson plots (sites 6, 10, 13, 21, 25, 66, and an additional location near site 25), and the remaining three were in hot-burn locations. The first plot was surveyed June 22-23, 2012, and was located at 39° 02.123 N, 79° 41.979 W, with an elevation of 748 m a slope of 19 degrees, and faced sixty degrees from northeast (Figure 4.46). The dominant plot 1 trees were sugar maple (2 trees) and red oak (2 trees), which were also the dominant seedlings and saplings, respectively (Figure 4.46). The ectomycorrhizal species found in plot 1 were red oak and beech, where red oak was the dominant tree and also dominated the seedlings and saplings (Figure 4.47). The 6 to 12 cm size class had the largest number of stems (~100); the next highest size class was 2 to 6 cm (just over 40 stems), which was followed by 12 to 20 cm (~35 stems), 20 to 60 cm (<20 stems), and 60 to 100 cm (<5 stems) size classes (Figure 4.48).

The second plot, located at 39° 02.274 N, 079° 41.025 W, had an elevation of 774 m, a slope of 21 degrees, faced ninety degrees from northwest, and was sampled on June 20, 2012 (Figure 4.49). The dominant tree species in plot 2 was chestnut oak (3 trees), then beech (2 trees), and finally, red oak (1 tree) (Figure 4.49). The dominant seedlings and saplings were maples, with a large number of red oaks also present (Figure 4.49). The dominant ectomycorrhizal tree was chestnut oak, but the largest number of seedlings and saplings were red oak (Figure 4.50). Over 200 stems were found in the 2 to 6 cm size class, with a considerable drop to just over 50 in the 6 to 12 cm size class, near 30 in the 12 to 20 cm size class, and well below 30 in the 20 to 60 cm size class (Figure 4.51).
The third plot, located at 39° 02.273 N, 079° 41.029 W, had an elevation of 833 m, a slope of 22 degrees, faced one hundred twenty degrees northeast, and was sampled on June 19, 2012 (Figure 4.52). The dominant trees, seedlings, and saplings within plot 3 were red oak and maple, although there were a large number of black gum and beech seedlings and saplings present (Figure 4.52). The ectomycorrhizal species in plot 3 were red oak, chestnut oak, and beech with the dominant trees, seedlings, and saplings belonging to red oak (Figure 4.53). The most abundant size class was 2 to 6 cm with over 600 stems, followed by a drop below 100 stems in the 6 to 12 cm size class, only a few stems found in the 20 to 60 cm size class, and none in the 12 to 20 cm size class (Figure 4.54).

The fourth plot, located at 39° 02.123 N, 079° 41.979 W, had an elevation of 722 m, a slope of 50 degrees, faced one hundred twenty degrees south, and was sampled during June 22, 2011 (Figure 4.55). There were no trees within the 5x5 m plot, but there were several seedlings and saplings that were dominated by chestnut oak and maple, respectively (Figure 4.55). The most abundant ectomycorrhizal species was chestnut oak, with a notable number of red oak and beech also found (Figure 4.56). The size class with the largest number of stems was 2 to 6 cm (~110), followed by a nearly equal number of stems in the 6 to 12 cm (~100) and 12 to 20 cm (~90) size classes; the 20 to 60 cm (~50) size class was the least abundant (Figure 4.57).

The fifth plot, located at 39° 02.007 N, 079° 41.781 W, had an elevation of 747 m, a slope of 17 degrees, faced one hundred sixty degrees south, and was sampled during June 20, 2012 (Figure 4.58). The fifth plot had only one hickory tree and was dominated by ash and red oak seedlings and saplings, respectively (Figure 4.58). The dominant ectomycorrhizal species was red oak, which was all seedlings and saplings (Figure 4.59). The size class with the most abundance was 6 to 12 cm (near 60 stems), with 2 to 6 cm (~35) and 12 to 20 cm (~30) having
close to the same number of stems and 20 to 60 cm (<20) having the least (Figure 4.60).

The sixth plot, located at 39° 83.293 N, 079° 41.248 W, had an elevation of 799 m, a slope of 24 degrees, faced thirty-five degrees northwest, and was sampled during June 21, 2012 (Figure 4.61). The dominant trees in plot 6 were maple (3 trees), which were also the dominant seedlings and saplings (Figure 4.61). Red oak and beech ectomycorrhizal species were also in the plot, with red oak being the dominant tree species and beech dominating the seedlings and saplings (Figure 4.62). The largest number of stems were in the 6 to 12 cm (near 180) size class, with half that number in found in the 2 to 6 cm (~80) size class (Figure 4.63). The two least abundant size classes were the 12 to 20 cm (~60) and 20 to 60 cm (<20) (Figure 4.63).

The seventh plot, located at 39° 02.088 N, 079° 41.490 W, had an elevation of 801 m, a slope of 9 degrees, faced sixty degrees northeast, and was sampled during June 23, 2012 (Figure 4.64). The dominant tree species in plot 7 was beech (4 trees), with red oak dominating the seedlings and saplings, although a large number of maple seedlings and saplings were also seen (Figure 4.64). The ectomycorrhizal species were dominated by beech trees and red oak seedlings and saplings (Figure 4.65). The size classes with the greatest number of stems were the 6 to 12 cm (near 160) and 12 to 20 cm (near 180), with the 2 to 6 cm (~20) and 20 to 60 cm (~40) having much lower stem counts (Figure 4.66). The 60 to 100 cm and 100 to 140 cm size classes were very small, with less than 10 stems found in each class (Figure 4.66).

The eighth plot, burn plot 1, was located at 39° 02.089 N, 079° 41.488 W, had an elevation of 799 m, a slope of 30 degrees, faced ninety degrees northwest, and was sampled during June 23, 2011 (Figure 4.67). Plot 8 was dominated by maple trees, seedlings, and saplings (Figure 4.67). Although much lower in abundance, large numbers of tulip poplar, red oak, and ash stems were also present (Figure 4.67). The ectomycorrhizal species in plot 8 were all seedlings and
saplings of red oak, chestnut oak, and beech, with red oak being the dominant of the three (Figure 4.68). The 6 to 12 cm and 2 to 6 cm size classes had the most number of stems, with ~120 in the former class and ~110 in the latter (Figure 4.69). The next two size classes have stem counts below 20 and are from 12 to 20 cm and 20 to 60 cm (Figure 4.69).

The ninth plot, burn plot 2, was located at 39° 04.383 N, 079° 40.325 W, had an elevation of 799 m, a slope of 49 degrees, faced ninety degrees northwest, and was sampled during June 24, 2012 (Figure 4.70). There were no trees in plot 9, but the plot was dominated by maple, beech, and tulip poplar seedlings and saplings, respectively (Figure 4.70). The ectomycorrhizal species in plot 9 were red oak, chestnut oak, and beech, with beech dominating the seedlings and saplings (Figure 4.71). The two size classes with the largest number of stems were 2 to 6 cm (almost 200) and 6 to 12 cm (~150), with the smallest number of stems in the 12 to 20 cm (~20) and 20 to 60 cm (~20) size classes (Figure 4.72).

The tenth plot, burn plot 3, was located at 39° 04.116 N, 079° 40.291 W, had an elevation of 709 m, a slope of 43 degrees, faced ninety degrees southwest, and was sampled during June 24, 2012 (Figure 4.73). The only tree, and therefore, the dominant tree, in plot 10 was maple, which also dominated the seedlings and saplings (Figure 4.73). There were very few ectomycorrhizal stems in plot 10, but there were some red oak and beech seedlings and saplings, with red oak being the dominant species (Figure 4.74). The size classes with the largest number of stems were 6 to 12 cm (~65) and 2 to 6 cm (~60), and the two size classes with the least number of stems were 12 to 20 cm (~20) and 20 to 60 cm (<20) (Figure 4.75).
Figure 4.46. Distribution of stems within plot 1 at the Fernow Experimental Forest. Trees are designated with large legend symbols and in the legend text (e.g., Large red triangles represents red oak trees.) For ease of reading within the legend, saplings and seedlings have been combined into one small legend symbol and label (common name for each species), e.g., small red circles represent red oak seedlings and saplings.
Figure 4.47. Distribution of ectomycorrhizal stems within plot 1 at the Fernow Experimental Forest. Trees, saplings, and seedlings have the same designations as plot 1 within the Fernow Experimental Forest.
Figure 4.48. Abundance of tree seedlings and saplings by 10 cm increments within plot 1 at the Fernow Experimental Forest.
Figure 4.49. Distribution of stems within plot 2 at the Fernow Experimental Forest. Trees, saplings, and seedlings have the same designations as plot 1 within the Fernow Experimental Forest.
Figure 4.50. Distribution of ectomycorrhizal stems within plot 2 at the Fernow Experimental Forest. Trees, saplings, and seedlings have the same designations as plot 1 within the Fernow Experimental Forest.
Figure 4.51. Abundance of tree seedlings and saplings by 10 cm increments within plot 2 at the Fernow Experimental Forest.
Figure 4.52. Distribution of stems within plot 3 at the Fernow Experimental Forest. Trees, saplings, and seedlings have the same designations as plot 1 within the Fernow Experimental Forest.
Figure 4.53. Distribution of ectomycorrhizal stems within plot 3 at the Fernow Experimental Forest. Trees, saplings, and seedlings have the same designations as plot 1 within the Fernow Experimental Forest.
Figure 4.54. Abundance of tree seedlings and saplings by 10 cm increments within plot 3 at the Fernow Experimental Forest.
Figure 4.55. Distribution of stems within plot 4 at the Fernow Experimental Forest. Trees, saplings, and seedlings have the same designations as plot 1 within the Fernow Experimental Forest.
Figure 4.56. Distribution of ectomycorrhizal stems within plot 4 at the Fernow Experimental Forest. Trees, saplings, and seedlings have the same designations as plot 1 within the Fernow Experimental Forest.
Figure 4.57. Abundance of tree seedlings and saplings by 10 cm increments within plot 4 at the Fernow Experimental Forest.
Figure 4.58. Distribution of stems within plot 5 at the Fernow Experimental Forest. Trees, saplings, and seedlings have the same designations as plot 1 within the Fernow Experimental Forest.
Figure 4.59. Distribution of ectomycorrhizal stems within plot 5 at the Fernow Experimental Forest. Trees, saplings, and seedlings have the same designations as plot 1 within the Fernow Experimental Forest.
Figure 4.60. Abundance of tree seedlings and saplings by 10 cm increments within plot 5 at the Fernow Experimental Forest.
Figure 4.61. Distribution of stems within plot 6 at the Fernow Experimental Forest. Trees, saplings, and seedlings have the same designations as plot 1 within the Fernow Experimental Forest.
Figure 4.62. Distribution of ectomycorrhizal stems within plot 6 at the Fernow Experimental Forest. Trees, saplings, and seedlings have the same designations as plot 1 within the Fernow Experimental Forest.
Figure 4.63. Abundance of tree seedlings and saplings by 10 cm increments within plot 6 at the Fernow Experimental Forest.
Figure 4.64. Distribution of stems within plot 7 at the Fernow Experimental Forest. Trees, saplings, and seedlings have the same designations as plot 1 within the Fernow Experimental Forest.
Figure 4.65. Distribution of ectomycorrhizal stems within plot 7 at the Fernow Experimental Forest. Trees, saplings, and seedlings have the same designations as plot 1 within the Fernow Experimental Forest.
Figure 4.66. Abundance of tree seedlings and saplings by 10 cm increments within plot 7 at the Fernow Experimental Forest.
Figure 4.67. Distribution of stems within plot 8 (burn site 1) at the Fernow Experimental Forest. Trees, saplings, and seedlings have the same designations as plot 1 within the Fernow Experimental Forest.
Figure 4.68. Distribution of ectomycorrhizal stems within plot 8 (burn site 1) at the Fernow Experimental Forest. Trees, saplings, and seedlings have the same designations as plot 1 within the Fernow Experimental Forest.
Figure 4.69. Abundance of tree seedlings and saplings by 10 cm increments within plot 8 (burn site 1) at the Fernow Experimental Forest.
Figure 4.70. Distribution of stems within plot 9 (burn site 2) at the Fernow Experimental Forest. Trees, saplings, and seedlings have the same designations as plot 1 within the Fernow Experimental Forest.
Figure 4.71. Distribution of ectomycorrhizal stems within plot 9 (burn site 2) at the Fernow Experimental Forest. Trees, saplings, and seedlings have the same designations as plot 1 within the Fernow Experimental Forest.
Figure 4.72. Abundance of tree seedlings and saplings by 10 cm increments within plot 9 (burn site 2) at the Fernow Experimental Forest.
Figure 4.73. Distribution of stems within plot 10 (burn site 3) at the Fernow Experimental Forest. Trees, saplings, and seedlings have the same designations as plot 1 within the Fernow Experimental Forest.
Figure 4.74. Distribution of ectomycorrhizal stems within plot 10 (burn site 3) at the Fernow Experimental Forest. Trees, saplings, and seedlings have the same designations as plot 1 within the Fernow Experimental Forest.
Figure 4.75. Abundance of tree seedlings and saplings by 10 cm increments within plot 10 (burn site 3) at the Fernow Experimental Forest.
C. Analysis of the Forest Interior for Northwest Arkansas and the Fernow Experimental Forest

The types of tree species in the Fernow Experimental Forest plots were slightly different than those found in northwest Arkansas, but they both had representative endomycorrhizal and ectomycorrhizal species present. The dominant tree species in all of the Devil's Den State Park plots were white oak, red oak, and hickory. There was also a clear dominance of white oak, red oak, and hickory as seedlings and saplings, but it was shared with maple. The size class with the most number of stems in all the Devil's Den State Park plots was the 6 to 12 cm size class. Pea Ridge National Military Park was dominated by post oak trees, as well as white oak and elm. The plots were fairly evenly split on the size class distribution, but in two of the plots, the 6 to 12 cm size class had the largest number of stems. Plots in the Buffalo National River were dominated by red cedar, hickory, and white oak trees, respectively. The size class with the most stems in all the plots was the 12 to 20 cm size class.

Comparing the three site locations in northwest Arkansas—Devil's Den State Park, Pea Ridge National Military Park, and the Buffalo National River—the dominant trees within the plots from these locations were oak and hickory, with a large number of seedlings and saplings also belonging to those species. The seedlings and saplings in the study sites also were characterized by abundance data that would suggest that most have heights between 6 to 12 cm.

On the other hand, the combined plots in the Fernow Experimental Forest were dominated by maple, oak (mostly red oak and chestnut oak), and beech. The seedlings and saplings in the Fernow Experimental Forest were also dominated by maple, oak, and beech, although in the burned sites, tulip poplar was also present. The seedlings and saplings displayed abundance data that placed most of their stems in the 6 to 12 cm size class, which was the same as northwest Arkansas.
As mentioned in chapter 1, the upper northwest region of the Ozark Mountains is an area dominated by hardwoods, with *Quercus* (oak) and *Carya* (hickory) being the most dominant taxa present (Spetich 2002). The data obtained in the present study show that this is still the case, with the largest number of oak and hickory trees, saplings, and seedlings found in the study sites. Likewise, the Fernow is known to be dominated by second- and third-growth Appalachian hardwood forests, with *Quercus* (oak) and *Fagus* (beech) among the most dominant taxa present (Stephenson et al. 1994). Once again, the ten plot locations in the Fernow Experimental Forest confirmed the dominant trees species were oaks and beeches. However, maple was also a dominant tree found in many of the Fernow Experimental Forest plots from this study, which could suggest a shift in dominance to an oak-beech-maple forest. Thomas Schuler (2004) reported a similar situation in recent studies (1987-2001), in which the most abundant species in the Fernow Experimental Forest were sugar maple and red maple. The presence of maple in both regions provides another indication that oak regeneration is on the decline, and quite possibly, maple is taking its place, which is why good forest management practices need to be implemented in order to assist oak regeneration. The next few sections in chapter 4 will look at the potential for ectomycorrhizal influences on oak seedlings and saplings between themselves and from host trees to suggest that they may be a key component of oak regeneration that needs to be further addressed.

4.3 Community and Species Analysis within the Forest Interior

A. Introduction to Community Analysis

Coefficient of community and percent similarity indices (Mueller-Dombois and Ellenberg 1974, Gauch 1982, Stephenson 1988) were used to compare community composition among plots and within study sites in the Ozark Mountains and the Fernow Experimental Forest. As
described in Stephenson (1988), coefficient of community is based on the presence or absence of a species and is calculated with the use of the equation,

\[
\text{Coefficient of community (CC)} = \frac{2c}{a + b}
\]

where \(a\) is the total number of species in the first community, \(b\) is the total number of species in the second community, and \(c\) is the number of species common to both communities. The values of CC range between 0 and 1, where 0 indicates there are no species in common between the communities and 1.0 indicates that the species in the two communities are the same (Stephenson 1988).

Percent similarity is an index that considers the relative abundance of each species in two communities rather than just their presence or absence (Stephenson 1988). Percent similarity is calculated through the use of the equation,

\[
\text{Percent similarity (PS)} = \sum \min (a, b, ..., x)
\]

where \(a\) represents a species in the first community, \(b\) represents the same species in the second community, and \(\min\) is the smaller relative abundance percentage for each \((a, b, ..., x)\) species shared between the two communities. The values of PS range between 0 and 1.0, where 0 is the result of no species in common and 1.0 indicates "communities identical both in species composition and in quantitative values for the species" (Stephenson 1988).

Coefficient of community and percent similarity indices were calculated for all plots in the northwest Arkansas study sites (Devil's Den State Park, Pea Ridge National Military Park, and the Buffalo National River Park). For example, calculations for CC and PS indices for Devil's Den State Park were carried out using the method outlined below. First, the total number of species present in the first plot (community \(a\)) was determined and then added to the total number species in plot 2 (community \(b\)). Then, \(2c\) was divided by this number (the number of
species common to both communities), giving the CC index between these two plots. Continuing this example, the next step involved calculating the percent relative abundance for all species in the two communities (plots 1 and 2) by adding the counts obtained for each species separately and then combining these values to obtain a final species count. The final species count was divided by each species and multiplied by 100 to derive their percent relative abundance. The PS index was then derived by taking the smaller relative abundance percent from the total number of species in common between the two plots. This method was repeated for the remaining plots at Devil's Den State Park, thus yielding CC and PS indices indicated in Table 4.1. In a similar fashion, CC and PS indices for Pea Ridge National Military Park and the Buffalo National River Park were calculated, as indicated in Tables 4.2 and 4.3, respectively. A comparison among the three northwest Arkansas study sites was carried out using a similar method, except that the communities used in the calculations of CC and PS indices were, instead of a sum of the species present in a single plot, pooled data of all species within a site (Table 4.4). Additionally, species richness was determined for the three sites (Table 4.5), with the total number of species provided for each size class and all seedlings, saplings, and trees.

The CC indices, PS indices, and species richness were calculated in a similar manner for plots in the Fernow Experimental Forest (Tables 4.6 and 7.7). In addition, this was followed by deriving overall comparisons of CC and PS indices between the two regions, northwest Arkansas and the Fernow Experimental Forest, with communities represented by an average number of species per region.

**B. Results of Community Analysis**

Coefficient of community and percent similarity indices in all pairwise combinations for the plot locations (1-8) within Devil's Den State Park are presented in Table 4.1, in which it can be
noted that the overall values for CC are higher than those for PS. The relatively narrow range of values (between 0.6 and 0.9) for CC shows that the species composition of the plots was fairly similar. The overall values (0.42) for the PS indices indicate that the relative abundance of particular species in the plots varied rather considerably (Table 4.1). There were a few exceptions (bold indices in Table 4.1) where CC and PS indices were high, 0.9 and nearly 0.6, respectively. Plots 1 and 2 had the highest overall coefficients of community (0.60), indicating that the species within those plots had the greatest similarity to the other six plots (Table 4.1). Plot 1 had the highest overall percent similarity (0.54) among the plots, giving it the highest relative abundance for all the plots.

Coefficient of community and percent similarity indices for all pairwise combinations for the plot locations (1-4) within Pea Ridge National Military Park are given in Table 4.2. The lowest values for coefficient of community and percent similarity were between plot 2 and the remaining three plots (Table 4.2). These results could be due to plot 2 being surveyed a year earlier and that it was the only unburned location in the Pea Ridge National Military Park plots. The remaining three plots all had high values for CC and PS (Table 4.2). The highest coefficient of community, 1.0, was found between plot 1 and plot 4, indicating they had the same species present (Table 4.2). The highest percent similarity (0.80) was found between plot 3 and plot 1 (Table 4.2), showing that there was little difference in the abundance of species among them.

The CC and PS indices in all pairwise combinations for the plot locations (1-3) within the Buffalo National River Park are given in Table 4.3. The CC indices for plots in the Buffalo National River Park were relatively high, especially between plot 1 and the two other plots (Table 4.3). The high CC indices indicate that the species among the plots were similar.
However, the PS indices were rather low (highest at only 0.33), indicating that the abundances of species displayed considerable variation within this site (Table 4.3).

Comparisons of CC and PS indices in all pairwise combinations for the northwest Arkansas locations—Devil's Den State Park, Pea Ridge National Military Park, and Buffalo National River Park—are presented in Table 4.4. The highest CC index (0.6), and therefore the site locations with the most similar species, was found between Devil's Den State Park and the Pea Ridge National Military Park (Table 4.4). The abundance of species in the three sites was quite different, as indicated by the low index values for PS; however, the Buffalo National River Park and Pea Ridge National Military Park did have a PS index of 0.51, indicating they had a fairly similar relative abundance of species (Table 4.4). In northwest Arkansas, Pea Ridge National Military Park had the highest CC index (0.50) among all of the sites, indicating that several of the species within Pea Ridge National Military Park were found in the other sites. The PS for each site in northwest Arkansas was the same (0.7), indicating that the relative abundance of species between site locations was not that different.

Overall, the composition of species in this region tends to lean towards higher species richness for seedlings than for saplings or trees (Table 4.5). Devil's Den State Park had an even number of species of seedlings and saplings (20 each) with fewer trees (12); the Buffalo National River Park had the same number of species richness (13) for seedlings and saplings with fewer trees (10); and Pea Ridge National Military Park had a higher species richness for seedlings (10) than saplings (7) or trees (8) (Table 4.5). Many of the species found in this region were in unburned plots, as indicated by the much larger species richness for seedlings (20), saplings (18), and trees (14) for unburned plots than for burned plots (Table 4.5).

Coefficient of community and percent similarity indices in all pairwise combinations for the
plot locations (1-10) within the Fernow Experimental Forest are presented in Table 4.6, which shows higher overall CC index values than for PS. The rather high average (0.7) and large values (between 0.6 and 1.0) of the CC indices indicate that the abundance of species among plots was similar, with the same values for plot 9 compared to plot 2 and plot 9 compared to plot 8 (Table 4.6). The overall low PS indices (0.54) in all of the plots indicate that there was a greater difference in relative abundance between species than species they had in common (Table 4.6). There were a few exceptions (bold indices Table 4.6) where both the CC indices and the PS indices were exceptionally high. In particular, plot 1 had the highest overall percent similarity (0.58) among all of the plots, giving it the highest number of species in common with the other plots (Table 4.6). Plot 2 had the highest CC index (0.8), indicating that the abundance of species was high for this plot when compared to the others. Overall, the composition of species in this region had a higher species richness for seedlings than for saplings or trees (Table 4.7). Interestingly, the unburned and burned sites had the same number of species for seedlings (6) with only one different species for saplings. The different species of trees within this region were mostly found in the unburned sites (Table 4.7).

The overall average CC indices and PS indices were calculated for northwest Arkansas and the Fernow Experimental Forest so that a region comparison could be also made. The two regions did not have a high number of species in common (CC = 0.3), nor did they share high relative abundances for those species (PS = 0.12). The CC value is not too surprising, as the values for species richness of seedlings (22), saplings (17), and trees (16) in northwest Arkansas (Table 4.5) were much higher than in the Fernow Experimental Forest (10, 6 and 5, respectively) (Table 4.7). It should be noted that the two regions do have a high proportion of ectomycorrhizal trees, seedlings, and saplings; however, they belong to different species, which
also accounts for the low CC index. The abundance of stems in the two regions and the underlying reason for the difference in plot size was considered during the survey (i.e., 10 x 10 m for northwest Arkansas plots and 5 x 5 m in the Fernow Experimental Forest). However, the possibility does exist that this correction was not enough to project a good comparison for species abundance, which could have resulted in the low PS index.

Surprisingly, despite a decline in stem counts, the burned plots in the two regions had higher overall indices for CC and PS. Pea Ridge National Military Park had three burn sites, which had an average CC index of 0.9 and an average PS of 0.66. The Fernow Experimental Forest displayed a similar pattern, although the CC (0.7) and PS (0.64) indices were not as high. The presence of fire in these sites could help to selectively remove some of the endomycorrhizal stems, therefore increasing the similarity between plots.

The next two chapters, chapters 5 and 6, will address the interactions between ectomycorrhizal (ECM) species in the plots at each study site within northwest Arkansas and the Fernow Experimental forest. These chapters will build on ECM linkages between trees, seedlings, and saplings and the potential spatial patterns, distance relationships, and clustering that occurs as a result.
Table 4.1. Coefficient of community (lower left) and percent similarity (lower right) indices in all pairwise combinations of the plot locations (1-8) within Devil's Den State Park.

<table>
<thead>
<tr>
<th></th>
<th>plot 1</th>
<th>plot 2</th>
<th>plot 3</th>
<th>plot 4</th>
<th>plot 5</th>
<th>plot 6</th>
<th>plot 7</th>
<th>plot 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>plot1</td>
<td>***</td>
<td>0.58</td>
<td>0.58</td>
<td>0.64</td>
<td>0.47</td>
<td>0.50</td>
<td>0.52</td>
<td>0.47</td>
</tr>
<tr>
<td>plot 2</td>
<td>0.9</td>
<td>***</td>
<td>0.34</td>
<td>0.52</td>
<td>0.39</td>
<td>0.35</td>
<td>0.51</td>
<td>0.62</td>
</tr>
<tr>
<td>plot 3</td>
<td>0.8</td>
<td>0.9</td>
<td>***</td>
<td>0.45</td>
<td>0.37</td>
<td>0.31</td>
<td>0.27</td>
<td>0.34</td>
</tr>
<tr>
<td>plot 4</td>
<td>0.6</td>
<td>0.7</td>
<td>0.7</td>
<td>***</td>
<td>0.49</td>
<td>0.41</td>
<td>0.28</td>
<td>0.48</td>
</tr>
<tr>
<td>plot 5</td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
<td>0.6</td>
<td>***</td>
<td>0.46</td>
<td>0.16</td>
<td>0.40</td>
</tr>
<tr>
<td>plot 6</td>
<td>0.9</td>
<td>0.9</td>
<td>0.8</td>
<td>0.7</td>
<td>0.8</td>
<td>***</td>
<td>0.26</td>
<td>0.35</td>
</tr>
<tr>
<td>plot 7</td>
<td>0.7</td>
<td>0.9</td>
<td>0.6</td>
<td>0.8</td>
<td>0.7</td>
<td>0.8</td>
<td>***</td>
<td>0.33</td>
</tr>
<tr>
<td>plot 8</td>
<td>0.7</td>
<td>0.7</td>
<td>0.7</td>
<td>0.6</td>
<td>0.7</td>
<td>0.8</td>
<td>0.8</td>
<td>***</td>
</tr>
</tbody>
</table>
Table 4.2. Coefficient of community (lower left) and percent similarity (lower right) indices in all pairwise combinations of the plot locations (1-4) within Pea Ridge National Military Park.

<table>
<thead>
<tr>
<th></th>
<th>plot 1</th>
<th>plot 2</th>
<th>plot 3</th>
<th>plot 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>plot 1</td>
<td>***</td>
<td>0.40</td>
<td>0.80</td>
<td>0.60</td>
</tr>
<tr>
<td>plot 2</td>
<td>0.5</td>
<td>***</td>
<td>0.43</td>
<td>0/16</td>
</tr>
<tr>
<td>plot 3</td>
<td>0.8</td>
<td>0.4</td>
<td>***</td>
<td>0.58</td>
</tr>
<tr>
<td>plot 4</td>
<td>1.0</td>
<td>0.5</td>
<td>0.8</td>
<td>***</td>
</tr>
</tbody>
</table>
Table 4.3. Coefficient of community (lower left) and percent similarity (lower right) indices in all pairwise combinations of the plot locations (1-3) within the Buffalo National River Park.

<table>
<thead>
<tr>
<th></th>
<th>plot 1</th>
<th>plot 2</th>
<th>plot 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>plot 1</td>
<td>***</td>
<td>0.33</td>
<td>0.26</td>
</tr>
<tr>
<td>plot 2</td>
<td>0.8</td>
<td>***</td>
<td>0.22</td>
</tr>
<tr>
<td>plot 3</td>
<td>0.6</td>
<td>0.5</td>
<td>***</td>
</tr>
</tbody>
</table>
Table 4.4. Coefficient of community (lower left) and percent similarity (lower right) indices in all pairwise combinations of pooled data for the three Northwest Arkansas locations: Devil's Den State Park (DD), Pea Ridge National Military Park (PR), and the Buffalo National River Park (BNR).

<table>
<thead>
<tr>
<th></th>
<th>DD</th>
<th>PR</th>
<th>BNR</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD</td>
<td>***</td>
<td>0.37</td>
<td>0.34</td>
</tr>
<tr>
<td>PR</td>
<td>0.6</td>
<td>***</td>
<td>0.51</td>
</tr>
<tr>
<td>BNR</td>
<td>0.3</td>
<td>0.4</td>
<td>***</td>
</tr>
</tbody>
</table>
Table 4.5. Species richness for each site in northwest Arkansas along with the total species richness in the region and average species richness in unburned and burned plots.

<table>
<thead>
<tr>
<th>Site</th>
<th>Devil's Den State Park</th>
<th>Pea Ridge National Military Park</th>
<th>Buffalo National River Park</th>
<th>Total northwest Arkansas</th>
<th>Unburned plots</th>
<th>Burned plots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seedlings (&lt; 20 cm)</td>
<td>20</td>
<td>10</td>
<td>13</td>
<td>22</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>Saplings (&gt; 20 cm)</td>
<td>20</td>
<td>7</td>
<td>13</td>
<td>17</td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td>Trees</td>
<td>12</td>
<td>8</td>
<td>10</td>
<td>16</td>
<td>14</td>
<td>4</td>
</tr>
</tbody>
</table>
Table 4.6. Coefficient of community (lower left) and percent similarity (lower right) indices in all pairwise combinations of the plot locations (1-10) within the Fernow Experimental Forest.

<table>
<thead>
<tr>
<th></th>
<th>plot 1</th>
<th>plot 2</th>
<th>plot 3</th>
<th>plot 4</th>
<th>plot 5</th>
<th>plot 6</th>
<th>plot 7</th>
<th>plot 8</th>
<th>plot 9</th>
<th>plot 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>plot 1</td>
<td>***</td>
<td>0.66</td>
<td>0.76</td>
<td>0.49</td>
<td>0.32</td>
<td>0.63</td>
<td>0.66</td>
<td>0.61</td>
<td>0.50</td>
<td>0.62</td>
</tr>
<tr>
<td>plot 2</td>
<td>0.9</td>
<td>***</td>
<td>0.48</td>
<td>0.53</td>
<td>0.22</td>
<td>0.58</td>
<td>0.48</td>
<td>0.77</td>
<td>0.55</td>
<td>0.88</td>
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<tr>
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<td>0.9</td>
<td>***</td>
<td>0.46</td>
<td>0.43</td>
<td>0.65</td>
<td>0.80</td>
<td>0.43</td>
<td>0.52</td>
<td>0.44</td>
</tr>
<tr>
<td>plot 4</td>
<td>0.8</td>
<td>0.9</td>
<td>0.8</td>
<td>***</td>
<td>0.17</td>
<td>0.51</td>
<td>0.46</td>
<td>0.50</td>
<td>0.51</td>
<td>0.48</td>
</tr>
<tr>
<td>plot 5</td>
<td>0.5</td>
<td>0.6</td>
<td>0.5</td>
<td>0.6</td>
<td>***</td>
<td>0.58</td>
<td>0.53</td>
<td>0.68</td>
<td>0.54</td>
<td>0.43</td>
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<tr>
<td>plot 6</td>
<td>0.7</td>
<td>0.6</td>
<td>0.7</td>
<td>0.7</td>
<td>0.5</td>
<td>***</td>
<td>0.58</td>
<td>0.53</td>
<td>0.68</td>
<td>0.54</td>
</tr>
<tr>
<td>plot 7</td>
<td>0.8</td>
<td>0.7</td>
<td>0.8</td>
<td>0.6</td>
<td>0.5</td>
<td>0.9</td>
<td>***</td>
<td>0.43</td>
<td>0.43</td>
<td>0.45</td>
</tr>
<tr>
<td>plot 8</td>
<td>0.6</td>
<td>0.8</td>
<td>0.6</td>
<td>0.8</td>
<td>0.9</td>
<td>0.5</td>
<td>0.4</td>
<td>***</td>
<td>0.62</td>
<td>0.83</td>
</tr>
<tr>
<td>plot 9</td>
<td>0.8</td>
<td>1.0</td>
<td>0.8</td>
<td>0.9</td>
<td>0.8</td>
<td>0.5</td>
<td>0.7</td>
<td>1.0</td>
<td>***</td>
<td>0.48</td>
</tr>
<tr>
<td>plot 10</td>
<td>0.7</td>
<td>0.6</td>
<td>0.7</td>
<td>0.5</td>
<td>0.5</td>
<td>0.6</td>
<td>0.7</td>
<td>0.5</td>
<td>0.7</td>
<td>***</td>
</tr>
</tbody>
</table>
Table 4.7. Species richness in the Fernow Experimental Forest for unburned and burned plots.

<table>
<thead>
<tr>
<th>Site</th>
<th>Total species richness in the Fernow Experimental Forest</th>
<th>Unburned plots</th>
<th>Burned plots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seedlings ( &lt; 20 cm)</td>
<td>10</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Saplings ( &gt; 20 cm)</td>
<td>6</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Trees</td>
<td>5</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>
Chapter 5. Spatial Distribution Patterns for ECM Trees, Seedlings, and Saplings

5.1 Introduction

Studies of the symbiotic relationship that exists between ectomycorrhizal (ECM) fungi and plants show an increased uptake of nutrients (carbon, phosphorus, and nitrogen), enhanced reproduction, increased disease resistance, and generally better health in plants (Hartnett and Wilson 2002, He et al. 2009). This symbiotic relationship manifests in an underground network formed along the root tips of the host plant. The fungal hyphae spread throughout the soil to reach other ectomycorrhiza-forming plants, establishing spatial linkages and making the proximity to other plants important. This symbiotic relationship, in turn, affects population and demographic patterns of distribution of plants in the communities in which they occur (Hartnett and Wilson 2002). Furthermore, ectomycorrhizal associations are more prominent on more established trees, and the presence of these trees provides a symbiotic host that spatially extends to and assists in the survival and development of seedlings and saplings by creating linkages between them. How these spatial linkages have impacted the ectomycorrhizal trees, seedlings, and saplings in the Ozarks and the Fernow Experimental Forest is still not understood. The section that follows will evaluate the potential impact these linkages have on the growth and distribution of trees, seedlings, and saplings in these two regions.

5.2 Methods and Data Analysis

The linkages between ECM trees and seedlings or saplings in the Ozarks and the Fernow Experimental Forest were evaluated by examining the spatial distributions of large trees and nearby seedlings and saplings. This was done by calculating the distance from the tree to a particular stem to determine if there was a change in stem height as the distance between the two increased. To check for significant differences in stem height away from a tree, a linear
regression of stem height versus Euclidean distance was calculated. The Euclidean distance was calculated using the equation $\sqrt{(X_T - X_i)^2 + (Y_T - Y_i)^2}$, where $X_T$ and $Y_T$ are the tree position within the plot and $X_i$ and $Y_i$ are the stem position. Joon Jin Song, a professor at the University of Arkansas (personal communication), created a script in the statistics programming language “R” (Gentleman and Ihaka 1993) that would run through the calculations for significance.

A. Spatial Distribution Patterns for the Ozarks

The Ozarks had 15 plots, as mentioned previously, that occurred in Devil's Den State Park (8 plots), Pea Ridge National Military Park (4 plots), and the Buffalo National River (3 plots). The spatial distribution of seedlings and saplings relative to trees was computed for these locations using a Euclidean distance measured in R. Initial calculations for significance included all seedlings and saplings with distances between 1 to 5 m from a host tree, but was later limited to only include stems between 1 and 4 m due to a lack of significance found for stems above 4 m.

i. Devil’s Den State Park

The data compiled for plot 1, in Devil's Den State Park, is shown in Figures 5.1 and 5.2, whereas Table 5.1 provides information for the trees and corresponding seedlings and saplings within the plot. Figure 5.1 indicates the trend and linear regression of white oak, black oak, and red oak seedling and sapling height with increasing distance from two red oak trees, one with a DBH of 25.9 cm and the other with a DBH of 52.1 cm. Significance (p-value = 0.0207, Table 5.1) was found only for red oak tree #1 with white oak seedlings and saplings, but not for the other ectomycorrhizal smaller stems within the plot.

Figure 5.2 shows the spatial distribution patterns from plot 1 with two white oak trees,
white oak tree #1 with a DBH of 99.8 cm and white oak tree #2 with a DBH of 56.4 cm. Both white oak trees had taller black oak seedlings and saplings closer to the trees, but there was not any significant trend in this spatial relationship. White oak tree #1 displayed declining red oak seedling and sapling height as the distance from the tree increased, without significance, but the trend reversed for white oak saplings and seedlings. The opposite arrangement was found for white oak #2, which had red oak seedlings and saplings being taller with increasing distance from the tree and white oak saplings being taller closer to the tree. In both instances, the red oak saplings and seedlings had a few stems that were much taller than the rest, causing the linear regression to be skewed towards those stems. In contrast, the white oak saplings and seedlings had little variability in their heights with tree distance, which leads to most appearing to be relatively uniform in height and not dependent on distance from the host trees, although the second white oak tree showed a slight height increase for closer stems.

The spatial distribution and significance for plot 2 are shown in Figure 5.3 and Table 5.2. There was no significance in black oak and red oak seedling and sapling height from distance to the host red oak tree (DBH of 72.1 cm). However, there was a slight trend (Figure 5.2) shown in stem height being taller closer to the tree and shorter farther out.

Figure 5.4 and Table 5.3 show a distribution pattern in plot 3 similar to that observed in plot 2, with seedling and sapling heights being taller closer to the tree. In this case, the host tree was a red oak (DBH of 64.3 cm), with red oak and white oak seedlings and saplings. Like plot 2, plot 3 displayed no significance in the spatial distribution of these seedlings and saplings.

Devil's Den State Park plot 4 had several trees (Table 5.4), four white oaks and one red oak, within the plot. The red oak tree and white oak tree #1 are shown in Figure 5.5, white oak # 2 and #3 are in Figure 5.6, and white oak tree #4 is shown in Figure 5.7. The red oak tree (Figure
and white oak seedlings and saplings were the only stems in plot 4 that showed a significant trend of white oak seedlings and saplings being taller closer to the tree (p-value = 0.0088, Table 5.4). In some cases, the significance of both white oak and black oak seedlings and saplings (Table 5.4) was not calculated because their distances were further from the host trees, white oak #1 and #2, than being analyzed by this study.

The red oak tree (Figure 5.5) had taller white oak and red oak seedlings and saplings closer to the tree, with black oak seedlings and saplings showing the opposite trend. The reverse is seen for white oak #1 in the accompanying graph (Figure 5.5), having taller black oak seedlings and saplings closer to the tree than red oak seedlings and saplings, which were shorter as the distance from the white oak tree decreased.

The two white oak trees, white oak #2 (DBH = 73.7 cm) and #3 (DBH = 15 cm), in Figure 5.6 displayed what were essentially opposite arrangement for most of their smaller stems-white, red, and black oak. Both trees displayed no significant relationship between seedling and sapling stem height and proximity to a potential host tree. In the case of white oak tree #2, all of the seedlings and saplings tended to be taller the father they were from the tree. The heights of the red oak seedling and saplings in relation to white oak tree #3 were also taller farther from the tree. Alternatively, white oak tree #3 had black and white oak seedlings and saplings taller closer to the tree.

The last tree, white oak #4 (DBH = 38.1 cm) had the least number of seedlings and saplings in close proximity to the tree. There were only two black oak and white oak seedlings and saplings next to the tree (Figure 5.7), which provided too few data points to calculate significance (Table 5.4). However, there were several red oak seedlings and saplings within 4 m of white oak tree #4. The trend of the red oak seedling and sapling heights in relationship to
the tree was for shorter heights to be closer to the host tree then to decrease with increasing distance from the tree (Figure 5.7).

Thus far, Devil's Den State Park plot 5 (Figures 5.8 and 5.9) had the greatest diversity in ectomycorrhizal trees within the plot, with post oak, red oak, and white oak present. However, none of the trees displayed any significant spatial relationship between seedling and sapling heights away from the trees (Table 5.5). The post oak tree (DBH = 90.2 cm, Figure 5.8) displayed a spatial relationship to both black oak and white oak seedlings and saplings, in which stem heights were shorter closer to the tree, and a majority of the taller seedlings and saplings were 2 to 4 meters away from the tree. The white oak tree (DBH = 19.1 cm), although it had only a few black oak seedlings and saplings in close proximity, displayed a trend in which the stems were taller closer to the tree. Figure 5.9 showed the spatial relationship between two red oak trees, red oak #1 (DBH = 69.9 cm) and red oak #2 (DBH = 17.8 cm), which showed opposite trends with respect to seedling and sapling height. The first red oak tree showed that the tallest black oak seedlings and saplings were farthest from the tree, and the second red oak tree showed taller seedling and sapling heights close to the tree.

Plot 6 at Devil's Den State Park showed no evidence of a significant relationship between seedling and sapling height and distance from host trees (white oak #1 [DBH = 11.4 cm], white oak #2 [DBH = 118.9 cm], and black oak [DBH = 58.4 cm]) within plot 6 (Table 5.6). The spatial distribution between white oak tree #1 and the ectomycorrhizal seedlings and saplings in plot 6 was one in which shorter stems were closer to the tree and taller ones were farther out (Figure 5.10). However, white oak tree #2 had an opposite configuration, where the white oak and black oak seedlings and saplings were taller closer to the tree and became shorter as the distance increased. The black oak tree in plot 6 (Figure 5.11) had a similar arrangement as
white oak tree #1 in the same plot, with all of the ectomycorrhizal seedlings and saplings showing increasing height as distance away from the tree increased.

Plot 7 in Devil's Den State Park had a large number of trees, seven in total, which were predominately white oaks with the exception of one red oak tree. Two out of the six trees in plot 7 showed a significant relationship between seedling and sapling height and distance from the host trees within the plot (Table 5.7). Seedling and sapling height in relation to distance from two white oak trees, white oak #1 (DBH = 81.3 cm) and white oak #1 (DBH = 50.8 cm), for plot 7 are shown in Figure 5.12. White oak tree #1 had several smaller white oak and red oak stems near the tree, but most were 3 to 4 meters from the tree, with heights greater than the smaller stems close to the tree. In the case of white oak tree #2, the red oak and black oak seedlings and saplings showed opposite arrangements, with smaller red oak stems being significantly (p-value = 0.0218, Table 5.7) taller closer to the tree. The regression line for smaller white oak stems in relation to white oak tree #2 is close to horizontal, showing that the smaller stems had a relatively constant average height.

White oak tree #3 and #4 (Figure 5.13) had similar seedling and sapling heights in relation to distance, as each ectomycorrhizal smaller stem, white oak, black oak, and red oak, had similar linear regression trends. Both the smaller black oak and white oak stems were taller farther from the two host trees, and the smaller red oak stems had a nearly constant height, with the exception of a slight tendency towards taller smaller stems near white oak tree #4.

The next two white oak trees, white oak #5 and #6, (Figure 5.14), showed similar relationships between smaller red oak stems and the trees. The seedling and sapling heights were greater near the trees and even were significantly taller near the sixth white oak tree. Both trees had white oak seedlings and saplings that were taller farther away from the trees but
displayed an opposite arrangement for black oak seedlings and saplings. White oak tree #5 had taller black oak seedlings and saplings closer to the tree, whereas for white oak tree #6, they were taller farther away from the tree.

The last white oak tree, white oak tree #7 (DBH = 88.4 cm), did not exhibit any significance between seedling and sapling height and distance to the host tree (Table 5.7). Both black oak and white oak seedlings and saplings showed a trend for taller stems farther from the tree and shorter stems closer to the tree (Figure 5.15). The red oak seedlings and saplings showed an opposite trend, with a slight increase in average stem heights closer to the tree than farther out.

The last plot (plot 8) in Devil's Den State Park had three white oak trees-white oak #1 (DBH = 53.3 cm), white oak #2 (DBH = 104.6 cm), and white oak #3 (DBH = 29.2 cm) (Figure 5.16 and 17, Table 5.8). For all three white oak trees, there was no significance between seedling and sapling heights and distance to the trees (Table 5.8). The black oak seedlings and saplings showed a nearly constant average height with increasing distance from the first white oak tree (Figure 5.16) but exhibited an increasing height with increasing distance from both white oak tree #2 and #3 (Figure 5.16 and 17). White oak tree #1 (Figure 5.16) showed a trend of white oak seedling and saplings being shorter closer to the tree and taller farther away. The significance of the white oak seedlings and saplings (Figure 5.16) was not calculated because their distances were further from the host tree, white oak #2, than being analyzed by this study. White oak tree #3 (Figure 5.17) had an opposite arrangement of white oak and black oak seedlings and saplings in relation to the tree, with white oaks decreasing in height as distance increased from the tree and black oaks increasing in height as distance increased from the tree.

ii. Pea Ridge National Military Park

Applying the same method as outlined earlier, calculations to determine spatial significance
between host ECM trees and seedlings and saplings for plots in Pea Ridge National Military Park were completed for each of the four plots surveyed at this site. The first plot had four post oak trees, with DBH values ranging from 9.7 cm to 16.1 cm (Table 5.9). There was no apparent significance for seedling and sapling heights shown in relation to distance to any of the four post oak trees (Table 5.9). The linear regression line trends (Figure 5.18) for post oak seedlings and saplings in relation to the first two post oak trees (post oak #1 DBH = 16.1 cm and post oak #2 DBH = 9.7 cm) showed that the seedlings and saplings were taller closer to the trees than those farther out. The blackjack oak seedlings and saplings had a nearly constant linear regression line for height in relation to post oak #2 (Figure 5.18 right graph) as distance from the tree increased.

This same trend for blackjack oak seedlings and saplings was seen with post oak tree #3 (Figure 5.19), which had a nearly flat regression line, although the line does project a slightly greater, 1 or 2 cm, trend in stem height with a distance of approximately 4 m from the host tree. Post oak seedlings and saplings in relation to post oak tree #3 also showed a decrease in stem height with increasing distance from the host tree. In the case of post oak tree #4 (Figure 5.19, right graph), the opposite trend was apparent when compared to the data for post oak tree #3, with post oak saplings and seedlings displaying a nearly constant height with increasing distance from the host tree. Blackjack oaks had taller stems closer to post oak tree #4 than farther out.

Plot 2 (Figures 5.20 and 5.21 and Table 5.10) in Pea Ridge National Military Park had three trees, two white oaks and one black oak, which did not display any significant relationship between the height of seedlings and saplings and the distance to trees in the plot. The two white oak trees (Figure 5.20), white oak #1 (DBH = 60.0 cm) and white oak #2 (DBH = 72.6 cm), showed opposing trends for black oak seedlings and saplings, where white oak tree #1 had taller
stems closer to the tree. Both trees had taller stems of blackjack oak closer to the tree, but height declined as distance from the trees increased, albeit a less drastic decline in height was apparent for white oak tree #2. The significance of the blackjack oak seedlings and saplings (Table 5.10 and Figure 5.21) was not calculated because there were too few stems to do the calculations. The black oak tree (DBH = 28.7 cm) in plot 1 (Figure 5.21) had both taller blackjack oak and black oak seedlings and saplings closer to the tree, whereas white oaks displayed an opposite trend.

The third plot in Pea Ridge National Military Park had one blackjack oak tree and five post oak trees, all of which showed no significant relationship between the trees and the seedlings and saplings in the plot (Table 5.11). The blackjack oak tree (Figure 5.22) had a DBH = 12.7 cm, and there were both blackjack and post oak seedlings within 4 m of the tree. Both blackjack oak and post oak seedlings and saplings showed a trend of taller stems being closer to the tree, with a substantial decline in stem height out to 4 m. Adjacent to the data for the blackjack oak tree in Figure 5.22 (right side) is the linear regression graph for post oak tree #1 (DBH = 73.7 cm). Post oak tree #1 had only two post oak seedlings and saplings within 4 m of the tree, which created a shifted regression line displaying a trend towards shorter stems being closer to the tree than farther out.

Post oak trees #2 and #3 (Figure 5.23) show trends for taller blackjack oak seedlings and saplings closer to the trees than farther out. The two trees have opposing trends for post oak seedlings and saplings, which show taller heights farther from post oak tree #2 and taller heights closer to post oak tree #3.

The last two post oak trees, post oak tree #4 (DBH = 49.8 cm) and post oak tree #5 (DBH = 96.3 cm), in plot 3 (Figure 5.24) had post oak seedlings and saplings displaying different trends.
of growth patterns near the trees. For post oak tree #4, post oak seedlings and saplings were taller closer to the tree and had much shorter (nearly 5 cm to as much as 20 cm) heights near 3 to 4 meters from the tree. Post oak seedlings and saplings were shorter closer to post oak tree #5 and increased in height as distance from the tree increased.

The last plot in Pea Ridge National Military Park, plot 4, had a larger number of trees (five blackjack oak and three post oak trees) than the other plots in this site (Table 5.12). The blackjack oak trees did not show any significant relationship to blackjack and post oak seedling and sapling heights in the plot. However, all three post oak trees showed a significant relationship with post oak seedling and sapling heights in the plot.

The seedling and sapling height in relation to distance to blackjack oak tree #1 (DBH = 28.4 cm) and blackjack oak #2 (DBH = 13.7 cm) had similar trends, with shorter post oak seedlings and saplings found closer to the trees and taller farther out (Figure 5.25). The two trees had an opposite trend, with taller post oak seedlings and saplings found closer to blackjack oak tree #1 and shorter close to blackjack oak tree #2.

The next two blackjack oak trees, blackjack oak #3 (DBH = 33.0 cm) and #4 (DBH = 20.3 cm), in plot 4 (Figure 5.26) had several post oak seedlings and saplings, but showed opposite trends. The smaller post oak stems tended to be far from blackjack oak tree #3, but these smaller stems became taller with increasing distance from the tree. In the case of blackjack oak tree #4, the smaller post oak stems were taller closer to the tree and shorter as distance increased. Blackjack oak tree #4 also had taller blackjack oak seedlings and saplings closer to the tree, which decreased in height as distance from the tree increased. The last blackjack oak tree, blackjack oak #5 (DBH = 14.7 cm), had shorter post oak seedlings and saplings closer to the tree and taller as distance increased (Figure 5.27).
All of the post oak trees in Pea Ridge National Military Park plot 4 displayed spatial significance to the seedlings and saplings within the plot (Table 5.12). The first post oak tree (DBH = 29.2 cm) showed a significant (p-value = 0.0251) number of smaller stems that were taller closer to the tree, which declined as distance between smaller stems and tree increased (Table 5.12, Figure 5.27 right side). For post oak tree #2 (DBH = 116.8 cm) and post oak tree #3 (DBH = 37.8 cm), post oak seedlings and saplings had significantly (p-value = 0.0189 and p-value = 0.0005) greater heights closer to the trees that declined with increasing distance (Table 5.12, Figure 5.28).

iii. Buffalo National River Park

The last study site in northwest Arkansas was the Buffalo National River Park, which had three plot locations. The first plot location had a large number of trees, one black oak tree, two southern red oak trees, and three white oak trees (Table 5.13). The first two red oak trees, red oak #1 (DBH = 48.3 cm) and red oak #2 (DBH = 62.2), did not show a significant relationship between seedling and sapling heights and distance to either tree. For both red oak tree #1 and #2, there was only only one chestnut oak seedling within 4 m of each tree, which was not enough to show a relationship between height and distance to the trees (Figure 5.29). However, there were several white oak, black oak, and southern red oak seedlings and saplings within 4 m of these trees. Overall, shorter white oak and southern red oak seedlings and saplings were found closer to the two trees (Figure 5.29), and this height increased as the distance from the trees increased. The black oak seedlings and saplings were shorter closer to red oak tree #1, but they had greater heights closer to red oak tree #2.

In plot 3 white oak tree #1 (DBH = 84.1 cm) and white oak tree #2 (DBH = 7.4 cm) were surrounded by several species of oak seedlings and saplings: white oak, black oak, southern red
There were only a few chinquapin oak seedlings and saplings within 4 m of the white oak trees, resulting in skewed linear regression lines and causing only a best guess estimate that the stem heights tended to be shorter closer to the two trees and increased in height with increased distance. White oak tree #1 had shorter southern red oak and black oak seedlings and saplings close to the tree and had a trend towards taller heights as distance from the tree increased (Figure 5.30). White oak seedling and sapling heights in relation to distance from white oak tree #1 were significant (p-value = 0.0014, Table 5.13), with shorter stem heights found closer to the tree and taller stems heights found farther out. White oak tree #2 had a similar trend, with white oak seedlings and saplings being shorter closer to the tree, but this was not significant. Red oak seedlings and saplings were taller closer to white oak tree #2.

White oak tree #3 (DBH = 28.4 cm) and white oak tee #4 (DBH = 2.8 cm) had a large number of species, seedlings, and saplings of oak within 4 m of the two trees (Figure 5.31). White oak, southern red oak, and black oak seedlings and saplings were taller closer to white oak tree #3, which then tapered off to shorter heights as 4 m was approached. Chinquapin seedlings and saplings had an opposite relationship with white oak tree #3, where shorter heights were found closest to the tree and taller were found farther out. White oak tree #4 had an interesting relationship between seedling and sapling (white oak, black oak, southern red oak, and chinquapin oak) heights and distance from the tree in that all stems showed a trend towards greater heights near the tree, with heights that declined by approximately 10 cm out to 4 m (Figure 5.31).

The last two trees in plot 1 within the Buffalo National River Park were white oak tree #5 (DBH = 28.4 cm) and the black oak tree (DBH = 2.8 cm). Both trees had nearly identical linear
regression lines for the relationship between black oak and southern red oak seedling and sapling heights and distance from the trees, which displayed a trend towards taller stem heights closer to the trees that declined farther out (Figure 5.32). The chinquapin oak seedlings and saplings exhibited similar trends in relation to the two trees, with heights being shorter closer to the trees and increasing with distance (Figure 5.32). This trend was significant (p-value = 0.0495, Table 5.13) for the white oak tree. The white oak seedlings and saplings had an opposite relationship between the two trees, with seedlings and saplings having shorter heights closer to white oak tree #5 but taller heights closer to the black oak tree.

The second plot in the Buffalo National River Park had all white oak trees and very few seedlings and saplings within 4 m of the trees (Table 5.14), displaying no significant relationship between seedling and sapling height and distance to the trees. White oak tree #1 (DBH = 99.8 cm) and white oak tree #2 (DBH = 41.9 cm) had only a few seedlings and saplings within 4 m of the trees, with so few near white oak tree #2 that a test for significance could not be made (Figure 5.33, Table 5.14). The trend of seedlings and saplings for both trees showed them being shorter closer to the trees, which was observed for four white oak and two southern red oak seedlings and saplings near white oak tree #1 and #2, respectively.

White oak tree #3 (DBH = 34.3 cm) and white oak tree #4 (DBH = 52.1 cm) had both white oak and southern red oak seedlings and saplings within 4 m of the trees, but not enough to test for significance, with the exception of white oak seedling and sapling heights in relation to distance from white oak tree #3 (Figure 5.34, Table 5.14). The white oak and southern red oak seedling and sapling heights in relation to white oak tree #3 showed very little variation in height with increased distance from the tree, making the linear regression line nearly horizontal (Figure 5.34). The lack of seedlings and saplings within 4 m of white oak tree #4 resulted in an
inability to test for significance (Table 5.14), and any estimates based on the relationship between seedling and sapling heights and distance from the tree using the regression line could not be made as these lines were offset due to the low number of stems (Figure 5.34).

The last Buffalo National River Park plot, plot 3, had one post oak tree (DBH = 85.1 cm) with red oak seedlings and saplings within 4 m of the tree, but with no significance shown (Table 5.15, Figure 5.35). The red oak seedlings and saplings had an overall trend towards shorter stems closer to the tree and taller stems out to 4 m.

**B. Spatial Distribution Patterns for the Fernow Experimental Forest**

The next region, the Fernow Experimental forest, had seven plots in undisturbed areas and three plots in burn site locations. The seedling and sapling heights and spatial distribution relative to the trees in each plot were calculated using the same Euclidean distance measure and statistical software program "R" that was done in northwest Arkansas. There were no regression graphs or significant spatial relationships provided for the three burn sites due to a lack of seedlings and saplings within 4 m of the trees in the burn plots.

The spatial relationships between seedlings, saplings, and trees within the remaining seven plots in the Fernow Experimental Forest were calculated and are shown in Figures 5.36-5.44 and Tables 5.15-5.20. The first plot had two red oak trees, red oak #1 (DBH = 22.9 cm) and red oak tree #2 (DBH = 10.7 cm), with red oak seedlings and saplings within 4 m of the two trees. The first red oak tree had taller red oak seedlings and saplings closer to the tree that gradually decreased out to 4 m (Figure 5.36), but without significance (Table 5.16). The second red oak tree also had no significant spatial relationships with red oak seedlings and saplings (Table 5.16), with the overall trend of seedling and sapling heights being nearly constant with increasing distance from the tree (Figure 5.36).
The second plot in the Fernow Experimental Forest had several trees (two beech, three chestnut oak, and one red oak), but none of the calculations for significance between seedling and sapling heights in relation to distance from the trees were significant (Table 5.17). The first chestnut oak tree (DBH = 97.8 cm) and chestnut oak tree #2 (DBH = 129.5 cm) had nearly identical trends in seedling and sapling stem heights in relation to distance from the two trees (Figure 5.38). For each tree the seedlings and saplings within 4 m were red oak and beech, with shorter red oak seedlings and saplings found closer to the trees than farther out, and beech seedlings and saplings had an opposite trend, with taller stems found closer to the trees and declined in height with distance from them.

The spatial distribution graphs of seedling and sapling heights in relation to distance from the third chestnut oak tree (DBH = 111.5 cm) and red oak tree (DBH = 113.0 cm) within plot 2 are shown in Figure 5.39. Both trees had red oak and beech seedlings and saplings within 4 m of the trees and displayed similar trends in stem heights as distance from the trees increased. Taller red oak seedlings and saplings were found closer to the trees than farther out. However, this trend had a more dramatic decline in red oak seedling and sapling stem heights in relation to distance from the red oak tree than in relation to distance from the chestnut oak tree. A similar situation was seen for the beech seedlings and saplings, with shorter stems found closer to the two trees and taller farther out, but the trend towards taller heights had a more dramatic increase in height with relation to distance from the red oak tree.

Plot 3 had the greatest number of seedlings and saplings (red oak, chestnut oak, and beech) within 4 m of the two red oak host trees in the plot (Figure 5.40). The first red oak tree (DBH = 139.7 cm) had a nearly horizontal linear regression trend line for the red oak and beech seedling and sapling heights in relation to distance from the tree (Figure 5.40). The red oak seedlings
and saplings tended to be shorter closer to the first red oak tree, with a slight shift toward taller stem heights out to 4 m (Figure 5.40), without significance. Beech had an opposite relationship to red oak tree #1 in that the seedlings and saplings had taller stem heights closer to the tree and had a slight shift toward shorter stem heights out to 4 m (Figure 5.40), again without significance. Although significance was not found, chestnut oak seedling and sapling stem heights in relation to distance from red oak tree #1 had the most steep linear regression trend line, which had much taller stem heights closer to the tree than those out to 4 m (Figure 5.40).

The second red oak tree in plot 3 had a similar stem height relationship to red oak and beech seedlings and saplings to those that were seen for the first red oak tree; the linear regression line was close to horizontal, with stem heights having only slight variation out to 4 m (Figure 5.40). Shorter seedlings and saplings were found closer to red oak tree #2 (Figure 5.40), with a significant increase in red oak seedling and sapling heights out to 4 m (p-value = 0.0261, Table 5.17) and an insignificant increase in beech seedling and sapling heights out to 4 m. Chestnut oak seedling and sapling heights in relation to distance from red oak tree #2 were similar to red oak tree #1, where a steep linear regression trend line was calculated, and taller stem heights were found close to the tree and decreased substantially (~15 cm) out to 4 m.

Plot 4 in the Fernow Experimental Forest had fewer seedlings and saplings than seen in plot 3 (Figure 5.41). There was one chestnut oak tree (DBH = 91.2 cm) in plot 4, with red oak and chestnut oak seedlings and saplings within 4 m of the tree. Both the red oak and chestnut oak seedlings and saplings had stem heights that were taller closer to the tree and declined as distance from the tree increased, but neither trend was significant.

Plot 5 did not have any trees within 4 m of seedlings and saplings within the plot, which prevented any calculations for significance or trends in stem heights to be made. Plot 6, on the
other hand, had one red oak tree (DBH = 161.3 cm) with significant results for seedling and sapling heights in relation to distance from the tree (Table 5.19). Red oak seedlings and saplings in plot 6 (Figure 5.42) had significantly (p-value = 0.0175, Table 5.19) taller stems closer to the red oak tree, with shorter stems out to 4 m. Likewise, beech seedlings and saplings had significantly (p-value = 0.0038, Table 5.19) taller stems closer to the tree that decreased as distance from the tree increased (Figure 5.42).

The last plot, plot 7, in the Fernow Experimental Forest had four beech trees, two of which had significant beech seedling and sapling heights in relation to distance from the trees. Beech tree #1 (DBH = 40.1 cm) and beech tree #2 (DBH = 3.8 cm) had both red oak and beech seedlings and saplings within 4 m of the trees. The red oak seedling and sapling heights varied little with increased distance from the trees out to 4 m (Figure 5.43). The red oak seedling and sapling heights had only a slight shift towards taller heights near 4 m and shorter near the trees, with a nearly horizontal regression line. The beech seedling and sapling heights in relation to distance from the two trees showed a significant (p-value = 0.0015, Table 5.20) relationship to beech tree #2 but no significant relationship to beech tree #1. However, both trees had beech seedlings and saplings with shorter stem heights closer to the trees that increased considerably (~60 cm from beech tree #1 and ~20 cm from beech tree #2) at a distance of 4 m from the trees (Figure 5.43).

Beech tree #3 (DBH = 46.2 cm) and beech tree #4 (DBH = 5.8 cm) in plot 7 had similar trends in red oak and beech seedling and sapling heights in relation to distance from the trees (Figure 5.44). The red oak seedlings and saplings had smaller stem heights closer to the trees, with a slight increase, although without significance, in stem heights as distance from the trees increased. Taller beech seedlings and saplings were found closer to the trees, which decreased
in height out to 4 meters, with significance (p-value = 0.0182, Table 5.20) found in relation to beech tree #4 but not in relation to beech tree #3.

5.3 Results from the Spatial Distribution Patterns

There were a total of four trees within Devil's Den State park that exhibited a significant relationship between seedling and sapling heights and distance from the trees. The trees were both red oak and white oak, with significance found for white oak and red oak seedlings and saplings, respectively. The trend of the seedling and saplings heights was to be taller closer to the trees and shorter farther out, except in one case where the opposite was found. The red oak and white oak trees had an average DBH of 34.4 cm.

Pea Ridge National Military Park had three trees, all post oak, with a significant relationship between post oak seedling and sapling heights and distance from the trees. In all cases of significance, the seedling and saplings heights had a trend of being taller closer to the tree and shorter out to 4 m. The post oak trees had an average DBH of 61.3 cm.

The Buffalo National River had two white oak trees with a significant relationship between white oak and chestnut oak seedling and sapling heights and distance from the trees. In both cases, the seedling and sapling stem heights were shorter closer to the trees, which declined as distance increased. The average DBH of the two trees was 56.3 cm.

For all of the plots within northwest Arkansas, there were nine trees with a significant relationship between seedling and sapling stem heights and distance from the trees within the plots. The seedling and sapling stem heights showed a greater tendency towards being taller closer to the trees (6 out of 9) and shorter farther out. The host trees also had rather large DBH measurements, with an average of 76.0 cm.

The Fernow Experimental Forest had five trees (three red oaks and two beeches) that had a
significant relationship between red oak and beech seedling and sapling heights and distance from the trees within the plots. In relation to the trees, the seedling and sapling stem heights also showed a greater tendency towards being taller closer to the tree and shorter farther out (3 out of 5). The host trees had larger average DBH measurements (92.5 cm) than those in northwest Arkansas.

The overall trend in significance for both regions was for seedlings and saplings to have taller heights closer to host trees (9 out of 14) and to have an association with trees of larger DBH measurements (average of 61.1 cm). However, when looking at the overall trend in the regions without significance, northwest Arkansas had 54% of seedlings and saplings with shorter heights closer to the trees and 46% having taller heights closer to the trees, whereas the Fernow Experimental Forest showed a 50:50 relationship between seedling and sapling heights and distance from the trees. Based on the results from the trees, seedlings, and saplings without significance, the average between both regions would be shorter stem heights for seedlings and saplings (52%) closer to host trees, with the remaining (48%) having taller heights closer to host trees.

Given these data, it would seem that in order for there to be a significant relationship between seedling and sapling heights and distance from trees, they would need to be taller closer to the host and within 4 m of a well-established (large DBH) tree. The lack of significance throughout the data appears to be a result several factors, one of those being a skewing effect caused by outlier stems with very different heights in comparison to the average stem height, which shifted the trend lines and removed significance for the remaining stems.

Another factor would be the fact that the number of host trees within the plots were not large enough to gauge the impact they have on the seedlings and saplings. However, this factor
may not be avoidable, as the plot selection, although chosen for comparable regions with little disturbance, were made as random as possible to provide a realistic model of the forest floor.

Finally, it is believed that one of the largest impacts to these forests is unavoidable, and in some instances necessary, disturbances that take place such as prescribed burns and herbivory from deer and other animals. Prescribed burns took place in both northwest Arkansas and the Fernow Experimental Forest; however, the most recent burn locations were at Pea Ridge National Military Park and the three burn plots in the Fernow Experimental Forest. Plots 1, 3, and 4 in Pea Ridge National Military Park were established in the summer of 2012 and had undergone prescribed burns in the fall of 2011. The plots still had evidence of the prescribed burns on the forest floor, as charred pieces of debris were still present, and the number of seedlings and saplings within those plots were much lower than in plot 2, which was visited in 2011, before the burn.

The trend of seedling and sapling height in relation to host trees within the Pea Ridge National Military park plots had ~51% of stem heights being taller closer to the trees and the other 49% being shorter, although significance was not found. However, three of the host trees within these plots did significantly impact the height of the surrounding seedlings and saplings, which also showed taller stem heights closer to the trees. It would appear that in the areas of prescribed burn, being closer to a host tree helps to assist the growth and survivorship of ectomycorrhizal stems, as they tend to be taller (in some cases significantly so) closer to the tree.

The Fernow Experimental Forest had three burn sites, which were hot-burns that took place every 4-5 years. In all three burn plots there were no trees within 4 m of ectomycorrhizal seedlings or saplings that could have an influence on their growth, which could be attributed to the type of burn (hot-burn), frequency of burn (4-5 years), and/or recency of the burn. In
addition, the Fernow Experimental Forest had an average of 16 (6,400/ha) ectomycorrhizal seedlings and saplings in the burned plots and 168 in the unburned plots (67,200/ha), which also could have attributed to the inability to calculate potential spatial relationships in the burn plots.

Pea Ridge National Military Park also experienced a decline in the number of seedlings and saplings within the burned plots, with an average of 56 (5,600/ha) ectomycorrhizal seedlings and saplings in the burned plots and 83 (8,300/ha) ectomycorrhizal seedlings and saplings in unburned plots. Although the plot size in Pea Ridge National Military Park was larger (10x10 m) than those in the Fernow Experimental Forest (5x5 m), based on the data, there is an apparent trend towards fewer ectomycorrhizal stems surviving in burn plots. However, for those that do survive, there is a spatial relationship between the seedlings and saplings and their host trees that either significantly (plot 3 Pea Ridge National Military Park) or insignificantly results in stem heights being taller closer to the tree.

To gauge the long-term impacts of ectomycorrhizal seedling survivorship and regeneration in response to prescribed burns, it would be advantageous to extend this type of study over several years. La Spana (2008) looked at the growth and development of seedlings and saplings in recently burned plots over three seasons in 2008 and found their growth was not negatively impacted by prescribed burns and that the opened canopy due to the fire actually assisted the growth of smaller stems. Alternatively, Lear (2004), says that the assistance of prescribed burns to oak regeneration is a site-dependent factor and only under poor site conditions, typically xeric locations, will oaks be persistent due to their ability to live under unfavorable conditions, which gives them an advantage over more susceptible species. In good site locations, oaks are being replaced due to their slower growth rate, shade intolerance, and succession strategies (Lear 2004, Johnson et al. 2009). Lear et al (2000) and Brose et al. (2012) ascertained that frequency of
prescribed burns can be an assistive factor in oak regeneration, with increased burns resulting in more favorable conditions, even in good site locations. The frequency of burns within Pea Ridge National Military Park and the Fernow Experimental Forest are also often and could yield positive results for oak regeneration. However, determining the long-term impacts of prescribed burns in a 4-5 year frequency cycle or even from 1 year to the next was out of the scope of this study. It would be interesting and beneficial to revisit the same sites to determine the long-term impacts of the burns taking place in these locations.

Herbivory, mostly from deer, is an additional factor that may have influenced the low number of seedlings and saplings within the burn sites and the lack of significant seedling and sapling heights in relation to distance from host trees in northwest Arkansas and the Fernow Experimental forest. The pressure deer place on oak seedling and sapling growth and regeneration has been so great that studies have found decreased stem heights, species richness, and poor development caused by deer browsing (Tilghman 1984, Campbell 2006).

This was also shown in a controlled experiment, lasting 6-8 years, where fences were used to block deer browsing on oak seedlings (Yuska et al. 2008). During the 6-8 year cycle these authors monitored the oak seedlings and found an average increase in seedling abundance, density, and height. Extrapolating the results of these studies to the results of the seedling and sapling stem heights in relation to distance from host trees in northwest Arkansas and the Fernow Experimental Forest could explain the lack of significance found within many plots and the increased short stem heights closer to the host trees. The lack of significance may be a result of herbivory preventing seedlings and saplings from reaching their height potential and thereby reducing the significant impact the host trees would have on them. The lack of significance from seedling and sapling heights in relation to distance from host trees could also be a result of
deer removing terminal shoots during browsing. Removal of terminal shoots leads to stunted growth and/or death of oak seedlings (Oswalt 2006) and would reduce oak regeneration as well as interfere with predictions of the potential assistance a host ectomycorrhizal tree may have on them.
Figure 5.1. Seedling and sapling heights in relation to distance from two red oak trees in plot 1 in Devil's Den State Park. Left graph shows the correlation with red oak tree #1, with a DBH of 10.2 in (25.9 cm), and the right with red oak tree #2 having a DBH of 20.5 in (52.1 cm).
Figure 5.2. Seedling and sapling heights in relation to distance from two white oak trees in plot 1 in Devil's Den State Park. Left graph shows the correlation with white oak tree #1, with a DBH of 39.3 in (99.8 cm), and the right with white oak #2, having a DBH of 22.2 in (56.4 cm).
Table 5.1. Significance data for seedling and sapling heights in relation to distance from trees in Devil's Den State Park plot 1.

<table>
<thead>
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<th>Tree Common name</th>
<th>DBH in (cm)</th>
<th>Location (along, within)</th>
<th>Seedlings &amp; Saplings Common name</th>
<th>p-value</th>
<th>Significance</th>
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<td>Red oak #1</td>
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<td></td>
<td></td>
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<td>Red oak #2</td>
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<td></td>
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<td>0.8079</td>
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Figure 5.3. Seedling and sapling heights in relation to distance from a red oak tree (DBH 72.1 cm) in plot 2 in Devil's Den State Park.
Table 5.2. Significance data for seedling and sapling heights in relation to distance from trees in Devil's Den State Park plot 2.

<table>
<thead>
<tr>
<th>Tree</th>
<th>Location (along, within)</th>
<th>Seedlings &amp; Saplings</th>
<th>Common name</th>
<th>p-value</th>
<th>Significance</th>
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</thead>
<tbody>
<tr>
<td>Red oak</td>
<td>28.4 (72.1)</td>
<td>9.54, 8.92</td>
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<td>0.8516</td>
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<td>Red oak</td>
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Figure 5.4. Seedling and sapling heights in relation to distance from a red oak tree (DBH 64.3 cm) in plot 3 in Devil's Den State Park.
Table 5.3. Significance data for seedling and sapling heights in relation to distance from trees in Devil's Den State Park plot 3.

<table>
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<th>Tree</th>
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<td>Common name</td>
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<tr>
<td>Red oak</td>
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Figure 5.5. Seedling and sapling heights in relation to distance from a red and white oak tree in plot 4 in Devil's Den State Park. Left graph shows the correlation with a red oak tree with a DBH of 41.2 cm, and the right with white oak tree #1 having a DBH of 56.6 cm.
Figure 5.6. Seedling and sapling heights in relation to distance from two white oak trees in plot 4 in Devil's Den State Park. Left graph shows the correlation with white oak #2 tree, with a DBH of 73.7 cm, and the right with white oak #3 tree having a DBH of 15.0 cm.
Figure 5.7. Seedling and sapling heights in relation to distance from white oak tree #4 (DBH 38.1 cm) in plot 4 in Devil's Den State Park.
Table 5.4. Significance data for seedling and sapling heights in relation to distance from trees in Devil's Den State Park plot 4.

<table>
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<th>Tree Common name</th>
<th>DBH in (cm)</th>
<th>Location (along, within)</th>
<th>Seedlings &amp; Saplings</th>
<th>p-value</th>
<th>Significance</th>
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</thead>
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<td>0.2263</td>
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<td>White oak #1</td>
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<td>White oak #2</td>
<td>29 (73.7)</td>
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<td>White oak #3</td>
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<td>9.11, 6.88</td>
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<td>Black oak</td>
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<td>Red oak</td>
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Figure 5.8. Seedling and sapling heights in relation to distance from a post and white oak tree in plot 5 in Devil's Den State Park. Left graph shows the correlation with a post oak tree with a DBH of 90.2 cm, and the right with a white oak tree having a DBH of 19.1 cm.
Figure 5.9. Seedling and sapling heights in relation to distance from two red oak trees in plot 5 in Devil's Den State Park. Left graph shows the correlation with red oak tree #1 with a DBH of 69.9 cm and the right with red oak tree #2 having a DBH of 17.8 cm.
Table 5.5. Significance data for seedling and sapling heights in relation to distance from trees in Devil's Den State Park plot 5.

<table>
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<th>DBH in (cm)</th>
<th>Location (along, within)</th>
<th>Seedlings &amp; Saplings</th>
<th>Common name</th>
<th>p-value</th>
<th>Significance</th>
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<td>Post oak</td>
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<td>Black oak</td>
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<td>8.45, 7.53</td>
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<td>Red oak #2</td>
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Figure 5.10. Seedling and sapling heights in relation to distance from two white oak trees in plot 6 in Devil's Den State Park. Left graph shows the correlation with white oak tree #1, with a DBH of 11.4 cm, and the right with white oak tree #2 having a DBH of 118.9 cm.
Figure 5.11. Seedling and sapling heights in relation to distance from a black oak tree (DBH 58.4 cm) in plot 6 in Devil's Den State Park.
Table 5.6. Significance data for seedling and sapling heights in relation to distance from trees in Devil's Den State Park plot 6.

<table>
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<th>Tree Common name</th>
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Figure 5.12. Seedling and sapling heights in relation to distance from two white oak trees in plot 7 in Devil's Den State Park. Left graph shows the correlation with white oak tree #1, with a DBH of 81.3 cm, and the right with white oak tree #2 having a DBH of 50.8 cm.
Figure 5.13. Seedling and sapling heights in relation to distance from two white oak trees in plot 7 in Devil's Den State Park. Left graph shows the correlation with white oak tree #3, with a DBH of 40.6 cm, and the right with white oak tree #4 having a DBH of 66.0 cm.
Figure 5.14. Seedling and sapling heights in relation to distance from two white oak trees in plot 7 in Devil's Den State Park. Left graph shows the correlation with white oak tree #5, with a DBH of 113.0 cm, and the right with white oak tree #6 having a DBH of 30.0 cm.
Figure 5.15. Seedling and sapling heights in relation to distance from white oak tree #7 (DBH of 88.4 cm) in plot 7 in Devil's Den State Park.
Table 5.7. Significance data for seedling and sapling heights in relation to distance from trees in Devil's Den State Park plot 7.

<table>
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<th>Tree Common name</th>
<th>DBH in (cm)</th>
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<th>Significance</th>
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<td>Tree Common name</td>
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<td>p-value</td>
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<td>Red oak</td>
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Figure 5.16. Seedling and sapling heights in relation to distance from two white oak trees in plot 8 in Devil's Den State Park. Left graph shows the correlation with white oak tree #1, with a DBH of 53.3 cm, and the right with white oak tree #2 having a DBH of 104.6 cm.
Figure 5.17. Seedling and sapling heights in relation to distance from white oak tree #3 (DBH of 19.2 cm) in plot 8 in Devil's Den State Park.
Table 5.8. Significance data for seedling and sapling heights in relation to distance from trees in Devil’s Den State Park plot 8.

<table>
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<th>Tree Common name</th>
<th>DBH inches (cm)</th>
<th>Location (along, within)</th>
<th>Seedlings &amp; Saplings Common name</th>
<th>p-value</th>
<th>Significance</th>
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Figure 5.18. Seedling and sapling heights in relation to distance from two post oak trees in plot 1 in Pea Ridge National Military Park. Left graph shows the correlation with post oak tree #1, with a DBH of 16.1 cm, and the right with post oak tree #2 having a DBH of 9.7 cm.
Figure 5.19. Seedling and sapling heights in relation to distance from two post oak trees in plot 1 in Pea Ridge National Military Park. Left graph shows the correlation with post oak tree #3, with a DBH of 12.9 cm, and the right with post oak tree #4 having a DBH of 12.3 cm.
Table 5.9. Significance data for seedling and sapling heights in relation to distance from trees in Pea Ridge National Military Park plot 1.

<table>
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<th>Tree Location</th>
<th>Seedlings &amp; Saplings</th>
<th>p-value</th>
<th>Significance</th>
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<td>Common name</td>
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<td></td>
</tr>
<tr>
<td>Post oak #1</td>
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<td>Post oak #2</td>
<td>3.8 (9.7)</td>
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<td>Post oak #3</td>
<td>5.1 (12.9)</td>
<td>3.07,7.44</td>
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<td>Post oak #4</td>
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Figure 5.20. Seedling and sapling heights in relation to distance from two white oak trees in plot 2 in Pea Ridge National Military Park. Left graph shows the correlation with white oak tree #1, with a DBH of 66.0 cm, and the right with a white oak tree #2 having a DBH of 72.6 cm.
Figure 5.21. Seedling and sapling heights in relation to distance from a black oak tree (DBH of 28.7cm) in plot 2 in Pea Ridge National Military Park.
Table 5.10. Significance data for seedling and sapling height in relation to distance from trees in Pea Ridge National Military Park plot 2.

<table>
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<th>Tree</th>
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<th>Common name</th>
<th>p-value</th>
<th>Significance</th>
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<td>0.2363</td>
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<td>White oak #1</td>
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</tbody>
</table>
Figure 5.22. Seedling and sapling heights in relation to distance from a blackjack and post oak tree in plot 3 in Pea Ridge National Military Park. Left graph shows the correlation with a blackjack oak tree, with a DBH of 12.7 cm, and the right with post oak tree #1 having a DBH of 73.7 cm.
Figure 5.23. Seedling and sapling heights in relation to distance from two post oak trees in plot 3 in Pea Ridge National Military Park. Left graph shows the correlation with post oak tree #2, with a DBH of 10.2 cm, and the right with post oak tree #3 having a DBH of 73.2 cm.
Figure 5.24. Seedling and sapling heights in relation to distance from two post oak trees in plot 3 in Pea Ridge National Military Park. Left graph shows the correlation with post oak tree #4, with a DBH of 49.8 cm, and the right with post oak tree #5 having a DBH of 96.3 cm.
Table 5.11. Significance data for seedling and sapling heights in relation to distance from trees in Pea Ridge National Military Park plot 3.

<table>
<thead>
<tr>
<th>Tree Common name</th>
<th>DBH in (cm) (along, within)</th>
<th>Location</th>
<th>Seedlings &amp; Saplings Common name</th>
<th>p-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blackjack oak</td>
<td>5.0 (12.7)</td>
<td>6.26,6.7</td>
<td>Blackjack oak</td>
<td>0.3933</td>
<td>no</td>
</tr>
<tr>
<td>Post oak #1</td>
<td>29.0 (73.7)</td>
<td>2.1,0.28</td>
<td>Post oak</td>
<td>0.6657</td>
<td>no</td>
</tr>
<tr>
<td>Post oak #2</td>
<td>4.0 (10.2)</td>
<td>4.3,3.87</td>
<td>Blackjack oak</td>
<td>0.4707</td>
<td>no</td>
</tr>
<tr>
<td>Post oak #3</td>
<td>28.8 (73.2)</td>
<td>4.22,6.89</td>
<td>Blackjack oak</td>
<td>N/A</td>
<td>no</td>
</tr>
<tr>
<td>Post oak #4</td>
<td>19.6 (49.8)</td>
<td>1.08,9.83</td>
<td>Post oak</td>
<td>0.3933</td>
<td>no</td>
</tr>
<tr>
<td>Post oak #5</td>
<td>37.9 (96.3)</td>
<td>0.40,8.58</td>
<td>Post oak</td>
<td>0.4076</td>
<td>no</td>
</tr>
</tbody>
</table>
Figure 5.25. Seedling and sapling heights in relation to distance from two blackjack oak trees in plot 4 in Pea Ridge National Military Park. Left graph shows the correlation with blackjack oak tree #1, with a DBH of 28.4 cm, and the right with blackjack oak tree #2 having a DBH of 13.7 cm.
Figure 5.26. Seedling and sapling heights in relation to distance from two blackjack oak trees in plot 4 in Pea Ridge National Military Park. Left graph shows the correlation with blackjack oak tree #3, with a DBH of 33.0 cm, and the right with blackjack oak tree #4 having a DBH of 20.3 cm.
Figure 5.27. Seedling and sapling heights in relation to distance from two post oak trees in plot 4 in Pea Ridge National Military Park. Left graph shows the correlation with blackjack oak tree #5, with a DBH of 14.7 cm, and the right with post oak tree #1 having a DBH of 29.2 cm.
Figure 5.28. Seedling and sapling heights in relation to distance from two post oak trees in plot 4 in Pea Ridge National Military Park. Left graph shows the correlation with post oak tree #2, with a DBH of 116.8 cm, and the right with post oak tree #3 having a DBH of 37.8 cm.

<table>
<thead>
<tr>
<th>Tree Common name</th>
<th>DBH in (cm)</th>
<th>Location (along, within)</th>
<th>Seedlings &amp; Saplings Common name</th>
<th>p-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blackjack oak #1</td>
<td>11.2 (28.4)</td>
<td>1.02,0.32</td>
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<tr>
<td></td>
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<td></td>
<td>Post oak</td>
<td>0.4298</td>
<td>no</td>
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<tr>
<td>Blackjack oak #2</td>
<td>5.4 (13.7)</td>
<td>2.59,2.75</td>
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<td>0.7756</td>
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<td></td>
<td>Post oak</td>
<td>0.9001</td>
<td>no</td>
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<td>Blackjack oak #3</td>
<td>13.0 (33.0)</td>
<td>7.78,5.90</td>
<td>Post oak</td>
<td>0.4401</td>
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<td>Blackjack oak #4</td>
<td>8.0 (20.3)</td>
<td>2.25,5.66</td>
<td>Blackjack oak</td>
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<td>Post oak</td>
<td>0.7257</td>
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<td>Blackjack oak #5</td>
<td>5.8 (14.7)</td>
<td>2.92,6.99</td>
<td>Post oak</td>
<td>0.4647</td>
<td>no</td>
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<td>Post oak #1</td>
<td>11.5 (29.2)</td>
<td>4.27,9.13</td>
<td>Post oak</td>
<td>0.0251</td>
<td>yes</td>
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<tr>
<td>Post oak #2</td>
<td>46.0 (116.8)</td>
<td>4.78,9.17</td>
<td>Post oak</td>
<td>0.0189</td>
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<tr>
<td>Post oak #3</td>
<td>14.9 (37.8)</td>
<td>4.9,8.58</td>
<td>Post oak</td>
<td>0.0005</td>
<td>yes</td>
</tr>
</tbody>
</table>
Figure 5.29. Seedling and sapling heights in relation to distance from two red oak trees in plot 1 in Buffalo National River. Left graph shows the correlation with red oak tree #1, with a DBH of 48.3 cm, and the right with red oak tree #2 having a DBH of 62.2 cm.
Figure 5.30. Seedling and sapling heights in relation to distance from two white oak trees in plot 1 in Buffalo National River. Left graph shows the correlation with white oak tree #1, with a DBH of 84.1 cm, and the right with white oak tree #2 having a DBH of 7.4 cm.
Figure 5.31. Seedling and sapling heights in relation to distance from two white oak trees in plot 1 in Buffalo National River. Left graph shows the correlation with white oak tree #3, with a DBH of 85.1 cm, and the right with white oak tree #4 having a DBH of 3.8 cm.
Figure 5.32. Seedling and sapling heights in relation to distance from a white and black oak tree in plot 1 in Buffalo National River. Left graph shows the correlation with white oak tree #5, with a DBH of 28.4 cm, and the right with a black oak tree having a DBH of 2.8 cm.
Table 5.13. Significance data for seedling and sapling heights in relation to distance from trees in Buffalo National River plot 1.

<table>
<thead>
<tr>
<th>Tree Common name</th>
<th>DBH in (cm)</th>
<th>Location (along, within)</th>
<th>Seedlings &amp; Saplings Common name</th>
<th>p-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black oak</td>
<td>1.1 (2.8)</td>
<td>7.06,6.1</td>
<td>White oak</td>
<td>0.4054</td>
<td>no</td>
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<td></td>
<td></td>
<td></td>
<td>Black oak</td>
<td>0.2082</td>
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<td></td>
<td>Southern red oak</td>
<td>0.1310</td>
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<td>chinquapin oak</td>
<td>0.3965</td>
<td>no</td>
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<tr>
<td>Southern red oak #1</td>
<td>19.0 (48.3)</td>
<td>1.91,3.39</td>
<td>White oak</td>
<td>0.4249</td>
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<td>0.1093</td>
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<td>Southern red oak</td>
<td>0.5536</td>
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<td>Southern red oak #2</td>
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<td>1.10,7.41</td>
<td>White oak</td>
<td>0.0317</td>
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<td>0.7256</td>
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<td>Southern red oak</td>
<td>0.6759</td>
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<td>White oak #1</td>
<td>33.1 (84.1)</td>
<td>6.65,1.00</td>
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<td>0.2343</td>
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<td>White oak #2</td>
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<td>9.67,1.10</td>
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<td>Southern red oak</td>
<td>0.3924</td>
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</tr>
<tr>
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<td></td>
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<td>chinquapin oak</td>
<td>0.2228</td>
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</table>
Table 5.13. continued.

<table>
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<tr>
<th>Tree Common name</th>
<th>Location DBH in (cm) (along, within)</th>
<th>Seedlings &amp; Saplings Common name</th>
<th>p-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>White oak #3</td>
<td>33.5 (85.1) 6.22,4.67</td>
<td>Southern red oak</td>
<td>0.3924</td>
<td>no</td>
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<td>chinquapin oak</td>
<td>0.2228</td>
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</tr>
<tr>
<td>White oak #4</td>
<td>1.5 (3.8) 8.02,6.73</td>
<td>White oak</td>
<td>0.3298</td>
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<td>Black oak</td>
<td>0.2388</td>
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<td>Southern red oak</td>
<td>0.1234</td>
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<td>chinquapin oak</td>
<td>0.6899</td>
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<tr>
<td>White oak #5</td>
<td>11.2 (28.4) 6.53,8.81</td>
<td>White oak</td>
<td>0.4978</td>
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<td>Black oak</td>
<td>0.1703</td>
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<td>Southern red oak</td>
<td>0.6499</td>
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<td>chinquapin oak</td>
<td>0.0495</td>
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</table>
Figure 5.33. Seedling and sapling heights in relation to distance from two white oak trees in plot 2 in Buffalo National River. Left graph shows the correlation with white oak tree #1, with a DBH of 99.8 cm, and the right with white oak tree #2 having a DBH of 41.9 cm.
Figure 5.34. Seedling and sapling heights in relation to distance from two white oak trees in plot 2 in Buffalo National River. Left graph shows the correlation with white oak tree #3, with a DBH of 34.3 cm, and the right with white oak tree #4 having a DBH of 52.1 cm.
Table 5.14. Significance data for seedling and sapling heights in relation to distance from trees in Buffalo National River plot 2.

<table>
<thead>
<tr>
<th>Tree Common name</th>
<th>DBH in (cm)</th>
<th>Location (along, within)</th>
<th>Seedlings &amp; Saplings</th>
<th>p-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>White oak #1</td>
<td>39.3 (99.8)</td>
<td>1.51,1.2</td>
<td>White oak</td>
<td>0.4000</td>
<td>no</td>
</tr>
<tr>
<td>White oak #2</td>
<td>16.5 (41.9)</td>
<td>5.16,7.23</td>
<td>Southern red oak</td>
<td>N/A</td>
<td>no</td>
</tr>
<tr>
<td>White oak #3</td>
<td>13.5 (34.3)</td>
<td>1.34,6.73</td>
<td>White oak</td>
<td>0.9850</td>
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<td>Southern red oak</td>
<td>N/A</td>
<td>no</td>
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<tr>
<td>White oak #4</td>
<td>20.5 (52.1)</td>
<td>1.83,8.81</td>
<td>White oak</td>
<td>N/A</td>
<td>no</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Southern red oak</td>
<td>N/A</td>
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</table>
Figure 5.35. Seedling and sapling heights in relation to distance from a post oak tree (DBH of 85.1 cm) in plot 3 in Buffalo National River.
Table 5.15. Significance data for seedling and sapling heights in relation to distance from trees in Buffalo National River plot 3.

<table>
<thead>
<tr>
<th>Tree</th>
<th>Location</th>
<th>Seedlings &amp; Saplings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common name</td>
<td>DBH in (cm)</td>
<td>Common name</td>
</tr>
<tr>
<td>Post oak</td>
<td>33.5 (85.1)</td>
<td>1.73, 4.77</td>
</tr>
</tbody>
</table>
Figure 5.36. Seedling and sapling heights in relation to distance from two red oak trees in plot 1 in Fernow Experimental Forest. Left graph shows the correlation with red oak tree #1, with a DBH of 22.9 cm, and the right with red oak tree #2 having a DBH of 10.7 cm.
Table 5.16. Significance data for seedling and sapling heights in relation to distance from trees in Fernow Experimental Forest plot 1.

<table>
<thead>
<tr>
<th>Tree Common name</th>
<th>Location (along, within)</th>
<th>DBH in (cm)</th>
<th>Seedlings &amp; Saplings</th>
<th>p-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red oak #1</td>
<td>0.00, 0.23</td>
<td>9.0 (22.9)</td>
<td>Red oak</td>
<td>0.4747</td>
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<tr>
<td>Red oak #2</td>
<td>0.12, 0.48</td>
<td>4.2 (10.7)</td>
<td>Red oak</td>
<td>0.9461</td>
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</table>
Figure 5.37. Seedling and sapling heights in relation to distance from two beech trees in plot 2 in Fernow Experimental Forest. Left graph shows the correlation with beech tree #1, with a DBH of 15.2 cm, and the right with beech tree #2 having a DBH of 35.3 cm.
Figure 5.38. Seedling and sapling heights in relation to distance from two chestnut oak trees in plot 2 in Fernow Experimental Forest. Left graph shows the correlation with chestnut oak tree #1, with a DBH of 97.8 cm, and the right with chestnut oak tree #2 having a DBH of 129.5 cm.
Figure 5.39. Seedling and sapling heights in relation to distance from a chestnut and red oak tree in plot 2 in Fernow Experimental Forest. Left graph shows the correlation with chestnut oak tree #3, with a DBH of 111.5 cm, and the right with a red oak tree having a DBH of 113.0 cm.
Table 5.17. Significance data for seedling and sapling heights in relation to distance from trees in Fernow Experimental Forest plot 2.

<table>
<thead>
<tr>
<th>Tree Common name</th>
<th>DBH in (cm)</th>
<th>Location (along, within)</th>
<th>Seedlings &amp; Saplings Common name</th>
<th>p-value</th>
<th>Significance</th>
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</thead>
<tbody>
<tr>
<td>Beech #1</td>
<td>6 (15.2)</td>
<td>2.05,1.28</td>
<td>Red oak</td>
<td>0.4025</td>
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<td>Beech</td>
<td>0.9898</td>
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<tr>
<td>Beech #2</td>
<td>13.9 (35.3)</td>
<td>3.55,0.68</td>
<td>Red oak</td>
<td>0.0644</td>
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<td>Beech</td>
<td>0.7239</td>
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<td>Chestnut oak #1</td>
<td>38.5 (97.8)</td>
<td>1.35,2.51</td>
<td>Red oak</td>
<td>0.4355</td>
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<td>Beech</td>
<td>0.7464</td>
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<td>Chestnut oak #2</td>
<td>51 (129.5)</td>
<td>3.9,1.93</td>
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<td>Beech</td>
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<td>Chestnut oak #3</td>
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<td>Beech</td>
<td>0.2037</td>
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Figure 5.40. Seedling and sapling heights in relation to distance from two red oak trees in plot 3 in Fernow Experimental Forest. Left graph shows the correlation with red oak tree #1, with a DBH of 139.7 cm, and the right with red oak tree #2 having a DBH of 94.0 cm.
Table 5.17. Significance data for seedling and sapling heights in relation to distance from trees in Fernow Experimental Forest plot 3.

<table>
<thead>
<tr>
<th>Tree Common name</th>
<th>DBH in (cm)</th>
<th>Location (along, within)</th>
<th>Seedlings &amp; Saplings</th>
<th>p-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red oak #1</td>
<td>55.0 (139.7)</td>
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<td>Red oak</td>
<td>0.5090</td>
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<td></td>
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<td>Beech</td>
<td>0.7765</td>
<td>no</td>
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<td>Chestnut oak</td>
<td>0.1207</td>
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<tr>
<td>Red oak #2</td>
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<td>Beech</td>
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<td></td>
<td>Chestnut oak</td>
<td>0.1531</td>
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Figure 5.41. Seedling and sapling heights in relation to distance from a chestnut oak tree, with a DBH of 91.2 cm, in Fernow Experimental Forest plot 4.
Table 5.18. Significance data for seedling and sapling heights in relation to distance from trees in Fernow Experimental Forest plot 4.

<table>
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<th>Tree Common name</th>
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<th>Seedlings &amp; Saplings Common name</th>
<th>p-value</th>
<th>Significance</th>
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<tr>
<td>Chestnut oak</td>
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<td>Red oak</td>
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<td>Chestnut oak</td>
<td>0.1955</td>
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Figure 5.42. Seedling and sapling heights in relation to distance from a red oak tree, with a DBH of 161.3 cm, in Fernow Experimental Forest plot 6.
Table 5.19. Significance data for seedling and sapling heights in relation to distance from trees in Fernow Experimental Forest plot 6.

<table>
<thead>
<tr>
<th>Tree</th>
<th>Location</th>
<th>Seedlings &amp; Saplings</th>
<th>Common name</th>
<th>p-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red oak</td>
<td>63.5 (161.3)</td>
<td>1.00, 2.75</td>
<td>Red oak</td>
<td>0.0175</td>
<td>yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Beech</td>
<td>0.0038</td>
<td>yes</td>
</tr>
</tbody>
</table>
Figure 5.43. Seedling and sapling heights in relation to distance from two beech trees in plot 7 in Fernow Experimental Forest. Left graph shows the correlation with beech tree #1, with a DBH of 40.1 cm, and the right with beech tree #2 having a DBH of 3.8 cm.
Figure 5.44. Seedling and sapling heights in relation to distance from two beech trees in plot 7 in Fernow Experimental Forest. Left graph shows the correlation with beech tree #3, with a DBH of 46.2 cm, and the right with beech tree #4 having a DBH of 5.8 cm.
Table 5.20. Significance data for seedling and sapling heights in relation to distance from trees in Fernow Experimental Forest plot 7.

<table>
<thead>
<tr>
<th>Tree Common name</th>
<th>DBH in (cm)</th>
<th>Location (along, within)</th>
<th>Seedlings &amp; Saplings Common name</th>
<th>p-value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beech #1</td>
<td>15.8 (40.1)</td>
<td>1.07,4.18</td>
<td>Red oak</td>
<td>0.3851</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Beech</td>
<td>0.0015</td>
<td>yes</td>
</tr>
<tr>
<td>Beech #2</td>
<td>1.5 (3.8)</td>
<td>2.22,4.25</td>
<td>Red oak</td>
<td>0.7589</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Beech</td>
<td>0.3662</td>
<td>no</td>
</tr>
<tr>
<td>Beech #3</td>
<td>18.2 (46.2)</td>
<td>3.41,3.72</td>
<td>Red oak</td>
<td>0.9789</td>
<td>no</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>Beech</td>
<td>0.1085</td>
<td>no</td>
</tr>
<tr>
<td>Beech #4</td>
<td>2.3 (5.8)</td>
<td>4.92,4.54</td>
<td>Red oak</td>
<td>0.0763</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Beech</td>
<td>0.0182</td>
<td>yes</td>
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</table>
Chapter 6. Clustering

6.1 Introduction to Clustering and Methods

It is the objective of this section to address the potential for ectomycorrhizal linkages to influence the distribution of stems within a plot. The hypothesis is that ectomycorrhizal stems will tend to cluster together in response to increased access to nutrients provided by a mutual symbiotic partner. The null hypothesis is that the positions of ectomycorrhizal stems within a plot do not show any evidence of being clustered through an influence of their symbiotic partner and instead occur randomly within a plot. In order to test this hypothesis, an assessment of potential clusters would have to be carried out.

There are several methods for identifying clusters in a data set, and these include classifications of clusters using a hierarchical grouping method to optimize clusters (Ward 1963), a pure distance to nearest neighbor clustering method that uses Euclidean distances, a maximum likelihood approach using statistical modeling with the requirement of a normally distributed data set (Dempster, Laird, and Rubin 1977), and a model-based clustering algorithm (MCLUST) that uses Bayesian Information Criteria (BIC) and Expectation Maximum (EM) to perform several tests assuring optimum cluster assignment (Mclachlan and Krishnan 1996; Fraley and Raferty 2002, 2007).

Both the distance to nearest neighbor and the MCLUST model were applied to the plot data from the study sites. The first method used stem locations to create a cluster based on a chosen distance away from each stem to set the boundary of the cluster, which created a dependency on starting point position and resulted in several overlapping clusters. The MCLUST clustering method provided a less subjective approach by removing the dependency of a starting point through its use of multiple models and also uses functions that remove the dependence on
normally distributed data. Furthermore, MCLUST, which is provided as a user-friendly library, created by Fraley and Raferty, is included in the "R" statistical software package which makes data analysis quick, effective, and allows for graphical interpretation of results, which resulted in it being the method chosen to analyze the sites.

The MCLUST software uses an input data set, which consisted of the along and within plot positions of stems, and applies several modeling algorithms—BIC, hierarchal, normal mixture, density estimates, Gaussian mixture, and dimension reduction (see Fraley et al. 2012 for a complete review)—to the data and then, based on the best fit model, the results are displayed in first, a BIC graph, representing the various models used, a graph of the classification of clusters, and a final graph with the uncertainty in the clusters. The BIC graph shows the various models used to estimate the best fit model, with the highest (most negative) result corresponding to the best fit. The kinds of models include a spherical, diagonal, and ellipsoidal distribution, with a volume and shape that can be equal or variable. Each model is given an identifier—EII, VII, EEI, VEI, EVI, VVI, EEE, EEV, VEV, and VVV—that corresponds to a specific model name, distribution, volume, and shape, which are explained in detail by Fraley and Raftery (2002). The details of each model used during the analysis of the individual plots for each site are discussed in greater detail in the results section given below.

The clustering classification graph indicates the location of each ectomycorrhizal stem found within a plot, whose classifications are separated by different colors and symbols to designate the cluster with which they are associated. The boundary of the cluster occur as an elliptical, circular, or diagonal shape around the stems within the cluster.

The final graph, clustering uncertainty, is an overlay of the clustering classification graph but with the level of uncertainty of each stem’s placement in the cluster added in. The
uncertainty in the stem being in the cluster is shown by different sized circles of varying gray to black color, which corresponds to higher uncertainty for larger darker circles as opposed to smaller light gray. This allows for an additional interpretation on the placement and certainty of clustering results.

6.2 Classification of Clusters in the Ozarks

A. Devil’s Den State Park

The first plot in Devil's Den State Park had five clusters (Figure 6.2) that were calculated with the EEV, green cross box (Figure 6.1), multivariate BIC model in MCLUST. The EEV model states that the clusters were formed using an ellipsoid to make the cluster boundary and had equal area and volume. In the BIC versus number of components graph (Figure 6.1), the BIC y-axis values are the maximum log-likelihood that a model is a good fit to the clusters, and the number of components is the number of clusters fit to each model. MCLUST chose EEV as the best fit model (least negative BIC value) for plot 1 and found five components (aka, clusters) (Figure 6.1). Comparing the classification of the clusters (Figure 6.2) to the distribution of stems (refer to Figure 4.3 in the Forest Interior section), four (clusters with purple cross, green triangle, orange square, and blue circle) of the five clusters were near ectomycorrhizal trees. The uncertainty of the classification (Figure 6.2, right graph) indicates several stems fell outside the cluster boundary, which made them unlikely candidates to be included in the clusters (large black circles).

The second plot did not have any clusters associated with ectomycorrhizal stems in the plot, which undoubtedly is a result of the stems being spread out in the plot (refer to Figure 4.5 in the Forest Interior section ). However, plot 3 had a maximum likelihood of four clusters (Figure 6.1) that were calculated with the EEV, multivariate BIC model (Figure 6.3), which used an
ellipsoid shape to make the cluster boundaries and had equal area and volume. The classification of clusters in plot 2 is shown in Figure 6.4. Only one of the clusters had a tree along with seedlings and saplings in the cluster (purple crosses), whereas the other three were made up entirely of seedlings and saplings. Some uncertainty was found for smaller stems (red box symbols) inside one of the clusters, but most of the uncertainty was outside the cluster boundaries (black large circles) (Figure 6.4).

The fourth plot had two clusters (Figure 6) with the VII, open triangle, maximum likelihood, multivariate BIC model (Figure 6.5), which used a spherical shape to make the cluster boundary and had varying volume. The maximum likelihood BIC fit was not as good as the previous plots, which is evident in the low BIC y-axis values and low number of components that fit to the data. Therefore, when looking at the classification of clusters, a large amount of uncertainty exists (Figure 6.6), with several large dark gray and black circles found inside and around the two clusters. The clusters had boundaries that overlapped with two of the five ectomycorrhizal trees within plot 4 (refer to Figure 4.11 in the Forest Interior section), but mostly seedlings and saplings were found within the cluster boundaries.

The fifth plot had a maximum likelihood of four clusters corresponding to the EEI, multivariate BIC model, which produced diagonal clusters of equal area and shape (Figure 6.7). Fitting a model to this data was more difficult, which is indicated by the low BIC and incomplete components and values for several of the models (Figure 6.7). However, stems were placed close to one another within the clusters, and uncertainty was mostly found for stems outside the cluster boundaries (Figure 6.8). The clusters had boundaries that overlapped with two of the three ectomycorrhizal trees within plot 5 (refer to Figure 4.14 in the Forest Interior section), but the uncertainty of the trees belonging to the cluster was high (Figure 6.8). Therefore, the
remaining stems inside each cluster belonged to ectomycorrhizal seedlings and saplings.

The sixth plot had a maximum likelihood of three clusters corresponding to the VVI, multivariate BIC model, which produced diagonal clusters of varying volume and shape (Figure 6.9). Fitting stems to a model was also difficult for this plot as the BIC y-axis values were low (Figure 6.9), and the classification of clusters had high amounts of uncertainty (Figure 6.10). The three clusters did not have many stems within the cluster boundaries, but for those that were inside the clusters, there was a high certainty for them being there. The clusters had boundaries that overlapped only with ectomycorrhizal seedlings and saplings within plot 6 (refer to Figure 4.17 in the Forest Interior section). There were three ectomycorrhizal trees in the plot, but none that fell within the cluster boundaries, which resulted in a high level of uncertainty in at least two of the three trees belonging to a particular cluster (Figure 6.10).

The seventh plot had a maximum likelihood of two clusters corresponding to the EEI, multivariate BIC model, which produced diagonal clusters of equal volume and shape (Figure 6.11). There were several ectomycorrhizal trees, seedlings, and saplings within plot 7 (refer to Figure 4.20 in the Forest Interior section), although the seedlings and saplings were the dominant stems in the two clusters (Figure 6.12). All but one of the nine ectomycorrhizal trees fell outside the cluster boundaries, several of which had a large amount of uncertainty with respect to being within the clusters (Figure 6.12). The highest level of uncertainty in the designation of stems within cluster groups fell outside the clusters, on the cluster boundaries, and were greatest for stems that fell between the two clusters (Figure 6.12). The final plot (plot 8) in Devil's Den State Park did not have any associated clusters, which, just like plot 2, was a result of the stems within the plot being too dispersed.
B. Pea Ridge National Military Park

The locations of ectomycorrhizal stems from the four plots in Pea Ridge National Military Park were loaded into MCLUST to check for potential clusters, with MCLUST finding clusters within all the plots. Plot 1 had a maximum likelihood of four clusters corresponding to the EEV, multivariate BIC model, which produced ellipsoid-shaped clusters of equal volume and shape (Figure 6.13). The ectomycorrhizal seedlings and saplings within plot 1 (refer to Figure 4.23 in the Forest Interior section) are the dominant stems that made up the clusters shown in the classification of clusters graph (Figure 6.14). Only one of the ectomycorrhizal trees fell within a cluster (blue circles). It appears the proximity of two clusters near each other (blue circles and green triangles, Figure 6.13) creates a higher level of uncertainty for stems between the two cluster boundaries (Figure 6.14). There is also a high uncertainty in the cluster group with the red boxes, which could be a result of the low level of stems in the cluster or the two stems outside the cluster boundary (Figure 6.14).

Plot 2 in Pea Ridge National Military Park had a maximum likelihood of three clusters corresponding to the EVI, multivariate BIC model, which produced diagonally-shaped clusters of equal volume and varying shape (Figure 6.15). All three cluster groups (Figure 6.16) were made up of ectomycorrhizal seedlings and saplings, with the trees lying outside the cluster boundaries (refer to Figure 4.29 in the Forest Interior section). The level of uncertainty in the classification of stems within cluster groups resulted when stems were outside the clusters boundaries and was greatest when cluster groups were close to each other (Figure 6.16).

The third plot in Pea Ridge National Military Park had a maximum likelihood of three clusters corresponding to the VII, multivariate BIC model, which produced spherically-shaped clusters of varying volume (Figure 6.17). Just as in plot 2, all three cluster groups (Figure 6.18)
were composed of ectomycorrhizal seedlings and saplings, with trees lying outside the cluster boundaries (refer to Figure 4.32 in the Forest Interior section). The level of uncertainty in the classification of stems within cluster groups was the result of a low number of stems that fell into the cluster groups, but most of the uncertainty was due to stems that resided outside the clusters, and the greatest uncertainty fell between two cluster groups (Figure 6.18).

The fourth plot in Pea Ridge National Military Park had a maximum likelihood of four clusters corresponding to the EVI, multivariate BIC model, which produced diagonal clusters of equal volume and varying shape (Figure 6.19). Just as in the previous plots, all cluster groups (Figure 6.20) consisted predominately of ectomycorrhizal seedlings and saplings with trees residing outside the cluster boundaries (refer to Figure 4.35 in the Forest Interior section). There was one cluster (green triangles) that had three trees in the cluster with high certainty (Figure 6.20). The level of uncertainty in the classification of stems within cluster groups resulted from a few that fell into the cluster groups (blue circles and red boxes), but a majority of the uncertainty was due to stems that were outside the clusters or along the cluster boundary, and the greatest uncertainty occurred when the stems were between cluster groups (Figure 6.20).

C. Buffalo National River Park

The last study site in northwest Arkansas was the Buffalo National River Park. The ectomycorrhizal stems from these plots were loaded into MCLUST, and each plot had clusters with varying certainty. Plot 1 had a maximum likelihood of three clusters corresponding to the VEV, multivariate BIC model, which produced ellipsoidal clusters of equal shape and variable volume (Figure 6.21). Plot 1 had several ectomycorrhizal trees, seedlings, and saplings (refer to Figure 4.38 in the Forest Interior section) in the plot. However, the seedlings and saplings dominated the large clusters classified for this location (Figure 6.22) as only two of the eight
trees fell inside the cluster boundaries, which meant all cluster groups consisted predominately of ectomycorrhizal seedlings and saplings, with the trees lying outside the cluster boundaries (Figure 6.22). Just as in the previous plots, the uncertainty in the classification of stems within cluster groups resided outside the cluster boundaries, with greatest uncertainty found when stems were located between cluster groups (Figure 6.22).

The second plot in the Buffalo National River Park had a maximum likelihood of seven clusters corresponding to the EEV, multivariate BIC model, which produced ellipsoidal clusters of equal volume and shape (Figure 6.23). Plot 2 had only a few ectomycorrhizal trees, seedlings, and saplings (refer to Figure 4.41 in the Forest Interior section) that corresponded to the rather small clusters classified for this location (Figure 6.24). All but one of the four trees fell inside the cluster groups, which meant the cluster groups were made up of trees and ectomycorrhizal seedlings and saplings (Figure 6.24). The level of uncertainty was high in the classification of stems within all cluster groups, which can be seen in the classification uncertainty graph, with each stem’s uncertainty shown by large dark circles in the clusters (Figure 6.24).

The last plot in the Buffalo National River Park, plot 3, had a maximum likelihood of five clusters corresponding to the EEV, multivariate BIC model, which produced ellipsoidal clusters of equal volume and shape (Figure 6.25). Plot 3 had a similar density of ectomycorrhizal stems as plot 2, with only a few ectomycorrhizal trees, seedlings, and saplings (refer to Figure 4.44 in the Forest Interior section) found in the plot. The low number of stems, in turn, corresponded to the rather small clusters classified for this location (Figure 6.26). There were only two trees in the plot, one that fell inside a cluster group (red boxes) and one that was outside (purple cross). The majority of stems within the clusters were ectomycorrhizal seedlings and saplings (Figure
The level of uncertainty was high in the classification of stems within all cluster groups, which can be seen in classification uncertainty graph, as each stem had uncertainty shown by large dark gray and black circles in and around the clusters (Figure 6.26).

6.3 Classification of Clusters in the Fernow Experimental Forest

The Fernow Experimental Forest had ten total plots whose ectomycorrhizal stems were checked in MCLUST for potential clusters. MCLUST found clusters in all of the ten plots, but the last plot had a high level of uncertainty in the clusters produced. The first plot in the Fernow Experimental Forest had two clusters (Figure 6.27) that were calculated with the VII, multivariate BIC model in MCLUST, which produced spherical clusters of varying volume and equal shape (Figure 6.27). Plot 1 had several ectomycorrhizal trees, seedlings, and saplings (refer to Figure 4.47 in the Forest Interior section) that made up the clusters determined for this location (Figure 6.28). Both of the trees fell outside the cluster groups, which meant all cluster groups were made up of ectomycorrhizal seedlings and saplings, with the trees lying outside the cluster boundaries (Figure 6.28). The uncertainty in the classification of stems within cluster groups centered around the smaller cluster, with uncertainty inside and outside the cluster boundary, but the greatest uncertainty was found when stems were located between the two clusters (Figure 6.28).

The second plot had three clusters (Figure 6.29) that were calculated with the VVI, multivariate BIC model in MCLUST, which produced diagonal clusters of varying volume and shape (Figure 6.29). Plot 2 had a large number of ectomycorrhizal trees and a small number of seedlings and saplings (refer to Figure 4.50 in the Forest Interior section) that made up the clusters classified for this location (Figure 6.29). Two of the six trees fell inside the cluster groups, which meant most of the cluster groups consisted of ectomycorrhizal seedlings and
saplings with the trees lying outside the cluster boundaries (Figure 6.29). The uncertainty in the classification of stems within cluster groups centered around the two smaller clusters with uncertainty inside and outside the cluster boundaries, but the greatest uncertainty was found when stems were located between the clusters (Figure 6.29).

The third plot had eight clusters (Figure 6.31) that were calculated with the EEV, multivariate BIC model in MCLUST, which produced ellipsoid-shaped clusters of equal volume and shape (Figure 6.31). Plot 3 had two ectomycorrhizal trees with a large number of seedlings and saplings (refer to Figure 4.53 in the Forest Interior section) that made up the clusters classified for this location (Figure 6.32). Both trees fell outside the cluster groups, which meant all cluster groups consisted of ectomycorrhizal seedlings and saplings (Figure 6.32). The large number of smaller stems in plot 3 produced a great deal of uncertainty in the classification of stems within cluster groups (Figure 6.32). Most uncertainty was found in regions of cluster overlap, along cluster boundaries, and in regions with stems between the clusters, although there was an associated uncertainty for stems inside cluster boundaries of relatively isolated clusters (Figure 6.32).

The fourth plot had three clusters (Figure 6.33) that were calculated with the VEV, multivariate BIC model in MCLUST, which produced ellipsoid-shaped clusters of equal shape and variable volume (Figure 6.34). Plot 4 had no ectomycorrhizal trees but did have a large number of seedlings and saplings (refer to Figure 4.56 in the Forest Interior section) that made up the clusters classified for this location (Figure 6.34). The smallest cluster (green triangles) had the highest level of uncertainty in the classification of stems within its cluster group (Figure 6.34). The other two clusters (blue circles and red squares) had fairly high certainty in the classification of the stems within the clusters, but this declined for stems near cluster boundaries.
and between the two clusters (Figure 6.34).

The fifth plot had three clusters (Figure 6.35) that were calculated with the EII, multivariate BIC model in MCLUST, which produced spherical clusters of equal volume and shape (Figure 6.36). Plot 5 had no ectomycorrhizal trees but did have several seedlings and saplings (refer to Figure 4.59 in the Forest Interior section) that made up the clusters classified for this location (Figure 6.36). The clusters had equal volume, with a high level of certainty in the classification of stems within the cluster boundaries (Figure 6.36). The highest uncertainty in the classification of the stems was found for stems outside the clusters, particularly those that fell between clusters (Figure 6.36).

Plot 6 in the Fernow Experimental Forest had four clusters (Figure 6.37) that were calculated with the EII, multivariate BIC model in MCLUST, which produced spherical clusters of equal volume and shape (Figure 6.38). There was one ectomycorrhizal tree in plot 6 (refer to Figure 4.59 in the Forest Interior section), but it did not fall within any cluster boundary (Figure 6.38). Plot 6 did have a large number of seedlings and saplings that made up the clusters classified for this location (Figure 6.38). All the clusters had equal volume, but those with the highest level of certainty in the classification of stems within the cluster boundaries were for the top two clusters (green triangles and purple crosses) (Figure 6.38). The certainty in the other two clusters (blue circles and red boxes) was also high within the clusters except for stems residing on the cluster boundary (Figure 6.38). The highest uncertainty in the classification of stems was found in stems residing outside the clusters, particularly those that fell between clusters (Figure 6.38).

Plot 7 had three clusters (Figure 6.39) that were calculated with the EVI, multivariate BIC model in MCLUST, which produced diagonal clusters of equal volume and varying shape
There were four ectomycorrhizal trees in the plot (refer to Figure 4.65 in the Forest Interior section), but they did not fall within any cluster boundary (Figure 6.40). Plot 7 did have a large number of seedlings and saplings that made up the clusters classified for this location (Figure 6.40). The clusters had the highest level of certainty in the classification of stems inside the cluster boundaries (Figure 6.40). The highest uncertainty in the classification of stems was found in stems residing outside the clusters, particularly those that fell on or near cluster boundaries and between clusters (Figure 6.40).

The eighth plot, the first burn location, had five clusters (Figure 6.41) that were calculated with the EEI, multivariate BIC model in MCLUST, which produced diagonal clusters of equal volume and shape (Figure 6.42). Plot 8 had no ectomycorrhizal trees but did have a small number seedlings and saplings (refer to Figure 4.68 in the Forest Interior section) that made up the clusters classified for this location (Figure 6.42). The low number of stems, in turn, corresponded to the rather small size of the clusters classified for this location (Figure 6.42). The level of uncertainty was high in the classification of stems for the two central clusters (red boxes and orange squares), which can be seen in classification uncertainty graph, as each stem had uncertainty shown by large dark gray and black circles in and around the clusters (Figure 6.42). The remaining clusters had a high amount of certainty in the classification of stems within the clusters, as indicated by the small light gray circles in the uncertainty graph (Figure 6.42).

Plot 9, the second burn location, had four clusters (Figure 6.43) that were calculated with the VEI, multivariate BIC model in MCLUST, which produced diagonal clusters of variable volume and equal shape (Figure 6.44). Plot 9 had no ectomycorrhizal trees but did have a large number of ectomycorrhizal seedlings and saplings (refer to Figure 4.71 in the Forest Interior section) that
made up the clusters classified for this location (Figure 6.44). The clusters had equal shape with a high level certainty in the classification of stems within two of the clusters (blue circles and purple crosses) (Figure 6.44). The other two clusters (red boxes and green triangles) had an appreciable amount of certainty in the classification of stems inside the cluster boundaries, but for stems that fell on the cluster boundary, the certainty was low. The highest uncertainty in the classification of the stems was found for stems outside the clusters, particularly those that fell between clusters (Figure 6.44).

The final plot, the last burn location, had nine clusters (Figure 6.45) that were calculated with the EEI, multivariate BIC model in MCLUST, which produced diagonal clusters of equal volume and shape (Figure 6.46). Plot 10 had no ectomycorrhizal trees but did have a small number of seedlings and saplings (refer to Figure 4.74 in the Forest Interior section) that made up the clusters classified for this location (Figure 6.46). The low number and widespread distribution of stems in the plot corresponded to the small size and high uncertainty in the classification of clusters (Figure 6.46). The level of uncertainty was high in the classification of all clusters, which is evident in all stems having large black circles in the classification uncertainty graph (Figure 6.46).

6.4 Review of the Cluster Results

The maximum log-likelihood, multivariate BIC models projected by MCLUST for the clusters generated from the sets of data obtained in Ozarks of northwest Arkansas and in the Fernow Experimental Forest were relatively diverse. The most common BIC model that fit the stems in the plots for northwest Arkansas was the EEV (ellipsoid shaped clusters of equal volume and shape) model, which was the case for five of the fifteen plots. The Fernow Experimental Forest had more diversity in the type of model used in the cluster classification.
However, plots 5 and 6 had EEI (diagonal clusters of equal volume and shape) and plots 8 and 10 had EII (spherical clusters of equal volume and shape) as the best models for cluster classification.

In northwest Arkansas, Devil's Den State Park had an average number of three clusters per plot, which extrapolates to 3333 clusters/ha for the 10x10 m plot size used in this region. When looking at the average number of clusters (3) for a plot in Devil's Den State Park, there was an average of one tree that fell inside the cluster boundaries. The number of trees inside a cluster boundary was relatively small considering that there was an average of four trees per plot. However, most trees that did not lie inside a cluster boundary were within 1 m to 2 m of that boundary, which is likely to be ecologically significant as clusters were potentially a result of the spatial linkages formed between ECM seedlings and saplings.

Pea Ridge National Military Park had an average of four clusters per plot (3500 clusters/ha), with an average of two trees inside the cluster boundaries. The average number of trees within a plot was four, making an even distribution between trees inside and outside a cluster boundary. The trees that did not reside inside cluster boundaries were within 2 m of them.

The Buffalo National River Park had an average of five clusters per plot (5000 clusters/ha), with an average of two trees inside the cluster boundaries. Buffalo National River Park, like Pea Ridge National Military Park, had an average number of four trees per plot, making an even distribution of trees inside and outside cluster boundaries. The trees outside cluster boundaries were found to reside at least 2 m from the edge of the boundary.

Overall, for northwest Arkansas, there was an average of four clusters per plot (3944 clusters/ha), with an average of one tree inside a cluster boundary. There was an average of four trees per plot in northwest Arkansas, and the majority fell outside cluster boundaries but
within an average of 2 m of a nearby cluster.

The Fernow Experimental Forest had an average of four clusters per plot (1720 clusters/ha), with an average of less than one tree (0.20) within a cluster boundary. The average number of trees in a plot was two, with all trees falling outside cluster boundaries but within 2 m of a nearby cluster.

Despite the two study regions being in two different states, the results of the ectomycorrhizal clustering analyses were very similar. Both northwest Arkansas and the Fernow Experimental Forest had a low number of trees within a cluster boundary compared to the number of trees inside the entire plot. In addition, in both regions, the trees that were in the plots but not in a cluster boundary were found within 2 m of a cluster boundary. The two regions also had an average of four clusters per plot and, given the difference in plot size, this would average out to be 3944 clusters/ha for northwest Arkansas and 1720 clusters/ha for the Fernow Experimental Forest. For both regions, there appears to be tendency for clusters to form between seedlings and saplings, with several having trees within 2 m of their cluster boundaries. This correlates well with the results found in the section on spatial distribution, where the distance a host tree can have a significant impact on surrounding ECM seedlings and saplings that occurred within 4 m from the tree. This is important because the established tree can form a symbiotic relationship involving fungi, which is then manifested in an underground network formed along the root tips of the tree. This network can spread throughout the soil, reaching other ECM-forming plants.

The symbiotic relationship between plants and fungi has been shown to increase the uptake of nutrients, reproduction, and health of the plant (Hartnett and Wilson 2002, He et al. 2009), which would make it important for plants to be in close proximity to a fungus and to other
symbiotic plants. As mentioned previously, Hartnett and Wilson (2002) found that this symbiotic relationship alters plant populations and their demographic patterns of distribution in the communities in which they occur. Therefore, the associations between ECM fungi and plants that represented the primary focus of this study could be the underlying mechanism behind cluster formations.

In both regions, the classification of clusters correlated with seedlings and saplings that were, on average, within two meters of a host tree. It appears that the presence of these trees helps provide a symbiotic host that extends to and assists in the survival and development of seedlings and saplings within the plots. Furthermore, given the number of clusters found within the regions and the lack of trees inside the cluster boundaries, it can be assumed that seedlings and saplings are providing assistance to one another through their underground linkages, making their proximity to each other important and potentially being another underlying cause for their clustering.
Figure 6.1. Results of the multivariate BIC model in MCLUST for Devil's Den State Park plot 1 with EEV (ellipsoidal, equal shape and volume) corresponding to the model used for clustering.
Figure 6.2. Classification of clusters produced by MCLUST (left) with uncertainty in clustering (right) for plot 1, Devil's Den State Park. Larger and darker circles (right) indicate areas of greater uncertainty within the cluster groupings.
Figure 6.3. Results of the multivariate BIC model in MCLUST for Devil's Den State Park plot 3 with EEV (ellipsoidal, equal shape and volume) corresponding to the model used for clustering.
Figure 6.4. Classification of clusters produced by MCLUST (left) with uncertainty in clustering (right) for plot 3, Devil's Den State Park. Larger and darker circles (right) indicate areas of greater uncertainty within the cluster groupings.
Figure 6.5. Results of the multivariate BIC model in MCLUST for Devil's Den State Park plot 4 with VII (spherical with varying volume) corresponding to the model used for clustering.
Figure 6.6. Classification of clusters produced by MCLUST (left) with uncertainty in clustering (right) for plot 4, Devil's Den State Park. Larger and darker circles (right) indicate areas of greater uncertainty within the cluster groupings.
Figure 6.7. Results of the multivariate BIC model in MCLUST for Devil's Den State Park plot 5 with EEI (diagonal, equal volume and shape) corresponding to the model used for clustering.
Figure 6.8. Classification of clusters produced by MCLUST (left) with uncertainty in clustering (right) for plot 5, Devil's Den State Park. Larger and darker circles (right) indicate areas of greater uncertainty within the cluster groupings.
Figure 6.9. Results of the multivariate BIC model in MCLUST for Devil's Den State Park plot 6 with VVI (diagonal, varying volume and shape) corresponding to the model used for clustering.
Figure 6.10. Classification of clusters produced by MCLUST (left) with uncertainty in clustering (right) for plot 6, Devil’s Den State Park. Larger and darker circles (right) indicate areas of greater uncertainty within the cluster groupings.
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Chapter 7. Ectomycorrhizal Trees, Their Dominance, and Fungal Associations

7.1 Introduction

The study sites in the mountains of northwest Arkansas occupy a greater region known as the Ozark Plateau that is characterized by varying topography with mixed mesic (moist) and xeric (dry) forest communities (Braun 1950). This mixed system results in communities with varying degree of dominance for particular species. Examples include an increased dominance of post oak, blackjack oak, maple, some black oak, hickory, and elm on dry, south-facing slopes, whereas on more mesic, north-facing slopes, the dominance shifts to white oak, black oak, and some black gum and hickory (Braun 1950). Current studies have found the overall dominant species within this region of northwest Arkansas are white oak, red oak, post oak, sugar maple, pignut hickory, mockernut hickory, and black oak (Stephenson et al. 2007).

The Fernow Experimental Forest is also characterized by mixed mesic forest communities (Braun 1950), dominated by oak, along with beech and birch (Stephenson et al. 2007). There are a number of ectomycorrhizal species within the mixed mesic forest sites in the Fernow Experimental Forest, and a brief review of these shows a dominance of red oak, white oak, chestnut oak, scarlet oak, beech, and red maple in the less mesic sites, whereas in the more mesic sites, red oak, sugar maple, tulip poplar, black cherry, and white ash are present (Stephenson et al. 2007).

In summary, the dominant trees in these two regions are oaks, represented by white oak, red oak, blackjack oak, and black oak, along with hickory found in northwest Arkansas, and red oak, white oak, and chestnut oak, along with beech and birch found in the Fernow Experimental Forest. Of these species, the oaks and beech establish ectomycorrhizal linkages with fungi, which is the primary focus of the following section. Moreover, this section will address the
collecting methods and process used for determining the fungal species likely to be responsible for ectomycorrhizal linkages among several of the previously mentioned ectomycorrhizal tree species found in the two regions.

7.2 Methods

The fruiting season for fungi extends from spring to early fall when rainfall is prominent and temperatures are optimal for the growth and development of fungi. However, when the two regions, northwest Arkansas and the Fernow Experimental Forest, were visited, it was during early to late summer, which meant that collecting of fungal fruiting bodies was limited. Instead, the underground hyphae that are present year round were collected from the tree root tips to address the ECM linkages that presumably exist between stems within forests. Using a process similar to Smith et al. (2011), several large oaks-white oak, red oak, and black oak in northwest Arkansas and chestnut oak, red oak, and beech in the Fernow Experimental Forest were selected, and their lateral roots were traced from base of the tree down to the fibrous new growth roots (~1 to 3 m). The soil was carefully dug out around the roots, and roots, along with a small portion of soil, were collected in gallon-sized plastic bags and stored in a cool location. The roots were then rinsed with water, and using a compound microscope, the roots were examined individually for evidence of ECM colonization. Adopting a technique similar to one described by Smith et al. 2011, during examination, if any portion of the roots exhibited a mycorrhizal colonization, then that portion with the colonization was separated from the clean roots and placed for preservation in a eppendorf tube filled with CTAB. There were a total of 100 eppendorf tubes filled with colonized roots collected during the 2012 field season from the Fernow Experimental Forest. Similar techniques were carried out in the Ozarks during the spring of 2013, with the addition of collecting fungal fruiting bodies to supplement the root tip data.
Determining the species of fungi associated with the root tips was carried out using a DNeasy DNA extraction plant kit. To prepare the samples for DNA extraction, the preservative, CTAB (CetylTrimethylAmmonium Bromide) used to store root tip samples, was rinsed off a colonized root tip. The root tip was then dried off and prepared for amplifying and copying the DNA through the use of a Polymerase Chain Reaction (PCR) (Rogstad 1992). PCR is important because it provides enough DNA to create a template for DNA sequencing.

The technique for DNA extraction was performed by first preparing the samples with an extraction agent. A single root tip was placed inside a small eppendorf tube, and 25 µl of extraction buffer was added to the tube. The colonized root tip was then ground up with forceps, allowing the mycorrhizae to detach from the root tip and to be mixed with the buffer. The eppendorf tube was then placed in a Biometra® Tgradient thermal cycler (a heating unit) during an incubation stage for ten minutes with the temperature set at 95° C, which caused the cellular structure of the sample to break down and release DNA. The sample was then removed from the thermal cycler, and 25 µl of a dilution buffer was added to suppress the DNA from the tree root tip, allowing for amplification of only the mycorrhizal DNA to take place.

The sample was then prepared for DNA amplification and synthesis (replication). This stage required a "mastermix" of materials to be made that, when combined with the DNA and subjected to a thermal cycling replication phase, produced enough DNA for sequencing. The mastermix contained \((n + 1)\) times each of the following solutions, where \(n\) is the number of samples:

- primer 1 (ITS1F [CTTGGTCATTAGAGGAAGTAA]) (0.5 µl),
- primer 2 (ITS4R [TCCTCCGCTTATTGATATGC]) (0.5 µl) (White et al. 1990),
- 50:50 buffer and extraction solution (3.5 µl),

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RedExtract-n-Amp PCR reaction mix (10 μl),

and water (5.0 μl).

The mastermix (19.5 μl) was then combined with the DNA (0.5 μl) into small eppendorf tubes and ran in the thermal cycler with the following multiplexing routine: one cycle at 94°C for three minutes for denaturing, followed by thirty-five cycles of 94°C for thirty seconds, 55°C for thirty seconds, and 72°C for one minute for annealing, and finally 72°C for ten minutes for extension.

The next processing stage for the DNA was to check the accuracy of the PCR technique and, more importantly, to check for DNA amplification. This required the use of gel electrophoresis, commonly known as a "check gel", which passes an electrical current (negative to positive) through a gel (loaded with DNA) surrounded by a buffer, causing the DNA (negatively charged) to move down the gel towards the positive end. This process works because specific molecules exhibit different electrophoretic mobilities (Garner and Revzin 1981), causing them to move along the gel at different levels and thus allowing them to be distinguished and separated. These mobilities directly correlate to the size of the molecule, specifically the number of base pairs (bp) associated with the molecule (DNA in this study), where smaller molecules move faster, having different base pair markers (100 bp, 200 bp, etc.) along the check gel than larger molecules.

The gel was made by combining 2 g of agarose powder with 200 ml of 1xTA buffer and microwaving the mixture until clear. Then, 2 μl of SYBR® Safe (Life Technologies Corp. 2013) was added to the mixture, a stain used to the DNA to fluoresce in the gel; this represents a step used to visually inspect the DNA for amplification (no fluorescing means no DNA amplification). Once the mixture had cooled enough to touch, it was poured into a chamber inside an
electrophoreses box with loading trays that contained plastic inserts, making lanes and loading wells (small cells) to insert the DNA (Figure 7.1). After the gel cooled, the loading trays were removed, and the electrophoreses box was filled with 1xTA buffer to just over the top of the gel.

The loading trays come in different sizes and have different slit-widths that allow for different numbers and sizes of loading wells. When loading the wells, the first well was reserved for a "ladder" that amplified across several base pairs, providing a key, of sorts, to check for base pairs that correlate with known DNA. Typically, 5 μl of the ladder was added to the first well of each lane, two wells were skipped, and 10 μl of DNA product was added to every other well down the lane, except for the last two lanes, which were used for a control (DNA from a known fungus) and water (to check for contamination in the mastermix). After all wells were loaded, a lid was placed onto the electrophoreses box, and this was connected to the (negative and positive) female ends of the electrodes on the box sides (Figure 7.2). The opposite side of the lid contained the male ends (Figure 7.2 bottom image) with wires that were attached to a BioRad voltage and amplitude meter set at 120 V, which ran for approximately fifteen to twenty minutes. Both the mastermix and loading gel had bright colors, which were used to visually inspect the movement of the DNA along the gel and also to determine the running time necessary for the volt meter; monitoring the volt meter was ended when the DNA was halfway through the gel. The dye observed under a UV lamp caused the DNA to fluoresce and produce bright bands in the wells in which there was amplification. The entire gel was then taken to a BioRad Gel Doc™ XR + image Lab™ device for acquiring an image of the gel and amplified DNA (Figure 7.3).
Figure 7.1. Electrophoreses box (above) with chamber and two loading tray lanes (below) with several loading wells (small cells) for cooling the gel. Once the gel cooled, the plastic loading trays were removed, and wells were filled with DNA.
Figure 7.2. Electrophoreses box (top) with electrodes on the side, along with the Electrophoreses box lid shown fitting over the negative (black) and positive (red) electrodes (bottom) that were led into the voltmeter.
The next step was to process the amplified DNA for sequencing, which required cutting out the sections of amplified DNA from the gel and placing them into labeled mini-spin column centrifuge tubes that contained filters. The tubes were then spun in a centrifuge at 13,000 rpm for five minutes, which forced the DNA through the filter, separating it from the gel.

The DNA (5 μl) was then placed into eppendorf tubes, one with water (7 μl) and ITS1F (1 μl) primer and another with the same DNA (5 μl), water (7 μl), and ITS4R (1 μl) primer and sent to a lab for sequencing. The DNA came back with a sequence for the forward (ITS1F) and reverse (ITS4R) segments of the DNA strand associated with the sample. Each half of the DNA segment had an associated percent accuracy that was checked and modified (Figure 7.3).

The sequence files were loaded into the Sequencher (version 5.1) program, and sequences were visually inspected for regions that did not code the base pairs correctly during the cloning phase (replicating phase of PCR); these were represented by dark blue boxes and areas with unknown values, "N" (Figure 7.4). The number of base pairs was reduced during the process, but the accuracy of the strand match increased, making it possible for later recombination of the sequences. Sequencher also outputs a chromatogram (graphical representation of the sequence), where many of the dark blue error points were displayed under areas of overlapping peaks (Figure 7.5). The overlapping peaks in the chromatogram correlated with the areas where a single nucleotide (or many) could not be accurately matched along the DNA strand. Any area with bad coding or overlapping peaks (dark blue or "N" values) was deleted out of the sequence. Once the forward and reverse sequences were cleaned, they were automatically merged into a single file in Sequencher.

The file was then exported into a FASTA text document pre-formatted for use in online DNA databases that are used for determining a particular species from a sequence. Here, the
Figure 7.3. Image from the BioRad Gel Doc™ XR + image Lab™ software of the loading gel and amplified ladder (left column of bright bands) and amplified DNA (remaining bright columns).
Figure 7.4. Example of a forward sequence from DNA with the left indicating areas along the sequence that were unknown, "N", with several mismatched nucleotides (dark blue) and good matches (light blue). The same forward sequence is shown on the right after undefined nucleotides were removed, showing a more accurate sequence with a larger number of light blue nucleotides visible.
Basic Alignment Search Tool (BLAST®) was used to check DNA sequences for potential fungi matches against GenBank, which is a genetic sequencing database (US National Library of Medicine 1993). From the BLAST® homepage, the nucleotide blast query was chosen, and this was followed by uploading the FASTA file, choosing the "others" database search within the Nucleotide collection, and optimizing for highly similar sequences. Once a BLAST® search was carried out, BLAST® would output a results page with several rows and columns of research papers and the associated information on a known fungus (or organism) that matched the input sequence. Each BLAST® result had a different score for accuracy, identity, and amount of error associated in each match to the input sequence. The result with the lowest amount of error, but highest max score and accuracy in the identity of the match, was used to determine species.

The results from applying the above PCR, Sequencher clean-up, and BLAST® search techniques to the collection of root tips and fungi collected in northwest Arkansas and the Fernow Experimental Forest are described in the next section.
Figure 7.5. Chromatogram indicating areas of incorrect nucleotide matches along the DNA strand (top row near the "N" markers) where no "N" match has been made, and there are several peaks overlapping in the bad region. The second row shows a much better match along the sequence, with only one band and peak correlating with a nucleotide.
7.3 Results from the Ectomycorrhizal Root Tip and Fungi Fruiting Body Data

The colonized root tips from red oak, chestnut oak, and beech preserved from material collected in the summer of 2012 in the Fernow Experimental Forest were the first to undergo DNA analysis. The first run had only three root tips, all from beech, with a low amount of amplification and resulted in sequences with very low accuracy and not usable in a BLAST® search. Another eight samples taken from a different group of colonized beech root tips were processed but again produced no results. It was confirmed that the lack of results was not from PCR, since a control was used during each experiment that did not have any issues; instead, it was believed that many of the root tip samples, despite being stored in CTAB, had undergone degradation during the trip from West Virginia to Arkansas.

Another set of root tips was collected from the same species of trees from the Fernow Experimental Forest in June 2013. From the new collection, eight colonized root tips were extracted-four from chestnut oak and four from red oak-and after PCR, they all exhibited strong amplification markers in the check gel. Each DNA sample was prepared for sequencing, sent to a DNA sequencing lab at the University of Arkansas, and sequence results came back. Out of the eight samples, only two-one from each tree-had high enough confidence in the sequences that they could cleaned and used in a BLAST® search. The root tip extracted from the chestnut oak had a sequence whose closest match, 93% similarity, was only with the family Cundoniaceae, a member of the Ascomycetes, with no species match (Table 7.1). The other sample, from the red oak, had a much higher sequence match, 97% similarity, associating it as the species Oidiodendron citrinum (Table 7.1). However, it should be noted that in both of the samples, the forward and reverse segment of the sequences could not be paired up due to only half of the DNA sequence being determined accurately by the reverse primer. The results of this new
collection of root tips were still not as accurate or as efficient as the results reported by Smith et al. (2011), who had successfully identified 90% of the fungal species associated with the colonized root tips they sampled.

To check these findings, eight colonized root tip samples from fresh post oak root tips collected in northwest Arkansas were prepared for DNA analysis. All samples had amplification and were sent to be sequenced. The sequences came back with considerable variation in their quality from the forward and reverse primers. Three of the samples came back with sequence matches that were of too poor quality to work with, four had at least the reverse primer with a high enough quality (80% accuracy before clean-up) that they could be checked against GenBank in BLAST®, and one had high enough accuracy (+85% accuracy before clean-up) on both sequence segments. After clean-up and running (the reverse segment) through GenBank, three came back with DNA from a post oak tree and the other two came back with a 99% match to the fungal species *Cenococcum geophilum* (Table 7.1). These results, although successful in some instances, were not as accurate as hoped.

To check further, another set of colonized root tips (two from each tree) were taken from red oak and black oak roots along with a sample taken from the gills and hyphae of a fungus fruiting body found growing on a white oak tree root tip mass. In all instances, the samples showed amplification after PCR and were sent for sequencing. The sequences obtained from the fruiting body and hyphae found on the white oak tree root tip mass had accurate results for both the forward and reverse DNA segments; the red oak had both samples with high accuracy on the reverse primer, but only one with high accuracy on the forward primer; and the black oak had null results on one of the samples, but high accuracy for both the reverse and forward primers on the other. In the instances where both primers had high accuracy, the forward and
reverse segments were combined, whereas for the others, only the reverse was used to check against GenBank. The sequence from the fruiting body found on the white oak was matched, 95% sequence match, to *Russula lutea*, which was confirmed with a 92% sequence match for *Russula lutea* from the sequence returned for the hyphae collected off the same fungus (Table 7.1). The remaining sequences, when checked against GenBank, all had the same fungus associated with their root tips. This was *Cenococcum geophilum*, which was also found on the post oak (Table 7.1).

Given the results of these data, it appeared that sampling from the fungal fruiting bodies had a much higher accuracy than sequencing results from the root tips. Therefore, to supplement the root tip analysis, fungi from areas within the study sites in northwest Arkansas were collected and analyzed. Samples were taken from seventeen fungi that were then processed through the same PCR techniques as outlined above, with amplification found for all samples in the check gel. Sequences came back with varying levels of accuracy for both primers, although all samples had at least one segment (forward or reverse) with a high enough accuracy that it could be checked against GenBank. The sequences with the lowest match on GenBank (82% and 86%) had, at the very least, a genus they could be associated, and those with higher accuracy (88% and above) were matched to a particular species (Table 7.1). Although there were sequences on the lower end of the GenBank match (88%-93%), the results from the GenBank search had several papers associating the same species of fungus to the sequence.

Sampling from fungal fruiting bodies proved highly successful in northwest Arkansas, but access to fungal fruiting bodies in the Fernow Experimental Forest was problematic. However, in a personal communication with a fellow mycologist, Bill Roody, fruiting bodies were collected and analyzed with similar success as northwest Arkansas (Table 7.2). To aid in
future studies, improving the low accuracy in the DNA sequencing from the two primers, ITS1F and ITS4R, would require additional primers to be tested on root tips collected from the Fernow Experimental Forest. Through email correspondence with Dr. Matt Smith, lead author on the paper (Smith et al. 2011) and whose research this study was modeled after, two new primer sets were chosen. The first set, ITS1F and LR3 (CCGTGTTTCAAGACGGG) (White et al. 1990), was suggested by Dr. Matt Smith, and the second set of primers, ITS3F (GCATCGATGAAGAACGCAGC) and ITS4BR (CAGGAGACTTGTACACGGTCCAG) (Gardes and Bruns 1993), was one I selected. I chose the last two primers, as they had been developed for work with Basidiomycetes, and they were found in close proximity to each other on the ITS region, which was thought to increase accuracy in the PCR (limiting the DNA segment to be cloned and thus chance for error) and improving the ability to combine the sequences later.

Testing the primers began with the extraction of DNA from six colonized root tips collected from the Fernow Experimental Forest, two from each of the following trees: chestnut oak, red oak, and beech. The PCR was performed in duplicate with the DNA from the same six colonized root tips used, but differed by the primer sets used in the mastermix, with ITS1F and LR3 primers used in one and ITS3F and ITS4BR primers used in the other. The results of the check gel are shown in Figure 7.6, where the top row corresponds to amplification of the DNA with the use of the ITS1F and LR3 primers and the bottom row corresponds to the ITS3F and ITS4BR primers. There was no DNA amplification for the ITS1F and LR3 primers, but the amplification was very high (bright) for the ITS3F and ITS4BR primers. The DNA was prepared for the amplified samples and sent to be sequenced. Four of the six samples came back with highly accurate sequence matches (+90% before clean-up), which was much higher
Figure 7.6. Top row corresponds to the region where DNA amplification should be shown for the ITS1F and LR3 primers, but no amplification was found. The bottom row corresponds to the region where DNA amplification occurred for the ITS3F and ITS4BR primers.
than the previous primers. The sequences were cleared of bad base pair coding, increasing accuracy to around 98%, then loaded into BLAST®, and species were found for all four (Table 7.1). Russula favrei was found colonized on two separate root tips, and Cortinarius camphoratus and Laccaria bicolor were found on the other two (Table 7.1). The ITS3F and ITS4BR primers also had a highly accurate match (95-99%) during the BLAST® search (Table 7.1), which showed that these two primers were a better fit to the data.

An additional six root tips from the Fernow Experimental Forest, two for each of the previously mentioned trees, were processed using these primers, all of which had amplification. The sequences came back with high quality (90%+ accuracy before clean-up) and after clean-up, were checked against GenBank in BLAST®, with a high percentage of sequences matched to GenBank, with accuracy of 95-96% sequence match (Table 7.1). This new set of sequences resulted in four more species found in the Fernow Experimental Forest, the last four species listed in Table 7.1.

7.4 Summary of Results in Northwest Arkansas and the Fernow Experimental Forest

In northwest Arkansas, the most common fungi found colonized on tree root tips was Cenococcum geophilum, which was found twice on red oak and once on each of black oak and post oak. In both root tips and fungal fruiting bodies, Russula was the most common genus found in northwest Arkansas with Russula lutea found colonized on white oak root tips and growing near a white oak tree; Russula chameleontina was found growing near white oak and red oak trees; Russula crustosa was found near a white oak tree; and an unknown species of Russula was found near a white oak tree (Tables 7.1 and 7.2). For the fungal fruiting bodies, the most common genus was Amanita, with various species, Amanita flavoconia, Amanita rubescen, Amanita seplusae, Amanita lignitincta, and an unknown Amanita sp., growing near
white oak, red oak, and black oak trees (Table 7.2).

The most common genus found in the Fernow Experimental Forest was *Russula*, with *Russula faveri* found colonized on two separate beech root tips and on one red oak root tip (Table 7.1). The genus *Cortinarius* was also well-represented, with *Cortinarius camphoratus* found colonized on beech root tips and *Cortinarius torvus* found on red oak root tips (Table 7.1). In all primer sets for the Fernow Experimental Forest, chestnut oak had the greatest diversity of species colonizing root tips, which included *Oidiodendron citrinum*, *Laccaria bicolor*, *Trechisporales* sp., and an unidentified member of the family Cudoniaceae.

Given these results, the ECM associations undoubtedly involved numerous fungal taxa but appeared to be dominated by members of the genera *Amanita* and *Russula*. 
Table 7.1. Species of fungi associated with ectomycorrhizal trees in northwest Arkansas (NWA) and the Fernow Experimental Forest (FEF).

<table>
<thead>
<tr>
<th>Region</th>
<th>ECM taxonomic name and authority</th>
<th>% Similarity</th>
<th>GenBank Id.</th>
<th>Associated tree</th>
</tr>
</thead>
<tbody>
<tr>
<td>NWA</td>
<td><em>Cenococcum geophilum</em> Fr. 1829</td>
<td>99</td>
<td>EU057125.1</td>
<td>Post oak</td>
</tr>
<tr>
<td>NWA</td>
<td><em>Cenococcum geophilum</em> Fr. 1829</td>
<td>97</td>
<td>JX630567.1</td>
<td>Red oak</td>
</tr>
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<td>AY394919.1</td>
<td>Black oak</td>
</tr>
<tr>
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<td>97</td>
<td>AF062790.1</td>
<td>Chestnut oak</td>
</tr>
<tr>
<td>NWA</td>
<td><em>Russula lutea</em> Singer 1938</td>
<td>95</td>
<td>HQ604848.1</td>
<td>White oak</td>
</tr>
<tr>
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<td><em>Cenococcum geophilum</em> Fr. 1829</td>
<td>95</td>
<td>JX630567.1</td>
<td>Red oak</td>
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<td>FJ475669.1</td>
<td>Chestnut oak</td>
</tr>
<tr>
<td>FEF</td>
<td>Phylum: Ascomycota Whittaker 1959</td>
<td>95</td>
<td>FN298752.1</td>
<td>Beech</td>
</tr>
<tr>
<td>FEF*</td>
<td><em>Cortinarius camphoratus</em> Fr. 1838</td>
<td>95</td>
<td>HQ604694.1</td>
<td>Beech</td>
</tr>
<tr>
<td>FEF*</td>
<td><em>Russula favrei</em> M.M. Moser 1979</td>
<td>98</td>
<td>FJ627037.1</td>
<td>Beech</td>
</tr>
<tr>
<td>FEF*</td>
<td><em>Russula favrei</em> M.M. Moser 1979</td>
<td>99</td>
<td>DQ777975.1</td>
<td>Beech</td>
</tr>
<tr>
<td>FEF*</td>
<td><em>Laccaria bicolor</em> P.D. Orton 1960</td>
<td>98</td>
<td>GC994982.1</td>
<td>Chestnut oak</td>
</tr>
<tr>
<td>Region</td>
<td>ECM taxonomic name and authority</td>
<td>% Similarity</td>
<td>GenBank Id.</td>
<td>Associated tree</td>
</tr>
<tr>
<td>--------</td>
<td>---------------------------------</td>
<td>--------------</td>
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<td>-----------------</td>
</tr>
<tr>
<td>FEF*</td>
<td><em>Trechisporales</em> sp. K.H. Larss. 2007</td>
<td>95</td>
<td>JF691365.1</td>
<td>Chestnut oak</td>
</tr>
<tr>
<td>FEF*</td>
<td><em>Cortinarius torvus</em> Fr. 1838</td>
<td>96</td>
<td>AY669668.1</td>
<td>Red oak</td>
</tr>
<tr>
<td>FEF*</td>
<td><em>Xenasmatella aff. ardosiaca</em> Stalpers 1996</td>
<td>96</td>
<td>GQ268627.1</td>
<td>Red oak</td>
</tr>
<tr>
<td>FEF*</td>
<td><em>Russula favrei</em> M.M. Moser 1979</td>
<td>96</td>
<td>FJ627037.1</td>
<td>Red oak</td>
</tr>
</tbody>
</table>

*Indication of species found with second set of primers, ITS3F and ITS4BR. Taxonomic authority provided in the Index Fungorum (2013).
Table 7.2. Species of fungi associated with ectomycorrhizal trees in northwest Arkansas (NWA) and the Fernow Experimental Forest (FEF). The associated trees next to the ECM taxonomic name were ECM trees within 4 m of where the fungi were collected.

<table>
<thead>
<tr>
<th>Region</th>
<th>ECM taxonomic name and authority</th>
<th>% Similarity</th>
<th>GenBank Id.</th>
<th>Associated tree</th>
</tr>
</thead>
<tbody>
<tr>
<td>NWA</td>
<td><em>Amanita flavoconia</em> G.F. Atk. 1902</td>
<td>99</td>
<td>EU819463.1</td>
<td>Red and white oak</td>
</tr>
<tr>
<td>NWA</td>
<td><em>Laccaria proxima</em> (Boud.) Pat. 1887</td>
<td>99</td>
<td>GC994982.1</td>
<td>Red and white oak</td>
</tr>
<tr>
<td>NWA</td>
<td><em>Galiella rufa</em> (Schwein.) Nannf. &amp; Korf 1957</td>
<td>99</td>
<td>AF485073.1</td>
<td>White oak</td>
</tr>
<tr>
<td>NWA</td>
<td><em>Lactarius volemus</em> Fr. 1838</td>
<td>99</td>
<td>JQ358945.1</td>
<td>White oak</td>
</tr>
<tr>
<td>NWA</td>
<td><em>Ramaria subbotrytis</em> Corner 1950</td>
<td>99</td>
<td>AJ408363.1</td>
<td>White oak</td>
</tr>
<tr>
<td>NWA</td>
<td><em>Agaricus comtulus</em> Fr. 1838</td>
<td>99</td>
<td>AJ887992.1</td>
<td>White oak</td>
</tr>
<tr>
<td>NWA</td>
<td><em>Amanita rubescens</em> Pers. 1797</td>
<td>98</td>
<td>AJ889923.1</td>
<td>White oak and black oak</td>
</tr>
<tr>
<td>NWA</td>
<td><em>Russula chameleontina</em> Fr. 1838</td>
<td>96</td>
<td>JF834357.1</td>
<td>White oak and red oak</td>
</tr>
<tr>
<td>NWA</td>
<td><em>Amanita</em> sp. Dill. ex Boehm. 1760</td>
<td>95</td>
<td>JX029931.1</td>
<td>White oak and black oak</td>
</tr>
<tr>
<td>NWA</td>
<td><em>Amanita seplacea</em></td>
<td>94</td>
<td>AY436473.1</td>
<td>White oak and black oak</td>
</tr>
<tr>
<td>NWA</td>
<td><em>Lactarius piperatus</em> Pers. 1797</td>
<td>93</td>
<td>JF908270.1</td>
<td>White oak</td>
</tr>
<tr>
<td>NWA</td>
<td><em>Thelephora ganbajun</em> M. Zang 1987</td>
<td>93</td>
<td>EU696874.1</td>
<td>White oak</td>
</tr>
<tr>
<td>Region</td>
<td>ECM taxonomic name and authority</td>
<td>% Similarity</td>
<td>GenBank Id.</td>
<td>Associated tree</td>
</tr>
<tr>
<td>--------</td>
<td>---------------------------------</td>
<td>-------------</td>
<td>-------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>NWA</td>
<td><em>Russula lutea</em> Singer 1938</td>
<td>92</td>
<td>HQ604848.1</td>
<td>White oak</td>
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<tr>
<td>NWA</td>
<td><em>Cantharellus cibarius</em> Fr. 1821</td>
<td>92</td>
<td>HQ270123.1</td>
<td>Red and white oak</td>
</tr>
<tr>
<td>NWA</td>
<td><em>Thelephora palmata</em> Fr. 1821</td>
<td>92</td>
<td>EU819443.1</td>
<td>Red and white oak</td>
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<tr>
<td>NWA</td>
<td><em>Russula crustosa</em> Peck 1886</td>
<td>90</td>
<td>EU598194.1</td>
<td>White oak</td>
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<tr>
<td>NWA</td>
<td><em>Amanita lignitincta</em> Zhu L. Yang 1997</td>
<td>88</td>
<td>FJ441045.1</td>
<td>White oak</td>
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<tr>
<td>NWA</td>
<td><em>Cantharellus</em> sp. Adans. ex Fr. 1821</td>
<td>86</td>
<td>HQ270118.1</td>
<td>White oak</td>
</tr>
<tr>
<td>NWA</td>
<td><em>Russula</em> sp. Pers. 1796</td>
<td>82</td>
<td>EU819429.1</td>
<td>White oak</td>
</tr>
<tr>
<td>FEF</td>
<td><em>Lactarius peckii</em> Burl. 1908</td>
<td>100</td>
<td>EU598168.1</td>
<td>Mixed oak stands</td>
</tr>
<tr>
<td>FEF</td>
<td><em>Amanita flavoconia</em> G.F. Atk. 1902</td>
<td>100</td>
<td>JF313657.1</td>
<td>Broad leaf wood area</td>
</tr>
<tr>
<td>FEF</td>
<td><em>Amanita bisporigera</em> G.F. Atk. 1906</td>
<td>100</td>
<td>GQ486875.1</td>
<td>Mixed oak stands</td>
</tr>
<tr>
<td>FEF</td>
<td><em>Amanita muscaria</em> Lam. 1783</td>
<td>100</td>
<td>EU071921.1</td>
<td>Broad leaf wood area</td>
</tr>
<tr>
<td>FEF</td>
<td><em>Tylopilus sordidus</em> A.H. Sm. &amp; Thiers 1968</td>
<td>99</td>
<td>EU819450.1</td>
<td>Broad leaf wood area</td>
</tr>
</tbody>
</table>
Chapter 8. Concluding Remarks

The scientific study of mycorrhizal associations took root (pun intended) in the late 1880s when the German biologist A. B. Frank first coined the term “mycorrhizae” to describe the underground network of hyphae. What Frank failed to realize was that these hyphae were connected in intricate networks to the root tips (and cells) of plant roots. After the fossil record revealed that some 400 million year old plant (*Aglaphyton major*) had a symbiotic relationship with a zygomycete, it was clear that there was much more going on than anticipated.

The kingdom Fungi has gone through many changes, once considered a part of the kingdom Plantae, and now it is considered a separate kingdom containing four main phyla: Basidiomycota, Chytridiomycota, Zygomycota, and Ascomycota. Each of these contains very unique fungal species which would take the lifetimes of many researchers to understand completely. One key aspect of these groups is their associations with plants. The largest groups, the Ascomycetes and Basidomycetes, have the greatest mycorrhizal associations, as well as some of the most recognizable and beautiful fruiting bodies for fungi. The Ascomycetes are commonly known through the cup fungi and morels, which are macrofungi, but in general, the Ascomycetes are made up of microfungi, whereas the Basidiomycetes are made up of a large number of macrofungi, represented by such examples as the jelly fungi, boletes, stinkhorns, chantrelles, earth stars, puffballs, agarics, and bracket fungi.

The associations these fungi create with trees such as maple, elm, ash, magnolia, hickory, cypress, cherry, juniper, and many others are endomycorrhizal, and those formed with oak, birch, beech, chestnut, pine, spruce, hemlock, and fir are ectomycorrhizal (ECM). Both types are clearly advantageous to both parties as they have survived (with evolutionary changes) for millions of years. It is apparent that the role of mycorrhizal associations between plants and
fungi is an important one. The ectomycorrhizal association creates a symbiotic relationship, which is manifest in an underground network formed along the root tips of the host plant that then spreads throughout the soil to reach other ECM-forming plants. There are several advantages to this symbiotic relationship that have been addressed such as increased nutrient access, reduction in competition effects, the ability to withstand harsh environments, and the ability to transfer nutrients through hyphae from plant to plant. This makes establishing spatial linkages and the proximity to other plants important.

The leading issue with mycorrhizal associations is the lack of understanding of the spatial distribution of the advantageous mycorrhizal link between plants. Many studies have found that plants inoculated with fungal hyphae become well equipped to fight off disease and to better handle unfavorable weather conditions, but it is not known how fungal hyphae transfer from plant to plant. It is thought that the spatial relationship between ectomycorrhizal plants affects population and demographic patterns of distribution of plants in the communities in which they occur (Hartnett and Wilson 2002), but it is not known to what degree this occurs. Essentially the question that needs to be asked is, “Do parent plants provide an ecological advantage to their offspring if they have a mycorrhizal association, and if so to what extent?”

The two study areas used during this project, the Fernow Experimental Forest and the Ozark Mountains, were chosen for their similar topography, the presence of oak-dominated forests, and the overall dominance of trees with potential ectomycorrhizal linkages. Due to the decline in oak regeneration, the forests of northwest Arkansas and the Fernow Experimental Forest both regularly undergo management practices that researchers hope will aid in the reproductive success of young oak saplings and seedlings. One goal of this project was to address the potential benefit of ectomycorrhizal associations to these forests and how
ectomycorrhizal fungi may provide a potential resource in areas of particularly low regeneration.

Despite the decline in oak regeneration, in both regions, the dominant tree species are members of the genus *Quercus*, with codominance belonging to hickory in northwest Arkansas and beech in the Fernow Experimental Forest. The seedlings and saplings in the regions were also characterized by abundance data that would suggest that most have heights between 6 and 12 cm and belong to the genus *Quercus*. Although maple did show a noteworthy dominance in northwest Arkansas, it would seem that with the reduced number of larger stems, this would not mean an overall established dominance for the larger size classes or trees. However, maple was also a dominant tree found in many of the Fernow Experimental Forest plots sampled in this study, which could suggest a shift in dominance to an oak-beech-maple forest. The presence of maple in both regions provides another indication that oak regeneration is on the decline and that quite possibly, maple is taking its place, which is why good forest management practices need to be implemented in order to assist oak regeneration.

The potential positive impact ectomycorrhizal linkages could have on assisting the growth and distribution of ECM trees, saplings, and seedlings in these two regions would seem even more important given the decline in oak regeneration and the increased dominance of maple in the two forests. The linkages between ECM trees and seedlings or saplings in the Ozarks and the Fernow Experimental Forest were evaluated by examining the spatial distributions of large trees and nearby seedlings and saplings. Northwest Arkansas and the Fernow Experimental Forest did not have a high number of ectomycorrhizal species in common (coefficient of community = 0.3), nor did they share high relative abundances for those species (percent similarity = 0.12). The two regions do have a high proportion of ectomycorrhizal trees, seedlings, and saplings, but they belong to different species.
Euclidean distance calculations revealed that spatial relationships existed among ECM trees, seedlings, and saplings, in which seedlings and sapling displayed decreasing stem height with increasing distance from a tree and tended to have an association with trees of larger DBH measurements (average of 61.1 cm). In areas without significance, the average between both regions was for shorter stem heights to be characteristic for seedlings and saplings (52% of stems) closer to the host trees, with the remaining (48%) having taller heights closer to the host trees. It would seem that in order for there to be a significant impact on seedling and sapling heights in relation to distance from trees, they would need to be within 4 m of a well-established (large DBH) tree. The lack of significance throughout the data appears to be a result of several factors: a skewing effect caused by outlier stems with very different heights in comparison to the average stem height, the number of host trees within the plots not being large enough to produce an accurate measure of significance, and disturbances that take place such as prescribed burns and herbivory from deer and other animals.

Herbivory from animals would potentially remove terminal shoots during browsing, eliminating the stems from significance measures or plot data and making them an unmeasurable part of this study. However, this study did have sites that had undergone prescribed burn. The two regions experienced a decline in the number of ectomycorrhizal stems found in burned plots when compared to unburned locations. The Fernow Experimental Forest had an average of 16 (6,400/ha) ectomycorrhizal stems in the burned plots compared to 168 in the unburned plots (67,200/ha). The plots in northwest Arkansas, although larger in size, also showed a decline in ectomycorrhizal stem count in the burned plots, an average of 56 (5,600/ha) stems compared to 83 (8,300/ha) in the unburned plots.

However, the burned plots in the two regions had higher (> 0.50) overall indices for
coefficient of community (CC) and percent similarity (PS). Northwest Arkansas had an average CC index of 0.9 and an average PS value of 0.66, whereas the Fernow Experimental Forest had a CC index of 0.7 and a PS value of 0.64. The presence of fire in these sites could help to selectively remove some of the endomycorrhizal stems, therefore increasing the similarity between plots. For the ectomycorrhizal stems that do survive a fire, the smaller stems would need to be closer to a host tree so the tree could assist their growth and survivorship as indicated in spatial relationship data where the stems tended to be taller (in some cases significantly so) closer to the tree.

The potential for ectomycorrhizal linkages to influence the distribution of stems within a plot again showed that the ectomycorrhizal stems tended to be in close proximity to each other, where they clustered together, undoubtedly in response to increased access to nutrients provided by a mutual symbiotic partner. Both regions had an average of four clusters per plot, which was extrapolated to 3944 clusters/ha in northwest Arkansas and 1720 clusters/ha in the Fernow Experimental Forest. For both regions there appears to be a tendency for clusters to form between seedlings and saplings, with several having trees within 2 m of their cluster boundaries. This correlates well with the results found for the spatial distribution, where significance was found for ectomycorrhizal seedlings and saplings that were located within 4 m of a host tree. This is important because the established tree can form a symbiotic relationship with fungi that can then spread throughout the soil, reaching other ECM-forming plants, and this appears to help provide a host to these plants. Furthermore, given the number of clusters found within the regions and the lack of trees inside the cluster boundaries, it can be assumed that seedlings and saplings are providing assistance to one another through their underground linkages, thus making their proximity to each other important and potentially an underlying cause for their observed
ECM associations within the two regions undoubtedly involved numerous fungal taxa but, based on sequence data obtained from root tips and fruiting bodies, they appeared to be dominated by the members of the genera *Amanita* and *Russula*. Among the species identified were *Russula lutea*, *Russula chameleontina*, *Russula crustosa*, *Amanita flavoconia*, *Amanita rubescen*, *Amanita seplacea*, *Amanita lignitincta*, and additional unidentified *Russula* and *Aminina* species. It should be noted the species with the greatest colonization diversity was *Cenococcum geophilum*, which was found on red oak, black oak, and post oak tree root tips in northwest Arkansas.

Additional research still needs to be carried out on the acquisition of fungal species within the two regions. The low level of accuracy experienced in the DNA analysis of colonized root tips reduced the number of species observed in this study. In the future, it would appear that applying the set of primers, ITS3F and ITS4BR, would assist in solving this problem and provide a more accurate reflection of the fungi responsible for the ECM linkages.

This study contributes a detailed analysis of the occurrence of patterns of spatial distributions among ECM-forming trees, seedlings, and saplings in which the presence of the trees appeared to provide a symbiotic host effect in the survival and development of smaller stems. Additionally, the adoption of primers used in this thesis, ITS3F and ITS4BR, could assist in future DNA analysis of fungal types in forests to determine the species used to assist ECM stems and potentially increase colonization of host trees. Finally, future forest management practices should consider the results of this study to potentially use fungi to assist in the regeneration, development, and survivorship of smaller ECM stems, particularly those of oaks.
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