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John J. Rizza

University of Tennessee - Knoxville

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To the Graduate Council:

I am submitting herewith a thesis written by John J. Rizza entitled "The Influence of Different Ground Cover Treatments on the Growth of Outplanted Seedlings on Remined Sites in Eastern Tennessee." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Forestry.

Jennifer A. Franklin, Major Professor

We have read this thesis and recommend its acceptance:

David S. Buckley, John T. Ammons

Accepted for the Council:

Dixie L. Thompson

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Carolyn Hodges
Vice Provost and Dean
of the Graduate School

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The influence of different ground cover treatments on the
growth of outplanted seedlings on remined sites in
Eastern Tennessee

A thesis proposal presented for the Master of Science Degree

The University of Tennessee, Knoxville

John James Rizza

May 2007

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ABSTRACT

There is growing interest in the reforestation of surface mined lands for the production of valuable forest products and creation of quality wildlife habitat. These objectives can be met by planting native woody and herbaceous species on reclaimed surface mines. However, in this region, many of the common ground cover species used to reduce erosion, compete aggressively with tree seedlings, preventing successful establishment. A research project was designed with two main objectives: to investigate the growth and survival of tree seedlings across different herbaceous ground cover treatments, and to identify the relationship between the growth and function of tree seedlings and microsite variables. Five tree species, native to the eastern hardwood forest surrounding the mine site, were planted in 2005. They are: yellow poplar (*Liriodendron tulipifera*), sugar maple (*Acer saccharum*), northern red oak (*Quercus rubra*), eastern redbud (*Cercis canadensis*), and Virginia pine (*Pinus virginiana*). Five different ground cover treatments were applied within four replicated planting areas. Two treatments consisted of two different native warm season grass mixes, two were standard reclamation mixes, and one was an unseeded control. Growth and survival, seedling transpiration rate, light measurements, soil respiration, groundcover biomass, and soil chemical properties were measured and analyzed. Survival was significantly different across tree species, with sugar maple having the best overall survival and yellow-poplar the poorest. Seedling survival tended to be greatest within the native warm season grass treatments; however growth rates were variable between all treatments. Seedling survival and growth was related to the amount of herbaceous cover suggesting that tree species react differently to the conditions associated with the surrounding level of herbaceous cover. Moderate ground cover resulted in the best survival, while bare ground or full cover demonstrated the poorest survival rates for northern red oak and eastern redbud. Sugar maple transpiration rate was significantly greater in the moderate (50-75%) cover class during the second growing season. Soil

chemical concentrations differed significantly between years, but not between treatments. Soil respiration significantly increased during the two years of this study. The results suggest that moderate herbaceous cover is advantageous for the establishment tree seedlings.

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1. Introduction

A. Coal mining and reclamation in the Eastern United States

Surface mining in Tennessee

A significant amount of mining occurred in east Tennessee beginning in the 19th century as a consequence of the conclusion of the Civil War. This mining continued steadily for the next 100 years until a low point in coal production was reached in the 1950's. During the 1960's coal was becoming the major fuel source for the generation of electricity throughout the United States (US). In the 1970's the demand for coal surged and production has increased steadily ever since. More than one-half of Tennessee's coal production was from strip or surface mines until the mid 1970's (Fribourg *et al.*, 1981). Surface contour mining in the steep mountainous terrain of the Cumberland Mountains was difficult and much of the coal was inaccessible. However, with the increased interest in, value of, and technological advances made in surface coal mining, we have recently been able to access areas not previously mined.

The extraction of coal has had many deleterious effects on the environment. To access the coal, many different layers of overburden material are blasted out of the side of the mountain, which pulverizes and mixes the rock strata as it is moved. During this process, the layers that were the deepest (unweathered shale and sandstone) are deposited on the surface (furthest from where the coal seam was located). Sandstone is a clastic sedimentary rock made of layers of sand (Luther, 1959). Shales are finely bedded sedimentary rocks formed by extreme pressure exerted on clay and mud parent material. This is a soft type of rock which splits readily into thin layers and weathers rapidly once exposed to surface environmental conditions (Luther, 1959). Research has demonstrated that these properties of shale rocks can impact the ability of tree seedlings and ground covers to develop and survive (Burger and Zipper, 2002).

Pre-law reclamation

The first coal mines in the country opened in 1750 near Richmond, Virginia (Bowling, 1978). This began a long battle of controversy due to the degradation of the environment as a result of surface mining activity. The surge in coal production in the early 1900's led many to feel that there needed to be a regulatory agency to help promote responsible mining and reclamation of the disturbed sites. In 1939, West Virginia became the first state to regulate surface mines (Bowling, 1978). Soon after, in 1941, Indiana enacted a state law that required coal companies to plant trees on spoil banks (Rathfon *et al.*, 2003). Prior to 1977, 25 states regulated surface coal mining operations; however no regulations were in place for post-mining reclamation (Office of Surface Mining, 2007). In most states, no real efforts were made to reclaim the mined sites. Sites in Virginia were commonly hand seeded and planted with non-native exotic grass, shrub, and tree species (Holl and Cairns, 1994). In Tennessee, we have minimal records of any reclamation work within surface mined areas. Reports indicate that several hundred acres of pine were planted on Tennessee Valley Authority (TVA) and private mined lands by the Civilian Conservation Corps (CCC) prior to 1942 (Seigworth, 1948). Within University property, we do have records stating that mined lands were revegetated with several species of pine and black locust while no herbaceous ground covers were used (Kring, 1967). Plantation plantings consisting of species such as black locust and shortleaf pine (Ashby and Kolar, 1977) or monocultures of black locust (Ashby *et al.*, 1980) were commonly established on strip mines before 1977. However, the US Forest Service did implement research plots in Ohio on which they planted black locust, yellow-poplar, white pine and white ash (Zelevnik and Skousen, 1996). Few plantings consisting of multiple species were documented, and these techniques were not widely employed on reclamation projects. The revegetation and reclamation techniques used in the past have yielded variable results concerning the establishment of forested sites. The lack of records and follow-up research has made it difficult to assess the productivity of these sites.

Enactment of Federal Legislation

In 1977, the Surface Mining Control and Reclamation Act (Public Law 95-87, Federal Register, August 3, 1977, 445-532) was established to direct the restoration of lands following surface mining. The Surface Mining Control and Reclamation Act (SMCRA) currently requires the mined land be restored to a condition capable of supporting the pre-mining land use or higher and better uses. A surface mining proposal must address how the post-mining land use is to be achieved. Operators must design a realistic reclamation plan and supply any bond money required obtain a surface mine permit. Bond release is determined by an Office of Surface Mining (OSM) official who evaluates erosion, minimum ground cover requirements, and progression of the development of the post mining land use in accordance with the site permit.

Current production in Tennessee

The annual production value of coal has been recognized as the most important mineral commodity in Tennessee (Luther, 1959). Today, over 90% of the coal extracted in the United States is used for the generation of electricity (Freme, 2006) at clean-coal-burning plants. In eastern Tennessee, bituminous coal is located in various seams throughout 22 counties (Luther, 1959). This coal is located along the eastern edge of the Cumberland Plateau, and is accessible to current surface mining operations in twelve counties. Much of the mining occurring today is a result of remining activity where operators are extracting coal from seams deeper those that were mined in the past. The coal fields of Tennessee produced just under 3 million tons (short, US) of coal in 2004 (United States Dept. of Labor, 2005). This equates to a production value of over 75 million dollars per year, which results in a significant contribution to the Tennessee economy (Tennessee Dept. of Environmental Conservation, 2006). The Department of Energy expects coal production to continue to increase over the next several years due to higher demand and energy prices (United States Dept. of Energy, 2007). In the next several years TVA will increase electric

generation output in Tennessee which will demand new permits be issued and new mines opened (Richard Mann, OSM, personal communication, 2006).

SMCRA in Tennessee

Tennessee attempted to establish a state regulatory program with the Tennessee Coal Surface Mining Law of 1980, but was unsuccessful and in 1984, the Office of Surface Mining's Knoxville Field Office became the regulatory authority within the state (Victor Davis of OSM, personal communication, 2006). Mining permits dictate the activities occurring during the extraction of coal and the following rehabilitation program. The permit requires that revegetation meets standards written into the permit by OSM officials. In Tennessee, SMCRA holds the operator responsible for the site up to five years after surface rehabilitation has begun. Operators discovered that designating hayland, pasture, or wildlife habitat as the post mining land use was one way to achieve SMCRA requirements and obtain bond release in a timely fashion (Rodrigue *et al.* 2002, Burger *et al.* 1998). These post mining land use designations have helped reduce the amount of forest land reclamation due several factors. One reason identified is the high ground cover requirements necessary to meet regulations (Rodrigue *et al.*, 2002). Also, overburden materials on many sites were being heavily compacted in an effort to reduce erosion rates. The compacted site was then heavily seeded with non-native herbaceous ground covers. This created a significant barrier to the growth and establishment of trees, shrubs and other native forest species (Angel *et al.*, 2006). Another significant reason for reduced reforestation efforts can be attributed to the potential for planting failure of seedlings (Cunningham, 1988). On the steep slopes of the Tennessee coalfields, it has been common practice to compact the surface materials with large bulldozers which jeopardizes woody seedling establishment and native species invasion. Compared to pre-law and native forest sites, research shows that those sites reclaimed under SMCRA have lower woody species richness (Holl, 2002). These sites also express fewer native woody species, suggesting

an inability to colonize compacted mine spoils. Effective April 2007, OSM revisions in Tennessee will allow reduced competition with woody plants, stating herbaceous ground cover should be limited to that necessary to control erosion and support the post-mining land use (Tennessee Federal Regulatory Program; Final Rule 30, CFR Part 942, Federal Register, March 2, 2007, 9615-9637).

Reforestation status in Tennessee

National Coal Corporation (NCC) was founded in 2003 and continues to grow with the renewed interest in coal extraction for use in utility and industrial plants. National coal has demonstrated a commitment to the land and recognizes its responsibility to provide environmentally responsible surface mining practices. Surface mining is an increasingly controversial topic for a variety of reasons and NCC has made an effort to properly reforest mine sites during reclamation. They have been working with the University of Tennessee (UT) since 2003 to establish herbaceous plant and tree species ideal for successful reforestation of surface mines along the Cumberland Plateau. Ensuring the success of restoration work is an essential part of the mining operation. National Coal is funding research to develop and implement effective reforestation techniques that will produce an environmentally sustainable ecosystem after reclamation is completed.

Ecological restoration of reclaimed sites

Bohm and Ericksen (1979) describe reclamation as an integral part of mining itself. To ensure the development of natural communities, these restoration efforts must consider the larger landscape (Harker *et al.*, 1999), not just the area under direct impact. The importance of looking at competitive interactions between the species planted is paramount to site development. Utilizing planting materials that consider the interactions necessary to structure plant communities and create ecosystems must be understood (Callaway and Walker, 1997). This project considers several aspects of the complex associations occurring on drastically disturbed sites. It also emphasizes the reforestation ideas set forth in

the Forest Reclamation Approach (FRA) promoted by the Appalachian Regional Reforestation Initiative (ARRI). This partnership between seven state agencies, the OSM, industrial operators, land owners, and universities including UT, has formed to promote the reforestation of reclaimed coal strip mines using the latest research and technology available. I believe that ecological restoration, the assistance of the recovery of a degraded ecosystem function (Society of Ecological Restoration, 2004), is more than just a science. Ecologists have tried to promote that it also includes an artistic approach by individuals who wish to imitate the processes of the natural world (Harker et al., 1999).

Forestry as a post mining land use in Tennessee

There is growing interest into the reforestation of these surface mined lands for various land uses including forest products (Burger *et al.* 2002, Burger and Torbert 1999, Gorman *et al.* 2001). Property owners are becoming increasingly aware of the potential to return the land to a productive forest ecosystem (Burger *et al.* 2002, Rodrigue *et al.* 2002). When forestry is the post mining land use, federal regulations require the prevention of excess compaction which can be obtained by minimal pass grading. To achieve this requirement, the material is graded by dozer blades creating slopes which are much more uniform topographically than the undisturbed surrounding forest. Reducing the amount of grading and the number of passes with heavy equipment has been shown to reduce compaction of the minesoil surface (Daniels and Zipper, 1988). The method of loosely grading the final surface layer to create a non-compacted growth medium can produce soil that is conducive to tree growth and survival (Burger *et al.*, 2005a). Many researchers have been addressing the complex issues associated with planting trees on strip mine lands in surrounding states outside of Tennessee; we are looking specifically at the Cumberland Plateau ecosystem. Beyond that, this project attempts to shed light on the physiology of seedlings and competitive interaction between outplanted seedlings and herbaceous cover.

Importance of native species for restoration

The Southeast has a greater variety of native plant communities, native plant species, and rare and endemic native plants than anywhere in the US (Owen, 2002). The proportion of exotic plants on reclaimed sites has been shown to be more than 200% higher than on undisturbed sites (Martin *et al.*, 2005). These findings emphasize the need for increasing the native component on reclaimed sites through early planning. Holl (2002) emphasizes that reclamation efforts would benefit from planting a diverse mix of native species. There is a need for additional research on the ability of native plants to adapt to these drastically disturbed sites in the intricate ecosystem of the Cumberland Plateau (Ashby *et al.* 1989, Holl 2002). These native communities decrease the amount of time needed for forest recovery and establish a new, dynamic ecosystem similar to what was once present on site. In many situations, post mining activities focused on planting non-native herbaceous covers, such as annual rye and birdsfoot trefoil, to ensure bond release from the agency. However, there is considerable debate about the ability of these non-native species to add to the health of the ecosystem over the long term (Holl, 2002), and it is not known exactly how these species influence forest succession. The establishment of native pioneer species on newly reclaimed sites is vital to enhancing the ecological function of a site (Elmarsdottir *et al.*, 2003) and to promoting long-term natural forest succession (Burger and Zipper, 2002). This evaluation of native species interactions on reclaimed sites is the first of its kind in this area.

B. Outplanted Tree seedlings

Extensive research has been done on reforesting coal mine overburdens in the southeastern United States; however most of this work has been done outside of Tennessee (Daniels and Zipper 1988, Burger and Torbert 1999, Torbert and Burger 2000). The geologic formation of the area is one difference between the Tennessee coalfields and other coal-bearing regions. The Cumberland Plateau

is capped by resistant rocks of the Pennsylvanian geologic age. The dominant soils of the Plateau are about one meter deep over rock, well drained, loamy, strongly acid, and low in natural fertility (Moneymaker, 1981). The eastern boundary of the Plateau, where extractable coal is located, is defined by high topographic relief where the resistant sandstone gives way to softer shale carved by shifting faults and the Tennessee River system (Luther, 1959). Another difference is the specific vegetation found in the area. Analysis of the surrounding native species composition can help determine the proper species for planting on a site. Native local vegetation is well adapted to survive and grow in the above ground microclimates present on the post mining landscape. Studies show that choosing the proper tree species for reclamation appears to influence the rate of invasion and composition of the plant species naturally colonizing reclaimed mines (Holl *et al.*, 2001). Over time, the number of native species invading a site will increase and add to the overall diversity of the site (Harrington, 1999). Planting the proper species for reforestation as recommended by the Forestry Reclamation Approach (FRA) can produce a valuable forest that will support a variety of uses (Burger *et al.*, 2005a). Planting a diverse mix of native tree species on newly reclaimed sites can benefit the landowner by producing potentially marketable timber (Torbert and Burger, 2000), and habitat appealing to wildlife. Native plantings will also encourage increased biological diversity by promoting the invasion of nearby vegetation and benefit the long-term recovery of a natural forest (Holl, 2002). Various publications have documented the responses of some species well suited for planting on reclaimed sites. Torbert and Burger (2000) recommend Virginia pine, yellow poplar, and northern red oak among others to be planted on sites in the southern Appalachians. These species are native to the surrounding area and have grown well on newly reclaimed sites in Western Virginia.

Silvics of the species studied

The tree species selected for planting were those that are present in the surrounding forest. Understanding how these species commonly grow, reproduce, and respond to environmental changes can provide insight into the processes occurring on reclaimed mine sites.

Yellow-poplar (*Liriodendron tulipifera*)

Yellow-poplar is an opportunistic native species that is well adapted to moist, but well drained soil conditions (Hay *et al.*, 1987). However, this tree will tolerate a wide range of precipitation and temperature regimes and has an extensive geographic distribution. Soil physical properties can overshadow the chemical properties of the soil to determine the growth and survival of this species.

Liriodendron tulipifera is an integral part of the native forest in the southeast. It is characterized by a tall straight stem with a pyramidal shaped crown and showy spring flowers. Yellow-poplar is a moderately valued commercial species due to its wide range of uses and fast growth rate. Being shade intolerant helped to secure this tree as one of the species being selected for planting on the mine site. As a juvenile, a rapidly growing deep taproot forms to help this seedling to establish on harsh sites. *Liriodendron tulipifera* has thin bark in the seedling stage and is susceptible to a variety of damage including sunscald, insect, and mammal predation (Beck, 1990). Most insects that may attack yellow-poplar are not considered to cause significant damages or losses economically to the tree. This species is considered valuable to wildlife due to high seed production.

Liriodendron tulipifera seedlings have been shown to grow more rapidly than other associated tree seedlings when competition is present (Kolb and Steiner, 1990). To ensure success when first outplanted, this species has adapted by emphasizing shoot growth over root growth. However, in the absence of herbaceous competition, yellow-poplar grew taller and had higher rates of survival on old field sites in Indiana (Andersen *et al.*, 1989). Kolb and Steiner (1990) discuss how yellow-poplar demonstrates greater rates of resource capture

per unit plant weight than northern red oak. Iverson *et al.* (1999) documents yellow-poplar basal area growth rates reaching an average of 32.7 cm² per year for mature trees.

Sugar maple (*Acer saccharum*)

Sugar maple is a large deciduous tree that is an important species in the hardwood forest. Tennessee lies in the southern extent of the sugar maple range. This species is usually found growing slowly as a very shade tolerant species on moist fertile sites. *Acer saccharum* will grow on a variety of soil types and commonly grows on sites with a pH between 5.5 and 7.5 (Godman *et al.* 1990). This tree is sensitive to flooding and extreme drought during the growing season and can suffer from winter sunscald and ice damage. Sugar maple is most commonly known for its ability to produce maple syrup. It is also a valuable hard maple lumber species when grown in the Lake States. It is classified as a quality timber species and used in the production of various lumber products including cabinetry, flooring, furniture, veneer and pulp. *Acer saccharum* is not highly susceptible to damage by insects and seldom killed by insect attack. Diseases generally do not significantly impact the tree; fungus and canker infections can cause monetary losses but generally do not kill the infected tree. Various species of wildlife feed on the tree and seeds of this species. Leaf feeding insects and herbivores do not tend to cause any significant mortality to sugar maple trees (Godman *et al.* 1990).

It has been demonstrated that sugar maple can be a successful colonizer of newly planted mine sites. Torbert and Burger (1990) showed that 60% survival of sugar maple seedlings was obtained when planted on tracked-in minesoils. Controlling herbaceous competition on mine sites can provide increased survival and growth rates for this species (Salzberg and Burger, 2006). Their ability to successfully accumulate large amounts of biomass in full sun conditions regardless of soil nutrient status suggests that this tree species can be planted for reforestation on mine sites (St.Clair & Lynch, 2005).

Northern red oak (*Quercus rubra*)

Research suggests that red oak does well on unproductive soils, making it a great candidate for planting on the mine site, and it has been planted on various coal mine sites (Andersen *et al.* 1989, Burger *et al.* 2005b, Kolb and Steiner 1990, Tirmenstein 1991). It is a highly valuable timber species and is used in a variety of forest products including flooring and cabinetry. Large mature oaks produce a significant acorn crop every 2-5 years; under ideal conditions one tree may produce as many as 4000 acorns in one season. Wildlife use of this species is significant with animals utilizing many parts of the tree. Browsing by white tailed deer of young seedlings is well documented. Deer, various other mammals, waterfowl species, and several species of game birds consume the acorns produced by this species (Tirmenstein, 1991).

Tree survival on graded and tracked-in minesoils has been shown to be less than 40% in research conducted on 2:1 sandstone/siltstone overburden material in Virginia (Torbert and Burger, 1990). Red oak seedlings growing in these compacted soils were not able to grow in height rapidly and were often out competed by fast growing tall grasses and forbs. A significant amount of energy is used for root production of the seedling allowing it to withstand fire, drought, and repeated browsing events (Sander, 1990). Red oak seedling survival on three less compacted mine sites in western Pennsylvania averaged 65%, 76%, and 82% respectively, although early height growth of these seedlings was generally poor (Hughes, *et al.*, 1992). The Maryland DNR also presented research results indicating good survivability of northern red oak but very poor height growth (Bagley and Shaffer, 1992).

Eastern redbud (*Cercis Canadensis*)

Redbud has been identified as a native legume; however, this species does not appear to have an ability to fix nitrogen (McNiel and Carpenter, 1974). *Cercis canadensis* can tolerate a variety of soil conditions, including well drained soils. It is tolerant of nutrient deficiencies, and performs best at pH above 7.5. Under

optimum growing conditions, redbud may grow 30 to 45 cm in above ground height per year while developing a deep taproot. Although not valued as a timber producing species, it is common as an understory component of the forest system. Shotola *et al.* (1992) described redbud as a component of the woody understory in old-growth forests in Illinois. Carter and Ungar (2002) illustrated its importance as a volunteer species on reclaimed sites in Ohio. Wildlife use of the fruit by cardinals, ring-necked pheasants, rose-breasted grosbeaks, bobwhites, white-tailed deer, and gray squirrels has been documented (Dickson, 1990). This tree is listed as moderately preferred browse for deer (Sullivan, 1994). On sites in western Maryland, research indicated that planting redbud seedlings on lower elevation sites can increase their growth and survival (Bagley and Shaffer, 1992).

Redbud is classified as shade tolerant, but is characteristically less tolerant as age progresses and the species grows well in full sun when young. Redbud can make up a significant understory component on well-drained sites, and can grow well when not under intense competition (Iverson *et al.*, 1999). Its ability to tolerate drought conditions makes this tree well suited to live on the drier portions of newly reclaimed mine sites. This tree has been planted on various mine sites throughout the eastern US (Brothers, 1988). However, interest in establishing this tree on reclaimed sites has declined recently. Research suggests this species is a natural invader of abandoned mine lands in the southern Appalachian coal region (Skousen *et al.* 1994, Holl *et al.* 2001). Redbud is relatively short lived, but it is argued that this species is valued habitat for bird and other wildlife species and can play an important role in the accumulation of organic matter on the soil surface.

Virginia pine (*Pinus virginiana*)

Virginia pine is a commercially important tree species for both pulpwood and lumber production in the southeast. This pine thrives in well drained sandstone and shale soils with pH levels between 3.5 and 7.5 (Fribourg *et al.*, 1981).

Greenhouse research has shown Virginia pine grows best in mine spoil amended to reach a pH of 6.6 (Plass, 1969). Virginia pine is a native pioneer species with high wildlife value, mainly as a seed source. Deer browsing of this species has also been demonstrated. Virginia pine does best in full sun light conditions and thrives in a variety of environmental and soil conditions.

Research has indicated good success of Virginia pine on reclaimed mine sites (Sullivan, 1993). *Pinus virginiana* has demonstrated the ability to successfully compete for resources on abandoned fields and dry sites (Iverson *et al.*, 1999). As a seedling, the tree has thin bark and develops a shallow spreading root system enabling this species to rapidly dominate an area. Virginia pine has demonstrated a strong ability to establish on disturbed sites with bare mineral soil exposed. Results from western Maryland demonstrated poor growth characteristics when in the presence of vegetative competition (Bagley and Shaffer, 1992). This species can tolerate drought conditions better and is generally more successful on drier sites than other associated pine seedlings (Carter and Snow, 1990). Various projects have used Virginia pine for planting in restoration work on strip mines in the southeast. Torbert and Burger (1990) demonstrated that *Pinus virginiana* survived much better on compacted sites than white pine and several other hardwoods (including red oak and sugar maple). Torbert and Burger (1990) suggested that *Pinus virginiana* will outperform white pine on compacted soils.

C. Herbaceous Ground Covers for Reforestation

Designing and seeding a tree-compatible ground cover which will enable the site to have productive tree growth and survival rates has been demonstrated in West Virginia (Probert *et al.* 1992, Burger *et al.* 2005b). The concurrent establishment of both trees and herbaceous species is a desirable way to provide erosion control and contribute to the long-term goal of site reforestation (Vogel and Curtis, 1978). It is believed that the early height growth of many tree species is

often retarded due to competition with weeds (Andrews *et al.*, 1998). Sampling cover as a representation of herbaceous competition and photosynthetically active radiation reaching the seedling leaves can quantify the herbaceous influence upon each seedling. Research in West Virginia reported that seedlings demonstrated much higher survival rates when ground cover was less than 50%, with a rapid decline in survival above 70% cover (Skousen and King, 2004). Above ground vegetation can also influence the soil in a variety of ways including hydrologic modification, soil reinforcement, increased soil porosity, reduced runoff, and recycling of organic matter (Flege, 2000).

Commonly used cool season grass and forb species

The standard method for establishing herbaceous ground cover on mine sites consists of hydroseeding an annual nurse crop with perennial grasses and forbs (Daniels and Zipper, 1988), mixed with a predetermined fertilizer, lime, and mulch rate based on soil tests. This method of seeding helps the mining companies rapidly achieve the amount of ground cover required to retain bond release from OSM and reduces erosion by rooting herbaceous species quickly. Annual rye (*Lolium multiflorum*) is a hardy, fast growing annual grass that has been referred to as ‘throw and grow’ due to its abilities to colonize on poor sites without additional scarification, and grow in a variety of environmental conditions. When seeded with perennial grasses and forbs, this nurse crop can provide protection for the slower to establish perennial species on harsh sites (Skousen and Zipper, 1997). Orchard grass (*Dactylis glomerata*), a cool season grass, and birdsfoot trefoil (*Lotus corniculatus*), a forage legume, are two species commonly seeded in reclamation projects under the current regulations set forth by SMCRA (Hughes *et al.*, 1992). It is argued that seeding with cool season grasses will reduce competition with seedlings for moisture during the summer (Burger and Zipper, 2002). However, these cool season species are usually non-native and most will form dense cover that ensures rapid establishment to meet the minimum vegetation cover requirements mandated by the permit. Research

indicates that a healthy grass component is important to mitigate the impact of minesoil erosion and grasses are the most commonly seeded plants used in revegetation projects (Skousen and Zipper, 1997). The dense mats created by the cool season non-native species can have adverse effects on seedling development. Research in western Maryland showed that yellow poplar, sugar maple, and Virginia pine grew poorly on mine sites in the presence of heavy herbaceous competition (Bagley and Shaffer, 1992). Herbaceous competition has been identified as one of the most significant influences on tree survival and growth (Hughes *et al.* 1992, Ashby 1992). Chemical treatment of ground cover competition in Indiana was necessary to obtain the required tree stocking level for reforestation of mined sites (Andersen *et al.*, 1989). That study broadcast seeded K-31 fescue and red clover, two highly competitive cool-season species that are commonly used to revegetate mine sites. In another study, K-31 tall fescue was shown to be an example of an aggressive cover crop that can adversely impact the growth of certain tree species (Plass, 1968).

Restoration with native warm season grasses

Significant research has been conducted on restoring native warm season grasses in the western United States for grassland and prairie ecosystems and in the east for wildlife and erosion control enhancement. Recent research suggests that planting native warm-season grasses (NWSG) may help facilitate the success of the reforested area (Missouri DNR, 2003). Many native grass species are being seeded in rehabilitation projects because they ameliorate surface conditions, ensure revegetation success, and advance the biodiversity of the site. These species are known to tolerate the harsh conditions that exist on newly reclaimed minesoils. Research also suggests that NWSG and forbs will hold soil better than cool season species due to the extensive development of below ground rooting systems (Barnes and Washburn, 2000). Using these native bunch grasses allows other native woody and herbaceous species to establish and invade on mine sites to create the diverse ecosystem required for the

development of a natural system (Barns and Washburn 2000, Holl 2002, Torbert and Burger 2000). Interest in native warm season grasses has been demonstrated by the many wildlife projects involving these species (Holl 1996, Ledford 2005). Extensive acreages are being seeded with NWSG to promote wildlife use and aid in the overall progression of reclamation of mine sites. Currently, most native grass seed mixes are more expensive than conventional seed. These costs may be offset because native grasses are adapted to nutrient deficient soils thus requiring reduced liming and fertilization rates (Missouri DNR, 2003). Recommended application rates for the establishment of native grasses are significantly less than those of the common cool season species currently used (Burger *et al.*, 2005b) helping to further compensate for high seed costs. Research indicates that including less competitive legume species in the native seed mix can provide the important environmental conditions necessary to benefit trees during the early stages of development (Vogel and Curtis, 1978). The need for additional research on native ground covers is essential to understand the complex site development occurring on reclaimed sites. Seeding less competitive native species during the reclamation process can help expedite succession, native invasion, and biodiversity of reclaimed sites (Holl, 2002).

D. Microsite creation and influence on seedling growth and survival

Factors creating microsites

The reclamation process increases the variability of soils significantly from one location to another. Soil characteristics can vary significantly across a relatively small area (Holl and Cairns, 1994). Soil tests do have limitations on reclaimed sites due to significant variation across restored areas (Berg, 1978). Burg (1987) suggests several methods for sampling, but notes that meaningful guidelines can not be established due to soils varying from uniform to quite heterogeneous. Due to these variations, soil sampling at the site scale can determine nutrient and toxicity thresholds to aid in the recommendation of species for planting and

explanations for plant performance (Jones *et al.*, 2005). Other biotic and abiotic environmental conditions created within small scale microsites are important in the regeneration of vegetation on any disturbed site (Smith *et al.*, 1997). Microsite influence has been demonstrated to be most important in early stages of site development (Jones and del Moral, 2005). Grass survival and establishment has also been linked to the soil moisture and soil temperature conditions associated with microsites (Winkel *et al.*, 1991). For the purpose of this study, microsite refers to the small-scale localized environmental conditions with unique features created by factors such as slope, aspect, the mechanical alteration of the overburden materials, weathering of the minesoil, and development of vegetative competition. Characteristics such as irradiance, soil moisture, soil temperature, soil nutrients, and microtopography can influence microsite conditions. The favorable microsites created on reclaimed surface mines that meet the species-specific requirements for optimum growth and survival and have been termed “safe sites” (Elmarsdottir *et al.* 2003, Harper *et al.* 1965, Jones and del Moral 2005, Winkel *et al.* 1991, Young *et al.* 1990). Evaluating the characteristics of these microsites can allow interpretation of seedling responses (Oswald and Neuenschwander, 1993).

Factors influencing seedling growth and survival

Minesoil spatial variability is related to the mining and reclamation methods used and has been demonstrated to be high at the local scale (<10m) but not at the landscape scale (>500m) (Ammons and Sencindiver, 2000). This evidence suggests that the variation within a site on both the macro- and micro-levels can have a significant impact on the growth and survival of planted species. For this discussion macro-level refers to the overall site scale, while micro-level refers to the environment surrounding each seedling.

Macro-site influence

The overburden is replaced by heavy equipment creating uneven mixing of the shale and sandstone that is used as the growth media on these slopes. This site

variation can have significant influences on the overall health and success of the planted seedlings from one slope to the next. Research has shown that certain tree species do very well when the majority of topsoil substitute consists of sandstone. Burger and Torbert (1999) demonstrated significantly better growth of pine and oak tree seedlings on sandstone derived minesoils. These soils were more similar to the native forest soils than predominantly siltstone minesoils. Water relations were better suited for tree survival and growth within the sandstone dominated soil types for the tree species tested. Casselman *et al.* (2006) also reported that tree growth is often better when weathered sandstone is replaced on the surface rather than shale or siltstone. Sandstone soils tend to be coarse and droughty (Daniels and Zipper, 1999), which may facilitate the establishment of certain tree species. Additional research suggests that there are considerable differences between species in their response to soil type. Red oak seedling survival was significantly better on sites with higher amounts of siltstone than sandstone in Virginia (Zipper, 2005). The highest survival was on the 1:2 sandstone to siltstone (SS:SiS) minesoil mix. The highest growth rates were seen on sites with 1:1 SS:SiS ratio. Higher siltstone water retention levels (Daniels and Zipper, 1999) and greater nitrogen contents (McAfee and Edmonds, 2001) can provide an explanation for why certain species thrive in siltstone spoils. Soil chemical analysis can determine the properties that may influence reforestation efforts on these sites (Showalter *et al.*, 2006).

Micro-site influence

Additionally, the spatial heterogeneity in microenvironments may provide unique niches for trees and promote diversity (Beckage and Clark, 2003). On reclaimed minesoils, microsites have been shown to influence tree seedling survival (Casselman *et al.* 2006, Elmarsdottir *et al.* 2003). Research indicates that the above and below ground environment immediately surrounding a seedling can influence a single plant as well as the composition of the community as a whole (Elmarsdottir *et al.*, 2003). Variations in the fine scale topographic features of young mine soils will occur as the site develops and differential soil horizons

begin to form. The physical properties of the soil have been shown to exhibit the greatest impact on minesoil productivity (Sobek *et al.*, 2000). The formation of rills and gullies caused by precipitation events and weathering of the newly exposed overburden materials creates micro-topographic variation that can influence the growth and survival of outplanted seedlings. Erosion channel monitoring will increase the overall understanding of the complexity and changes that are occurring as the development of a site is underway. Newly reclaimed minesoils have high potential for unstable channelized flow and high erosion rates (Guebert and Gardner, 2001). However, these soils develop a network of macropores that will increase infiltration rates within a few years. The changes due to erosion were analyzed to determine if erosion channels significantly contributed to the overall success of the newly planted seedlings.

The formation of the characteristic depressions and mounds on a newly reclaimed site can result in the accumulation or deficiency of surface moisture dispersed throughout the site (Andersen *et al.*, 1989). On a minesoil in Indiana, seedling survival was affected by differences in plant-available water, and aeration (Andersen *et al.*, 1989). Seedling transpiration rate measurements can provide explanations of the soil water relations occurring on these sites (Ren and Sucoff, 1995). Minesoils characteristic of post-SMCRA reclamation can have a significant impact on soil water storage and the growth of above ground vegetation (Sharma *et al.*, 1995). Soil respiration, temperature, and moisture relations can provide additional insight into the processes occurring within the seedling growing space.

Importance of microsite consideration

When planting tree seedlings on mined sites, special attention should be focused on matching each species to the specific niche that provides the optimal conditions for its successful establishment and growth (Burger and Torbert 1999, Burger and Zipper 2002). These researchers cite factors such as spoil type, aspect, herbaceous vegetation, and wetness as being influential to species

selection, survival, and growth at a planting location. It is essential to understand the interactions between the planting space microsite and the specific seedling requirements to ensure optimal performance (Beckage and Clark, 2003). Properly utilizing these microsites can aid in the rapid recovery of a reclaimed site and development of a productive forest. To accomplish this, it is suggested that trained planting crews carry several different species and plant the right species on the right microsite (Burger and Torbert, 1999).

E. Objectives

This project has two main objectives. The first objective is to evaluate the competitive effects of five different native and non-native herbaceous cover treatments on the growth and survival of the five species of tree seedlings. The working hypothesis underlying this objective is that the native warm season grass treatments will allow for greater growth and survival of certain planted tree seedling species than the non-native ground cover treatments. The null hypothesis is that no significant differences in growth and survival of the seedlings will occur across the different seed mixes.

The second objective of this study is to identify the relationship between the growth and physiological function of five tree species planted as seedlings and the microsite characteristics associated with each seedling. The hypothesis pertaining to this objective is that different microsites on a newly reclaimed minesoil can illicit different responses from each of the species planted. The null hypothesis is that different species of seedlings will respond similarly to the various microsite conditions that exist.

2. Methods

A. Study site description

Physical description

In 2003, the Office of Surface Mining approved surface mining permit number 3132 which allowed UT to work in cooperation with National Coal to design and implement a forest restoration study. The research plots are located within the New River watershed in west Anderson County (36°08' N 84°21' W, elev. 800 m) (Figure 1). Following permit approval, the mining of three separate coal seams on Patterson Mountain began. The Pewee, the Pewee Rider, and the Walnut Mountain coal seams were excavated. Surface mining activities were employed by using surface and auger mining operations to extract the coal. The old coal bench near the top of the mountain was excavated to mine coal deeper into the mountain, a process called remining. An estimated 377,000 tons of coal were excavated from this 50 hectare surface mine during the operation. Work was completed in 2004 and the land was graded and ground cover seeded to meet the SMCRA requirements for bond release. The permit states the post-mining land use as undeveloped land and wildlife habitat. Commonly, this is achieved by planting grasses, legumes, and trees on the reclaimed mine bench. On this site, the permit requires 80% survival of ground cover, and 60% survival of trees (or 375 per hectare) every year following reclamation for bond release. The study site is located on the Photorevised 1979 Duncan Flats, Tenn. USGS 7.5 minute quadrangle map. The nearest TVA rain gauge is located within 10 kilometers of the study area (36°06' N 84°36' W) in the city of Wartburg, TN. The study region historically receives an average of 132 cm of rainfall each year (Tennessee Valley Authority, 2007). In 2005, this TVA rainfall gauge recorded 97.5 cm of precipitation during the year. From planting in April 2005 through the end of the year, the station recorded 83.6 cm of precipitation, which is 63% of normal. In 2006 the site recorded 73.9 cm of precipitation through October,

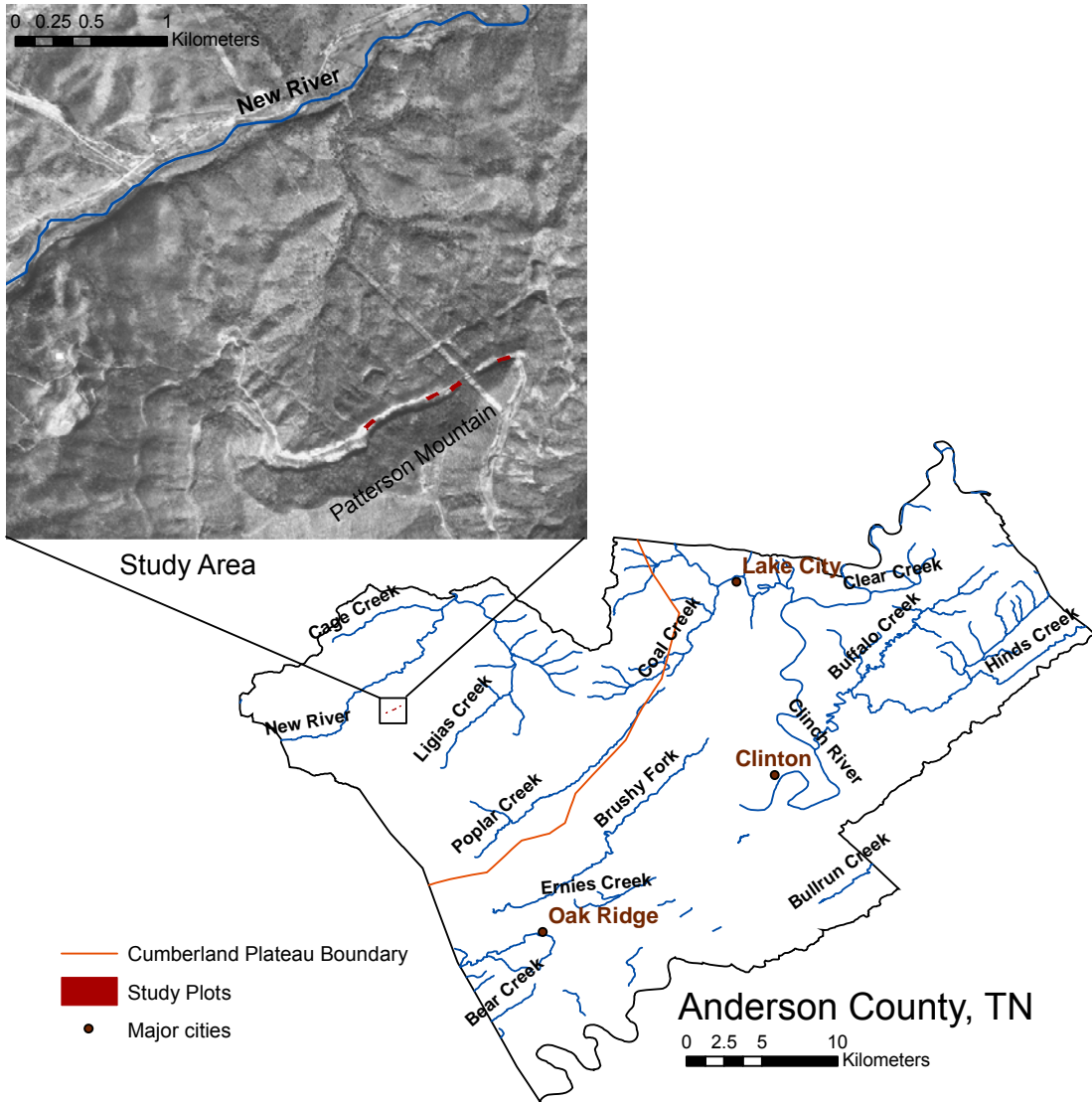


Figure 1: Map of study area within Anderson County, Tennessee. Major cities and major river systems are displayed and labeled. The small image is a digital elevation image of the Patterson Mountain mine study plots located within the New River watershed.

which is 56% of normal. The average annual temperature for the area is between 7°C and 18°C. The average high summer temperature is 28°C and the average low winter temperature is 1°C (Hoare, 2005).

Topography

This site, along the uplands on Patterson Mountain, is characterized by steep slopes with a north-northwest aspect. Percent slope was measured using a Suunto clinometer (Suunto USA, Inc., Carlsbad, CA). Slope aspect was measured on each treatment as an azimuth with a standard compass. The slope of the mined area ranges from 25% to 50% on the steepest part of the study area. The elevation of the study blocks ranges from 762 to 845 meters. The previous mine operator replaced the overburden in order to maintain the original contour of the mountain side. The previous mining operations removed soil and overburden material from the site to expose the coal seams. According to the mine permit, approximately 78% of the proposed site was previously mined and topsoil was not salvaged during these operations. The previous mining operations on Patterson Mountain occurred pre and post-SMCRA. Pre-law mining was done without regulation and no measures were taken to ensure that the contour be replaced to pre-mining conditions. During pre-law mining operations, the overburden was commonly cast over the mountain side and an exposed highwall was left behind. On this site, there is no way to fully restore the original contour and eliminate the highwall due to the past removal of soil and overburden materials. On this site, the engineers used an overburden swell factor of 15% to calculate the quantity of minesoil that will be available for final grading. Even with the swelling of the overburden, the material was not able to cover the exposed highwall. The mine operators used Caterpillar off-highway end dump trucks and bulldozers to reclaim the growth medium substitute by minimal pass grading. This method of reclamation was intended to leave the area rough and loosely compacted as recommended in the new reforestation guidelines (Burger *et al.*, 2005a).

Vegetation

Native vegetation

The forest cover surrounding the study site is classified as Oak-Hickory. The study area is located a southeastern mixed mesophytic hardwood forest as described by Braun (1950). Plant collections compiled within Anderson County, Tennessee show that this area has a significantly diverse native vascular plant collection including more than 832 different species (UT Herbarium, 2006). The surrounding forest above the active mine site was sampled in August of 2004 (Jordan Marshall and Brien Ostby, unpublished data, 2004). The herbaceous species and the woody species sampled above the mine area in the native forest are shown in Table 1 and Table 2. The species found surrounding this surface mine are similar to those found on other abandoned surface mines along the Cumberland Plateau (Stocum, 1980) and within the nearby Royal Blue Wildlife Management Area (Lupardus, 2005).

Table 1: Herbaceous species sampled above the reclaimed mine. Data unpublished; collected by Jordan Marshall and Brien Ostby in conjunction with this project in August 2004.

Common name	Scientific name
dolls eye	<i>Actaea pachypoda</i>
hog peanut	<i>Amphicarpaea bracteata</i>
American spikenard	<i>Aralia racemosa</i>
jack-in-the-pulpit	<i>Arisaema triphyllum</i>
aster	<i>Aster spp</i>
grape fern	<i>Botrychium spp</i>
hay scented fern	<i>Dennstaedtia punctilobula</i>
trefoil	<i>Desmodium spp</i>
bed straw	<i>Galium spp</i>
touch me not	<i>Impatiens spp</i>
whorled loosestrife	<i>Lysimachia quadrifolia</i>
indian cucumber root	<i>Medeola virginiana</i>
christmas fern	<i>Polystichum acrostichoides</i>
gall of the earth	<i>Prenanthes trifoliolata</i>
tall meadow rue	<i>Thalictrum pubescens</i>
stinging nettle	<i>Urtica dioica</i>
perfoliate bellwort	<i>Uvularia perfoliata</i>
violet	<i>Viola spp</i>

Table 2: Woody species sampled above the reclaimed mine site. Data unpublished; collected by Jordan Marshall and Brien Ostby in conjunction with this project in August 2004.

Common name	Scientific name
red maple	<i>Acer rubrum</i>
sugar maple	<i>Acer saccharum</i>
yellow buckeye	<i>Aesculus flava</i>
devils walking stick	<i>Aralia spinosa</i>
sweet birch	<i>Betula lenta</i>
mockernut hickory	<i>Carya alba</i>
bitternut hickory	<i>Carya cordiformis</i>
pignut hickory	<i>Carya glabra</i>
dogwood	<i>Cornus florida</i>
hawthorne	<i>Crataegus oxacantha</i>
green ash	<i>Fraxinus pennsylvanica</i>
yellow-poplar	<i>Liriodendron tulipifera</i>
cucumber magnolia	<i>Magnolia acuminata</i>
blackgum	<i>Nyssa sylvatica</i>
sourwood	<i>Oxydendrum arboreum</i>
Virginia creeper	<i>Parthenocissus quinquefolia</i>
black cherry	<i>Prunus serotina</i>
chestnut oak	<i>Quercus montana</i>
northern red oak	<i>Quercus rubra</i>
blackberry	<i>Rubus alleghaniensis</i>
sassafras	<i>Sassafras albidum</i>
basswood	<i>Tilia americana</i>
highbush blueberry	<i>Vaccinium elliotii</i>
mapleleaf viburnum	<i>Viburnum acerifolium</i>

Planted vegetation

National Coal seeded the site with an herbaceous mix compatible with the proposed post-mining land use prior to the study area designation. The ground cover seed mix applied consisted of four grass and legume species which were: orchard grass (*Dactylis glomerata*) (16.8 kg ha⁻¹), red clover (*Trifolium pratense*) (3.4 kg ha⁻¹), kobe lespedeza (*Lespedeza striate* var. Kobe) (12.3 kg ha⁻¹) and birdsfoot trefoil (*Lotus corniculatus*) (6.7 kg ha⁻¹). This was applied by a truck mounted hopper loaded hydroseeder on all the slopes within the mined area. A temporary cover crop was also used on material during the excavation process. This cover included winter wheat (*Triticum spp*) (45 kg ha⁻¹), annual rye (*Lolium multiflorum*) (45 kg ha⁻¹), and foxtail millet (*Setaria italica*) (16.8 kg ha⁻¹) which were also hydroseeded by truck mounted equipment. Bicolor lespedeza (*Lespedeza bicolor*) was also present on the post-mining revegetation species list seeded at a rate of 9 kg ha⁻¹. The hydroseed mixture included Liquid Lime Plus (Plant-Wise Biostimulant Company. Louisville, Ky) applied at the recommended liming rates that were determined based on soil tests of the area. However, exact application rates were unknown for this site. Kentucky Green Fertilizer (Ag/Gro Fertilizer Company. Winchester, KY) with an N-P-K rate of 15-15-15 was also applied in the seed mix. Fiber mulch, which acts as a tackifier and adds organic matter to the seeded area, was applied at 1680 kg ha⁻¹.

Soil and geologic characteristics

Soils of the area near the mining operation are texturally classified as shaly silty clay loams. The current minesoil classification system of the central Appalachian coal region maps the post mining soils as Typic Udorthents (Ammons and Sencindiver 1990, Galbraith 2004, Haering *et al.* 2005). This area had previously been mined before the enactment of SMCRA in 1977. The current mining permit allows for the overburden to be composed of a blend of topsoil, woody vegetation and weathered sandstone or shale as plant growth medium. The current minesoil is derived from overburden that has been mixed by blasting and

transportation by large excavation equipment. In some areas nearly one hundred feet of overburden exist above a valuable seam of coal. Shale, a sedimentary rock, comprises a significant amount of the overburden layers. Sandstone is the other major type of sedimentary rock that exists. Brown sandstone is mainly seen closest to the surface and makes up layers as thick as 10 meters. However, darker shale is the most common rock layer surrounding the three main coal seams being excavated on Patterson Mountain. Surface sandstones were not readily available to create a site that would not meet the criteria recommended by the Forestry Reclamation Approach (Burger *et al.*, 2005a). Reclamation of the Patterson Mountain site called for replacement of the overburden as the topsoil substitute, which in large part is shale. This led to a patchy poorly mixed distribution of shale with a limited sandstone component.

B. Experimental design

Block and treatment plot layout

Four rectangular blocks were installed along the overburden slopes which appeared to have the least amount of grading. These blocks were delineated above a service road and measured 64 meters by 19.8 meters. The area of each block is 0.13 hectares and was divided into five rectangular treatment plots of equal size (Figure 2). To create a randomized complete block design, each of the five treatments was randomly assigned to a plot within each of the four blocks (Table 3). A buffer column was established between each treatment plot where no seedlings were planted. This allowed for a 3.6 meter space between seedlings in adjacent treatment plots.

In an effort to help reduce soil erosion as well as ensure overall soil stabilization, National Coal hydroseeded the entire site using the seed, fiber mulch, fertilizer, and lime slurry mix before plot designation. Much of this work had to be done again the following spring after a lack of emerging vegetation suggested that heavy rains may have washed away seed before germination. Prior to the

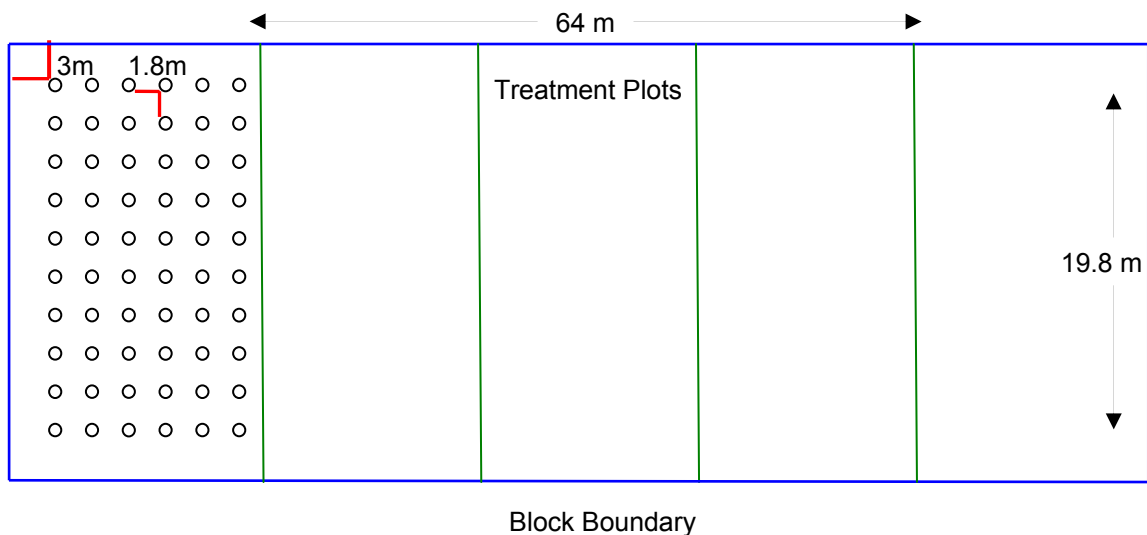


Figure 2: Diagram representing a single block and layout of planting locations within one of the five representative treatment plots. Each ground cover prescription was applied to the entire area within the treatment plot boundary. The green treatment line denotes a skipped column of seedlings allowing for a 3.7 meter distance between seedlings within two treatment plots. Treatment plots were seeded to the edge of the block boundary, 3m beyond the last seedling. o – represents a planted seedling, which are spaced 1.8m apart.

Table 3: Ground cover treatment assignment by block. Treatment one is the mesic native warm season mix, treatment two is the native warm season grass mix, treatment three is annual rye/birdsfoot trefoil, treatment four is perennial rye/red fescue, and treatment five is the control treatment plot. Refer to table 4 for a description of species used in each seed mix.

	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5
Block 1	4	3	2	1	5
Block 2	3	1	5	2	4
Block 3	5	2	3	1	4
Block 4	1	2	4	5	3

second seeding by the mine operator, the proposed study blocks were marked out and the crews were instructed to not seed within marked boundaries. This was successful, and very little overspray was observed within the marked plots. However, soon after the seedlings were planted, some of the annual rye grass they had seeded the previous year began to emerge. This created a need to eliminate the actively growing annual cover crop and any established vegetation (Casselmann *et al.* 2006, Davis *et al.* 1999). To ensure that the subsequent herbicide treatment would be successful, the waist high annual cover crop was cut using a Stihl FS-36 trimmer (Stihl Inc. Virginia Beach, VA) with 0.20 cm diameter nylon line. The annual rye grass was removed to within 3 cm of the ground surface within the study block boundaries including the 3 meter wide buffer strip around the entire block. Soon after the trimming, Roundup PRO (Scotts Miracle-Gro Co. Marysville, OH), a glyphosate herbicide, was applied based on the manufacturer's specifications. Application was accomplished using a 7.6 liter Roundup Herbicide Sprayer (Model RHS-2). This is a pump type hand held sprayer with an extension and fan nozzle. The herbicide was mixed in 5% solution as instructed in the manufacturers mixing instruction manual, 50.6 ml of herbicide per liter of water. It took approximately 11.4 liters of mixed herbicide to treat each one of the four blocks, totaling 45.6 liters of mix for the entire 0.5 hectare study area. A one meter section of perforated 10cm drainage pipe was cut and used to reduce the amount of overspray reaching the small seedlings with foliage near the ground level. The herbicide application was completed on June 3 and 4, 2005 in winds less than 3.2 kilometers per hour with sunny skies.

Tree seedling planting design

Twelve-hundred seedlings in total were planted; each was randomly assigned to a planting spot using a random number table. Within each plot, 300 seedlings were planted in rows on 1.8 by 1.8 meter spacing, this equates to an average of 3086 trees per hectare.

Five tree species, native to the eastern hardwood forest surrounding the mine site were planted on April 15, 2005. Yellow-poplar (*Liriodendron tulipifera* L.), sugar maple (*Acer saccharum* Marsh.), northern red oak (*Quercus rubra* L.), eastern redbud (*Cercis canadensis* L.), and Virginia pine (*Pinus virginiana* Mill.) were planted.

Herbaceous treatment design

For this study, I selected five different ground cover mixes for seeding into treatment plots (Table 4). Seeding was done by hand on June 7 and 8, 2005. The first mix, “Mesic prairie mix” (Shooting Star Nursery. Georgetown, KY), contains four native warm season species as well as 19 wildflower/forb species. This treatment mix was seeded at the recommend rate of 9 kg ha⁻¹ of pure live seed (PLS). Shooting Star Nursery mixed together the second seed treatment that I designed based on warm season grasses native to the area that are short in stature at maturity. This treatment was seeded at 9 kg ha⁻¹ also. Treatment three had two species: birdsfoot trefoil was seeded at 4.5 kg ha⁻¹ (Burger and Zipper, 2002) and the annual rye seeded at 16.8 kg ha⁻¹ (Burger and Torbert, 1999). Treatment four also had two species, perennial rye and creeping red fescue both seeded at 11.2 kg ha⁻¹ (Burger and Zipper 2002, Probert *et al.* 1992). Treatment five was a control, with no additional seeding done. Each treatment plot was seeded by hand or broadcast spreader. Treatment one and two, the mesic and warm season mixes, were spread using a five gallon bucket by hand. The hand broadcast method was employed for mix one and two because the seed was large and did not fit through the broadcast spreader. Mixes three and four were spread using the Scotts Handy Green broadcast spreader (Scotts Miracle-Gro Company. Marysville, Ohio). A three meter buffer around the treatment plots was seeded to reduce edge effect. All seeding was done in a similar pattern, across the slope between tree rows. Seed was spread one treatment plot at a time as dictated by the seeding pattern.

Table 4: Species list for each of the ground cover treatments. Common names (left column) and scientific names (right column) presented. * - indicates species that were seeded in both treatments one and two.

<i>Treatment 1</i>	
Mesic Prairie mix:	
Little Bluestem	Schizachyrium scoparium
Big Bluestem	Andropogon gerardii
Indiangrass	Sorghastrum nutans
Switchgrass	Panicum virgatum
Forb component:	
New England Aster	Aster novae-angliae
Tickseed Sunflower	Bidens aristosa
Partridge Pea	Cassia fasciculata
Lanceleaf Coreopsis	Coreopsis lanceolata
Illinois Bundleflower	Desmanthus illinoensis
Purple Coneflower	Echinacea purpurea
Ox-eye Sunflower	Heliopsis helianthoides
Downy Sunflower	Helianthus mollis
Prairie Blazingstar	Liatris pycnostachya
Spiked Blazingstar	Liatris spicata
Wild Bergamot	Monarda fistulosa
Smooth Beardtongue	Penstemon digitalis
White Prairie Clover	Petalostemum candidum
Purple Prairie Clover	Petalostemum purpureum
Prairie Yellow Coneflower	Ratibida pinnata
Black-eyed Susan	Rudbeckia hirta
Blue Sage	Salvia azurea
Rigid Goldenrod	Solidago rigida
Ironweed	Vernonia fasciculata
<i>Treatment 2</i>	
Warm season grass mix:	
Little Bluestem	Schizachyrium scoparium
Side Oats Grama	Bouteloua curtipendula
Eastern Gamagrass	Tripsacum dactyloides
<i>Treatment 3</i>	
Birdsfoot trefoil	Lotus corniculatus
Annual Rye	Lolium multiflorum
<i>Treatment 4</i>	
Perennial rye	Lolium perenne
Fescue, creeping red	Festuca rubra

C. Evaluation of seedling and treatment interactions

Soil analysis

Soil samples were taken on the site in July 2005 and July 2006. The soil was collected from five random spots per treatment plot, then mixed in a 5 gallon bucket and bagged in a Ziploc bag. These soil samples were crushed, sifted through a 2mm screen, and air dried in the laboratory in opened containers for three to five days to remove any soil moisture before analysis in the lab.

A fizz test rating was given to each of the samples to determine the neutralization potential of each sample. A few drops of 10% HCl solution was added to 5.0 grams of soil (Sobek *et al.*, 1978). The degree of reaction was observed and recorded, according to a four-tiered system where the reaction was judged to be none (0), slight (1), moderate (2), or strong (3).

Each of the 40 soil samples were analyzed for pH in a 1:1 water solution. This was accomplished by weighing 5.0 grams of soil and mixing with water until 10.0 grams of total solution were present. This mixture was allowed to stand for 30 minutes. Each mixture was then stirred and the electrode of an analogue soil pH meter, (Model 301, Orion Research, Boston, MA) was used to determine pH. At the beginning of sampling, and every 15 samples, the pH meter was calibrated using pH 4.0 and 7.0 buffer solutions to ensure accuracy (Sobek *et al.*, 1978). Potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), manganese (Mn), Zinc (Zn), and copper (Cu) levels were determined using the Mehlich 1 extractant. Mehlich extractant was made following the procedures published by Mehlich (1953) by mixing concentrated HCl, concentrated H₂SO₄, and deionized water. Then 5.00 grams of the screened soil and 50.0 ml of Mehlich 1 solution were mixed in a plastic container and placed on a reciprocating shaker for 30 minutes at 210 revolutions per minute (rpm). Solutions were then passed through Whatman No.1 filter paper and drained into test tubes. Samples were analyzed using a SPECTRO CIROS ICP-AES

(inductively coupled plasma-atomic emission spectroscopy) (SPECTRO Analytical Instruments, Kleve, Germany).

Seedling qualitative observations

Seasonal mortality was recorded on a bi-monthly basis. Throughout the season, each tree was observed and characteristics evaluated. Seedling survival, time of leaf flushing, evidence of browsing, and overall plant conditions were monitored throughout the 2005 and 2006 seasons. The soil around each seedling was observed for erosion channel development and other significant structural movement and characteristics recorded. This information was used to help determine the biotic as well as abiotic causes of mortality of each individual. Any seedling that did not flush or show any survival during the first season was determined to be killed by transplant stress. Seedlings were determined dead by using the scratch test to test for green inner bark (McCurry, 2006).

Seedling size

Root collar diameter (RCD) was measured with to the nearest mm a digital caliper on every seedling in May of the first growing season. Height of the seedling to the tallest main terminal bud was measured to the nearest cm with a meter stick. Measurements were recorded again in December 2005 and November 2006. Growth rates were determined by subtracting the end of season RCD and height from the beginning of season RCD and height for each seedling.

Seedling transpiration

Between 7:45 am and 10 am July 25 through July 29 seedling transpiration rates were measured in 2005, and again in 2006. Readings were taken using a Li-Cor LI-1600 Steady State Porometer with fixed aperture head (Li-Cor Biosciences Inc. Lincoln, NE). This machine computes stomatal conductance from a transpiring leaf on the plant, and displays leaf transpiration rate. Within each

treatment plot, three trees per species were selected using a random number chart. Across each block, 15 seedlings of a single species were sampled each day. A total of 60 seedlings of a single species were measured in a single day. To reduce sampling variability, each species of seedling was sampled per day over the five day sampling period. The humidity was adjusted at the beginning of the sampling period. While moving between blocks, the machine was left on and humidity reading adjusted for accuracy before the sampling of the next block began. The humidity remained relatively constant between treatment plots during the sampling period. At each seedling, the Porometer head was attached onto the uppermost fully expanded leaf. The narrow aperture sensor head was attached to the cuvette for sampling of the leaves. Each reading was obtained in less than three minutes allowing for a total sample of 300 seedlings over the week long sampling period.

Herbaceous cover within treatments

In August of the 2006 growing season, cover data were collected to determine the herbaceous composition of the treatment plots. The quadrat sampling method was employed to collect an accurate estimation of the percent cover within each of the plots (Elzinga, Salzer, and Willoughby; 1998). Two tapes measuring 18 meters were stretched diagonally within each treatment plot. Along each tape at 4 and 12 meters, 0.25 meter square clipping frames were used to visually estimate percent cover of the herbaceous vegetation. The total sample area was 1 square meter per treatment plot. This resulted in 1.5% sample of each treatment plot. Barbour *et al.* (1987) have demonstrated that sampling as little as one percent of the community can yield an accurate estimation of cover. The herbaceous vegetation was divided into four categories; white sweet clover (*Melilotus officinalis*), other forbs, grasses, and bare ground. For each category cover was estimated to the nearest five percentage points. Biomass collection on the site was done by clipping all vegetation within the 0.25 square meter clipping frames. Samples were collected in August to correspond

with the maximum standing crop (Fyles *et al.*, 1985). The same four clipping frames placed at 4 and 12 meters along each diagonal treatment plot transect were used to sample biomass in 2006 after cover was estimated. All the vegetation within the clipping frames was removed to within 2 cm of the ground surface (Andersen *et al.*, 1989), sealed in plastic bags, and returned to the lab where it was weighed, dried and reweighed to determine the dry biomass of each sample. The samples were placed in DKN900 Yamato Constant Temperature Oven (Yamato Scientific Co. Tokyo, Japan) and dried at 55° Celsius for a minimum of 48 hours. Sample weight was recorded to the nearest 0.1 gram on a Mettler Toledo PL3001-S digital scale (Mettler-Toledo, Inc. Columbus, OH).

D. Evaluation of seedling and microsite interactions

Herbaceous percent cover

In May 2005 and August 2006, percent cover of the herbaceous vegetation was measured and recorded around each of the 1200 seedlings. A circular area around each seedling with a radius of 0.5 meters was inspected, and herbaceous cover class recorded using the 25% sample scale developed by Braun-Blanquet (1932) (Table 5).

Photosynthetically active radiation

Photosynthetically active radiation (PAR) was measured in August 2006 during partly cloudy conditions. The PAR measurement was made 5 cm, 25 cm, and 1 meter above the ground level around solar noon (± 1 hr) using a Decagon Accupar Ceptometer (Decagon Devices. Pullman, WA). The same seedlings sampled for transpiration in 2006 were selected for PAR measurements. Each measurement was made within 6 cm of the seedling stem along the south side of the plant to avoid shading by the seedling leaves. A second stand-alone unit was set in full exposure, recording measurements every 30 seconds during the sample time, in order to calculate percent full PAR (Barwatt, 2004).

Table 5: Scale for visually estimating herbaceous percent cover classes around each seedling. This is based on the Braun-Blanquet (1932) cover classes for small sample areas.

Cover classes	0	1	2	3	4
Percent Cover	0-1%	1-25%	25-50%	50-75%	75-100%

Soil respiration

Soil respiration measures the CO₂ efflux rate of the soil in a dynamic chamber on the soil surface. This was done using the Li-Cor LI-6400 Portable Photosynthesis System (Li-Cor Biosciences Inc. Lincoln, NE) with soil respiration chamber attachment (IRGA). The respiration measurements were taken from July 25 to July 29, 2005 and 2006 from 10 am to 2 pm. Studies in Missouri have shown that the highest mean rate of CO₂ efflux occurs in July (Ponder, 2005). Sugar maple was the tree species chosen for measurement of respiration due to the survival and growth performance exhibited early in the first season of outplanting. As a result of time restrictions, it was only possible to sample respiration around this one species. However, this sampling scheme allowed for 20 readings per day during the week long sampling period. Each day, one tree per treatment plot was chosen from a random number chart. The block sampling scheme was also generated by a random number chart to reduce the effects caused by systematic sampling. The random generation of sample points meant that the same seedling would not necessarily be measured in both years. Measurements were taken by placing the chamber edge 6 cm from the stem of the maple seedlings. An area devoid of vegetation was selected to insert the chamber 1 cm into the soil to ensure an airtight seal was made. After placement, the chamber was left in place for 3 minutes before sampling started. This allowed the chamber CO₂ level to stabilize for accurate readings to be taken. At each seedling, three cycles were run and all results recorded.

Soil moisture

Percent soil moisture was collected using the Trase Mini soil moisture probe with TDR Technology (Soilmoisture Equipment Corp. Santa Barbara, CA) and Palm Pilot IIIc for display of probe data. Two 15 cm long wave guides were inserted into the minesoil to take moisture readings at the same seedlings, date, and time as the respiration measurements. The waveguides were inserted by hand within 0.6 meters of each maple seedling.

Soil temperature

Soil temperature can vary significantly from soil surface to greater depths. For this reason, I chose to record three separate soil temperature readings. Most seedling roots occur horizontally below the soil surface. Research on tree seedlings in various soil conditions has shown that most roots occur within the top 20 to 30 cm of the soil surface (Rindels, 1992). The temperature readings were taken along with the soil respiration and moisture measurements in 2005 and 2006. The first reading was collected at the soil surface using a Fisherbrand Traceable digital thermometer (Fisher Scientific Company, L.L.C. Pittsburg, PA) with an accuracy of $\pm 1^{\circ}$ C. The second reading was collected using the IRGA thermometer (Li-Cor Biosciences Inc. Lincoln, NE) at a depth of 6cm with an accuracy of $\pm 0.25^{\circ}$ C. The third reading was taken using a REOTEMP soil thermometer (REOTEMP Instruments Corp. San Diego, CA) at a depth of 15cm. REOTEMP states that the thermometer is accurate within $\pm 1\%$ and takes 40 seconds to stabilize once inserted into the ground. All three thermometers were placed in the soil for 3 minutes before readings were taken. These readings were taken during the time it took to record soil respiration, which took on average, 10 minutes per seedling.

E. Statistical analysis

The SAS program, version 9.1 (SAS Institute Inc. Cary, NC), and SPSS, version 14.0 (SPSS Inc. Chicago, IL), were used for all statistical analysis. Regression and ANOVA models were considered significant at an $\alpha \leq 0.05$. Significant differences between treatments and microsites were separated using Duncan's multiple range tests. This was used to determine the differences between group means for all significant relationships (StatSoft, Inc., 2006).

Analysis of treatment interactions

Analysis of variance (ANOVA) was used to test for treatment differences in survival, diameter, and diameter growth, total height, height growth, transpiration rate, soil respiration rate, herbaceous cover class, herbaceous percent cover, and biomass weight. When significant interactions were present, a main effect ANOVA was separately conducted.

Multiple ANOVA (MANOVA) was used to test for treatment differences in the soil chemical variables sampled including soil pH, potassium, phosphorus, calcium, magnesium, sodium, iron, manganese, Zinc, and copper. An independent samples t-test was used to test for soil chemical differences between year one and year two of the study period.

Survival of each species by initial root collar diameter at time of planting was also analyzed using ANOVA.

Analysis of microsite interactions

Mean transpiration rate of each tree species was analyzed separately, using ANOVA, due to the sampling scheme necessary to collect all the data.

Comparisons were made only within a single day, not across species or years. Multiple regression analysis was used to identify any relationship between root collar diameter and tree height growth with microsite properties (Andrews et al. 1998). Regression analysis was also conducted to determine whether soil

respiration rate was dependent upon treatments, herbaceous cover class, seedling growth (root collar diameter and height), or seedling transpiration rate. Multiple regression analysis using all possible regressions and the backwards selection process was utilized to establish the set of independent variables explaining a proportion of the variance in the dependent variable (soil respiration). Significant regressions were tested at $\alpha \leq 0.05$, which was used to establish the relative predictive importance of the independent variables (Kaye and Hart, 1998). Soil temperature and moisture were originally used as covariates in the regression analysis, but determined insignificant in the model and removed to determine the final regression model.

3. Results

A. General site differences

Soil chemistry

Soil chemical composition did not significantly differ between treatments, but did differ between years for Cu ($p < 0.000$), Fe ($p = 0.010$), Na ($p = 0.009$), and Zn ($p < 0.000$) (Table 6). The pH of the study area did not significantly change during the study period ($p = 0.637$) which averaged 7.6 (SE = 0.05). Although not all differences are significant, the general trend shows that concentrations increased from year one to year two for all chemical properties analyzed.

Soil moisture and temperature

There were no significant surface, 6cm, or 15cm soil temperature differences between treatments, or herbaceous cover classes. Soil temperatures significantly differed between year for surface readings ($p < 0.000$) and 6cm readings ($p < 0.000$), but not for 15cm readings ($p = 0.095$) (Table 7). There were no significant differences in soil moisture between treatment, cover class, or year.

Survival of planted seedlings

The ANOVA results indicate that there were several statistically significant survival relationships present at the end of this study (Table 8). There were no significant differences in overall species survival between treatments, but there were survival differences between species as well as herbaceous cover classes. There was also a treatment by cover and species by cover interaction effect present for seedling survival rates. Survival between blocks, used as a random factor in the ANOVA model, was also significantly different.

Table 6: Means and standard error of soil chemical properties (mg kg^{-1}) and pH during both first and second growing seasons. The four elements highlighted had significantly different concentrations from 2005 to 2006.

	2005		2006	
	Mean (mg kg^{-1})	SE	Mean (mg kg^{-1})	SE
Ca	3914.15	240.45	4012.05	228.53
Cu	6.488	0.14	8.1835	0.22
Fe	888.15	58.03	1091.3	47.65
K	214.585	6.79	217.22	4.98
Mg	618.23	35.11	676.425	33.25
Mn	163.025	8.97	183.17	7.77
Na	33.393	0.89	63.874	10.99
P	216.425	7.40	219.115	6.13
Zn	10.462	0.47	20.395	2.40
pH	7.6	0.06	7.7	0.04

Table 7: Mean soil moisture (%), soil temperature at the surface ($^{\circ}\text{C}$), 6 cm ($^{\circ}\text{C}$), and 15 cm ($^{\circ}\text{C}$) during both years of the study. Standard error (SE) and ranges of data presented.

Soil attribute	Mean 05	SE	Range	Mean 06	SE	Range
Moisture (%)	13.2	0.3	5.8 - 24.7	13.6	0.5	5.5 - 26.0
Surface $^{\circ}\text{C}$	34.6	0.6	26.1 - 50.0	29.6	0.6	20.0 - 44.0
6 cm $^{\circ}\text{C}$	27.8	0.3	23.8 - 36.7	24.8	0.3	19.6 - 31.1
15 cm $^{\circ}\text{C}$	25.6	0.2	20.6 - 30.0	25.2	0.2	20.6 - 30.0

Table 8: Summarized ANOVA table for survival. All main effects and two-way interactions were conducted. Significant differences are present at $\alpha = 0.05$ and highlighted.

Independent variable	Dep variable	df	F	p
Trt	Survival	4, 1136	1.397	0.233
Spp	Survival	4, 1136	44.372	< 0.000
Cover	Survival	4, 1136	7.778	< 0.000
Block	Survival	3, 1136	9.276	< 0.000
Trt*spp	Survival	16, 1136	1.702	0.082
Trt*Cover	Survival	16, 1136	1.788	0.041
Spp*Cover	Survival	16, 1136	1.529	0.028

Survival of each species

The five species of trees planted for this project have significantly different survival overall. Sugar maple seedlings survived significantly better through the first two seasons of growth than did any of the other species (Figure 3). The lowest survival rate was yellow-poplar at 24%; this was significantly lower than eastern redbud's 47% survival. Overall, 80% was the best survival, demonstrated by sugar maple seedlings; this was significantly higher than both northern red oak (59%) and Virginia pine (58%) survival rates during the two years of this study.

Survival by initial size

Seedling survival at the end of the second growing season was related to seedling root collar diameter at time of planting for sugar maple ($p = 0.050$), northern red oak ($p = 0.007$), eastern redbud ($p = 0.050$), and Virginia pine ($p < 0.000$) (Figure 4). Yellow-poplar seedlings with larger RCD at planting did not have significantly better survival in this study ($p = 0.394$).

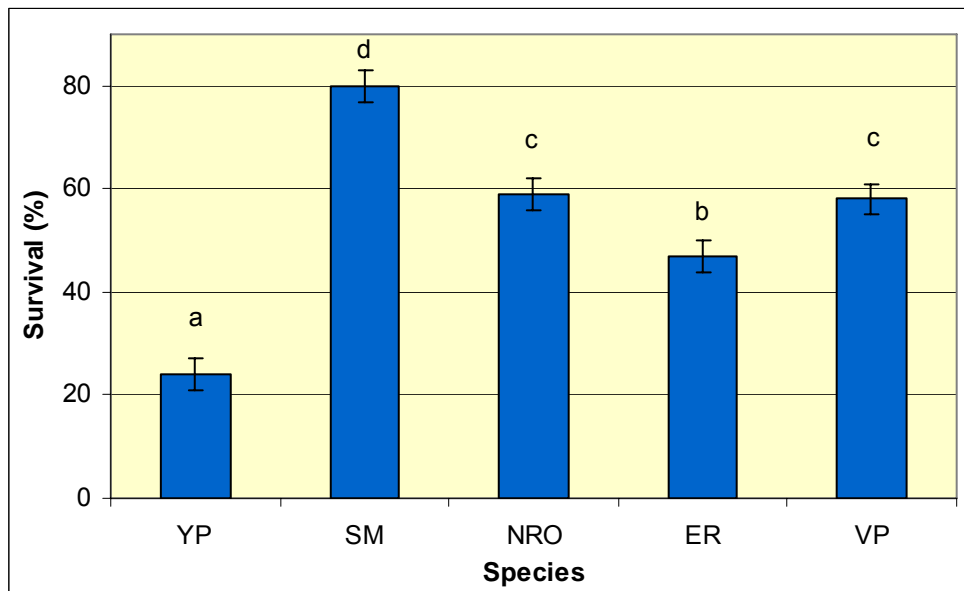


Figure 3: Percent survival of the five species of seedlings planted in this project over two full growing seasons. Bars represent standard error. (Means with the same letters are not significantly different at $\alpha = 0.05$ using Duncan's technique.) YP = yellow-poplar, SM = sugar maple, NRO = northern red oak, ER = eastern redbud, VP = Virginia pine.

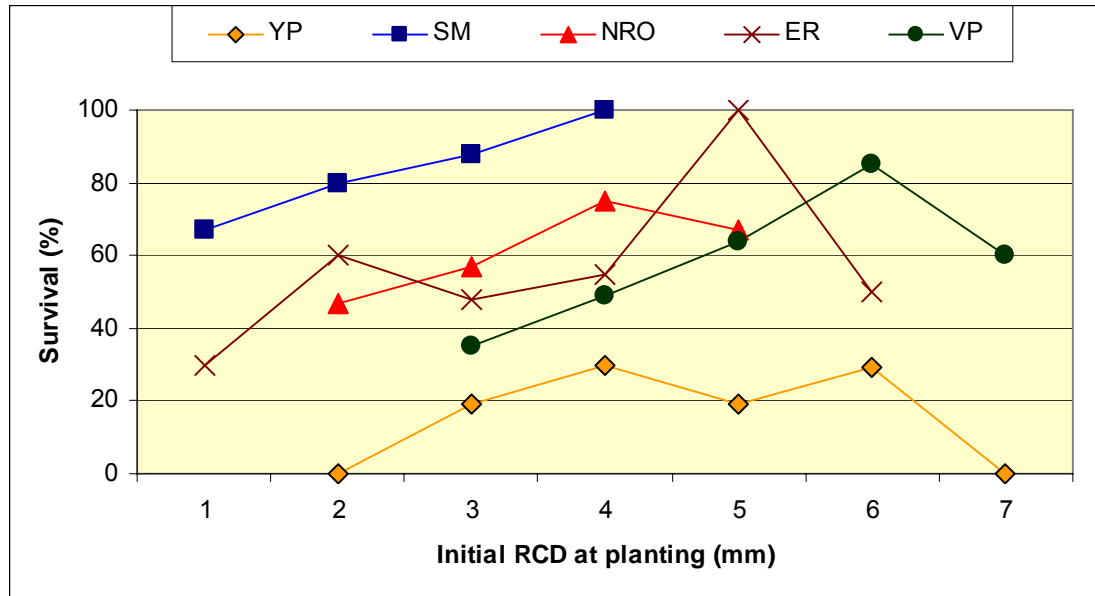


Figure 4: Percent survival at the end of the two year study of each species by RCD at time of planting. YP = yellow-poplar, SM =sugar maple, NRO = northern red oak, ER = eastern redbud, VP = Virginia pine.

Causes of seedling mortality

In this study, species mortality due to planting stress, winter stress, browsing, and erosion channel development was noted (Figure 5). Initial planting stress on the bare root seedlings accounted for an average of 12% of the overall mortality. Whitetail deer (*Odocoileus virginianus*) browsing accounted for just over 2% of the overall mortality. Virginia pine had the highest mortality related to browsing totaling 6%. Field observations determined that 85 Virginia pine seedlings were browsed during the second growing season. This was a significant increase ($p = 0.001$) from the 27 browsed during the first season. Virginia pine mortality due to browsing was greater than the other four species. In contrast, during the first year, red oak (65 browsed) and redbud (58 browsed) were browsed significantly less than during the second year (2 browsed, $p = 0.002$ and 8 browsed, $p = 0.007$, respectively). Overall, erosion channel development helped to explain an average of 8% of the mortality of the seedlings that were planted. Sugar maple

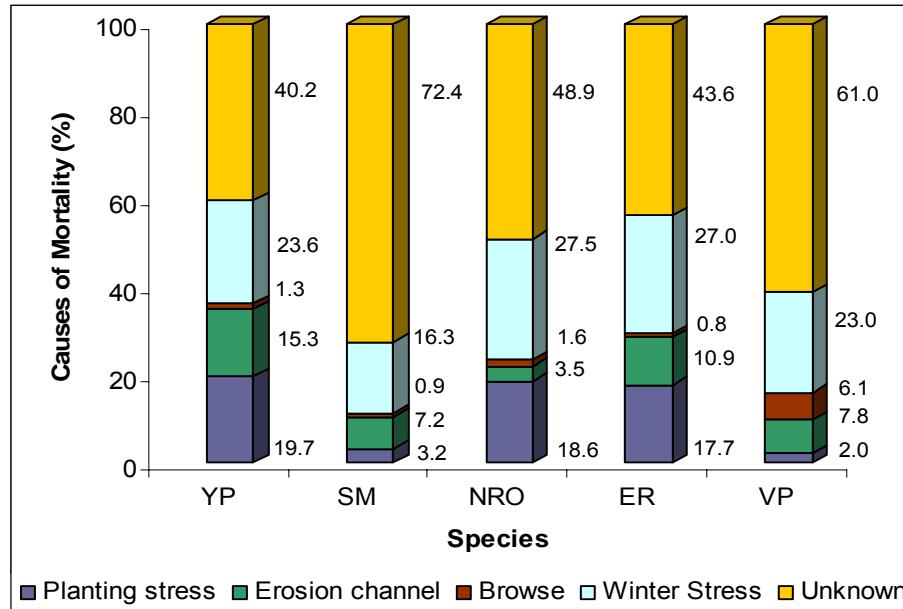


Figure 5: Percentage of seedling mortality explained by four main experimental observations including planting stress, erosion channel development, browsing, and winter mortality. Values are percent mortality. YP = yellow-poplar, SM = sugar maple, NRO = northern red oak, ER = eastern redbud, VP = Virginia pine.

and Virginia pine showed the least mortality from transplanting. The largest single factor in the death of the seedlings was winter mortality rate, which averaged 23.5% of the mortality overall. However, the cause of more than half of the total mortality could not be identified for sugar maple and Virginia pine seedlings.

B. Treatment and seedling interactions

Cover differences between treatments

The herbaceous cover class surrounding each seedling was significantly different between treatments during the second growing season ($p < 0.000$), but not the first ($p = 0.074$). Seedlings planted within the birdsfoot trefoil/annual rye seed mix (treatment three) were surrounded by significantly higher amounts of cover than in the other mixes (Figure 6). The control plot also had a higher average cover class than the NWSG/forb mix and the rye/fescue mix.

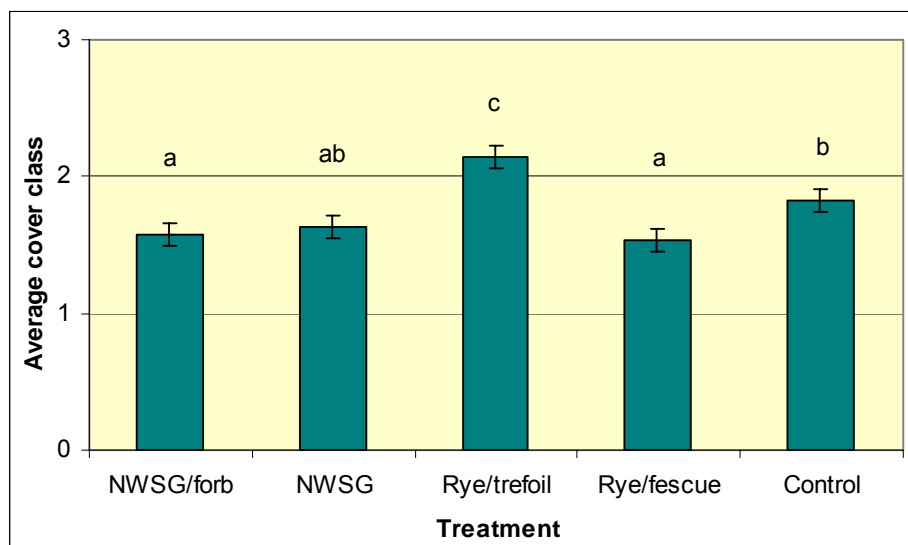


Figure 6: Mean herbaceous cover class differences by treatment for all seedlings in 2006. Herbaceous cover class zero is 0-1% cover, class one is 1-25%, class two is 25-50%, class three is 50-75% and class four is 75-100%. Bars represent standard error. (Means with the same letters are not significantly different at $\alpha = 0.05$ using Duncan's technique.)

Herbaceous differences between treatments

Herbaceous biomass did not significantly differ between treatments during the sampling period (Table 9). Treatment three had the highest mean above ground biomass at 967 kg ha^{-1} (SE = 158.3). Treatment four, the lowest mean biomass was 681.8 kg ha^{-1} (SE = 158.5). The average dry biomass production of the sample area was 879 kg ha^{-1} ranging from 4 kg ha^{-1} to 3290 kg ha^{-1} .

The overall percent herbaceous cover determined by quadrat sampling in August 2006 indicated an average of 70% cover on the site. The quadrat samples indicated that the forb percent cover differed between treatments (Table 9). The analysis shows that there were not any significant differences between the grass components between the treatments. Percent cover of forbs was highest in the annual rye/birdsfoot trefoil as compared with the other treatments (Figure 7). Across all treatments, the cover of grasses averaged 20%, with a maximum of 70%. Bare ground averaged 30%, with a maximum of 100% in one treatment plot.

Table 9: Summarized ANOVA table representing differences in herbaceous percent cover and biomass between each treatment. Percent cover sorted into white clover, forbs, grasses and bare ground. Biomass refers to total biomass removed from the treatment plot.

Independent variable	Dep variable	df	F	p
	<u>% cover</u>			
Treatment	White clover	4, 72	0.924	0.455
Treatment	Forbs	4, 72	4.287	0.004
Treatment	Grasses	4, 72	1.804	0.137
Treatment	Bareground	4, 72	1.366	0.254
	<u>Biomass</u>			
Treatment	Wet Weight	4, 98	1.871	0.122
Treatment	Dry Weight	4, 98	1.072	0.375
Treatment	% H ₂ O content	4, 98	1.658	0.166

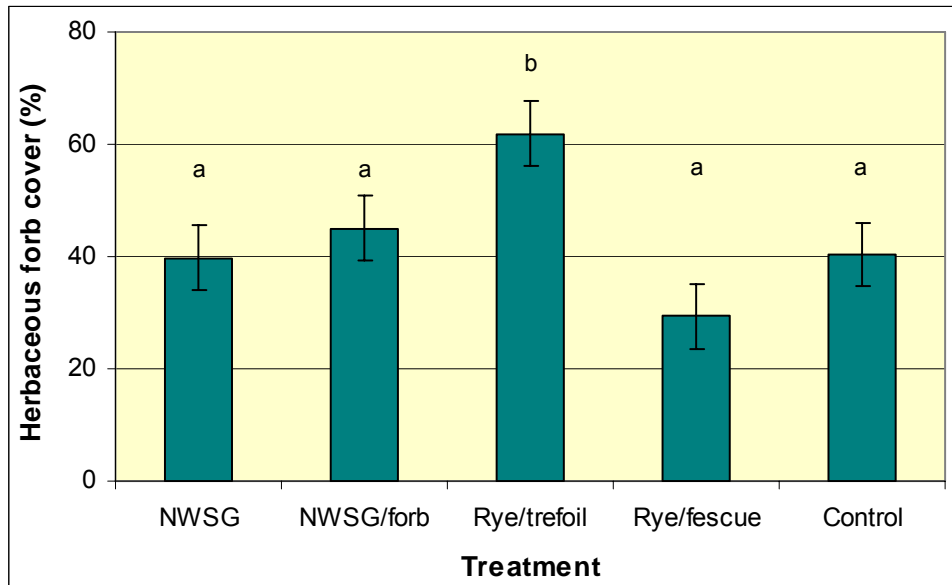


Figure 7: Mean percent cover determined by quadrat sampling of treatments. Within each of the five treatments, herbaceous cover of forbs was estimated to the nearest 5%. Bars represent standard error. (Means with the same letters are not significantly different at $\alpha = 0.05$ using Duncan's technique.)

Survival between treatments

Due to physiologic variation between each species of seedling, separate analyses were conducted to determine differences between treatments. There were no significant differences in overall survival between treatments for four of the species. Virginia pine was the only species with significantly different survival between treatments ($p = 0.002$). For this species, survival in the two NWSG treatments was significantly better than in the other three treatments. For all species except yellow-poplar, the trends suggest better survival in the two NWSG treatments than in the non-native treatments (Figure 8).

To further investigate this trend, two NWSG treatments were lumped against the non-native rye treatments for comparison. Significant differences indicate that the seedlings survive better in the NWSG treatments than the non-native and control treatments ($p = 0.026$). We found that sugar maple ($p = 0.122$) and eastern redbud ($p = 0.335$) tended to have higher survival rates in the native warm season grass treatments (Table 10). Northern red oak tended to survive better in the NWSG treatment than in the non-native treatment, but the highest survival rates tended to be in the control plots ($p = 0.273$). Virginia pine survival was significantly higher in the NWSG treatments ($p = 0.000$). Yellow-poplar was the only species which tended to have higher survival rates in the non-native grass treatments.

There was also a treatment by cover interaction ($p = 0.024$) present for redbud seedlings. In treatments two ($p = 0.009$), three ($p = 0.010$), and five ($p = 0.010$), survival was significantly different between the herbaceous cover classes (Figure 9). Treatments one ($p = 0.748$) and four ($p = 0.135$) did not have significantly different survival between the cover classes. There were no significant treatment differences in survival between the cover classes the other four species.

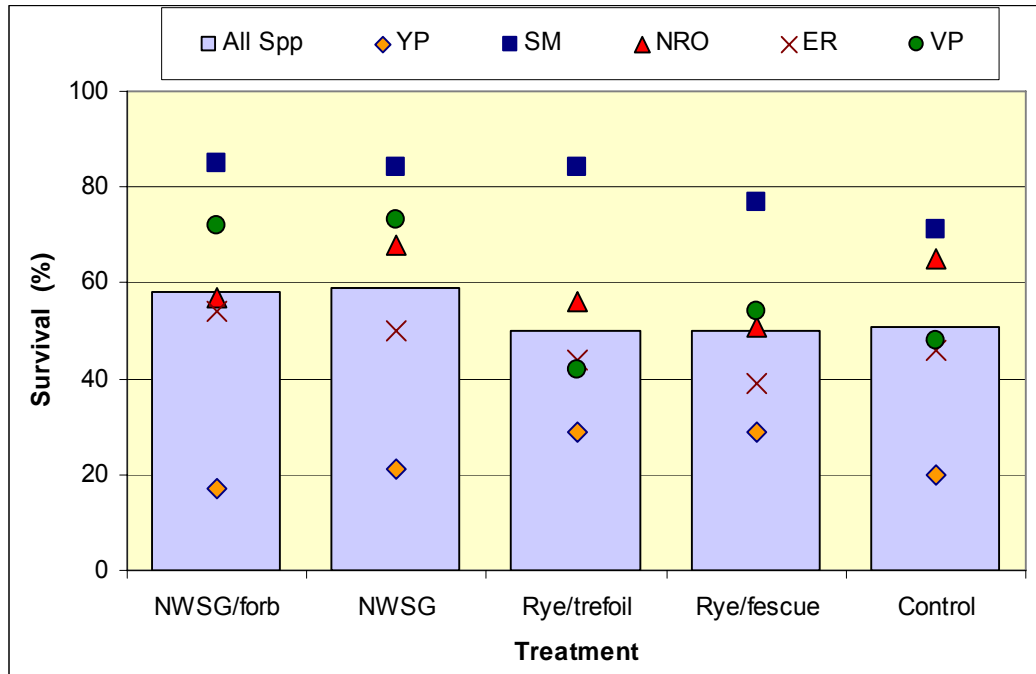


Figure 8: Overall seedling survival (bars) and the survival of each tree species (symbols) within each of the five herbaceous treatments. Standard error of means and significant differences using Duncan's technique presented in Appendix A table 13. YP = yellow-poplar, SM = sugar maple, NRO = northern red oak, ER = eastern redbud, VP = Virginia pine.

Table 10: Comparison of survival between native, non-native grass, and control treatments. Means and standard error presented. (Means with the same letters within each species are not significantly different at $\alpha = 0.05$ using Duncan's technique.) YP = yellow-poplar, SM sugar maple, NRO = northern red oak, ER = eastern redbud, VP = Virginia pine.

Species	Survival (%)		
	NWSG	Non-native grasses	Control
All Species	58 ± 2.3 A	50 ± 2.3 B	51 ± 3.2 B
YP	19 ± 4.2 A	29 ± 4.7 A	20 ± 6.2 A
SM	85 ± 3.9 A	80 ± 4.3 A	71 ± 6.5 A
NRO	63 ± 4.8 A	59 ± 4.9 A	65 ± 6.8 A
ER	52 ± 5.0 A	41 ± 5.2 A	46 ± 6.7 A
VP	72 ± 4.5 A	49 ± 5.0 B	48 ± 8.0 B

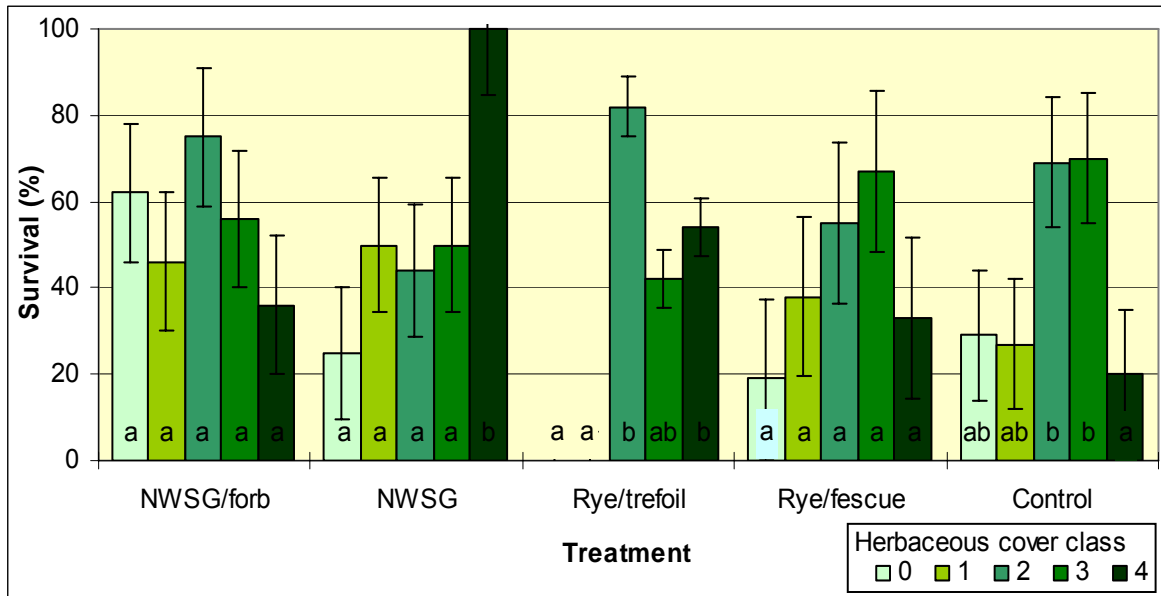


Figure 9: Mean survival of eastern redbud by herbaceous cover class within treatments. Bars represent standard error. (Means with the same letters, in each treatment, are not significantly different at $\alpha = 0.05$ using Duncan's technique.)

Seedling growth between treatments

Within species there were no significant differences in first year RCD growth between treatments. During the second growing season there were treatment effects on seedling RCD growth. Yellow-poplar ($p = 0.825$) and northern red oak ($p = 0.098$) did not have significantly different RCD growth, while sugar maple ($p = 0.040$), eastern redbud ($p = 0.006$), and Virginia pine ($p = 0.010$) growth was significantly different between treatments (Figure 10).

Photosynthetically active radiation differences between treatments

Measurements of PAR were only taken during the second growing season as light interception within treatments was not considered influential during the first season of growth. There were significant differences between treatments ($p = 0.001$) at the 5cm level (Figure 11). There were not any significant differences in PAR between treatments at the 25cm ($p = 0.337$) and 1m ($p = 0.756$) levels.

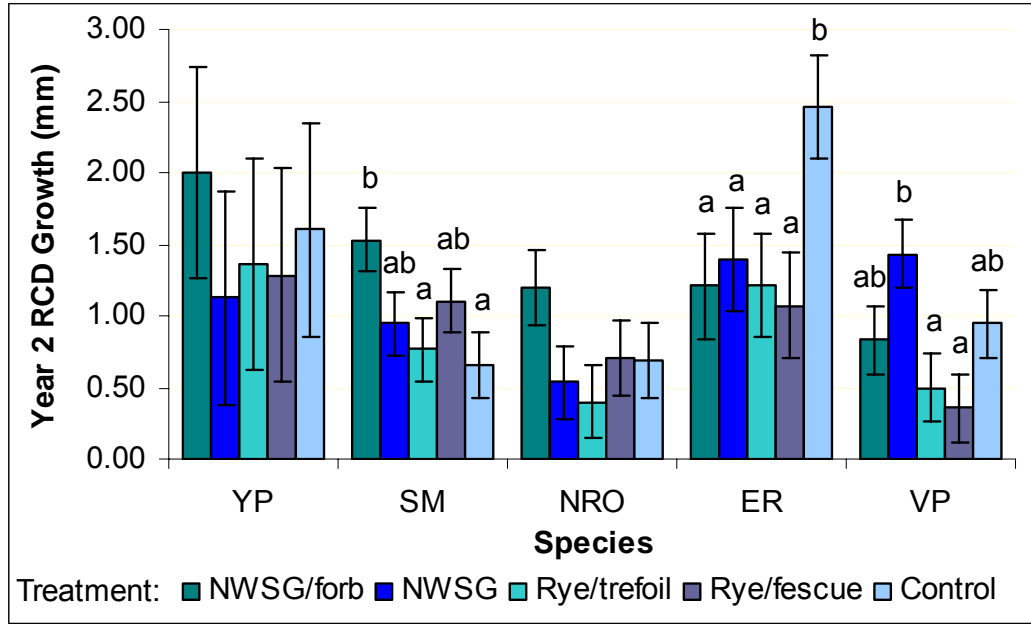


Figure 10: Seedling root collar diameter (RCD) growth in millimeters during the second growing season for each species within the five treatments. Bars represent standard error. Different letters represent significant differences between treatments within a species. YP = yellow-poplar, SM = sugar maple, NRO = northern red oak, ER = eastern redbud, VP = Virginia pine.

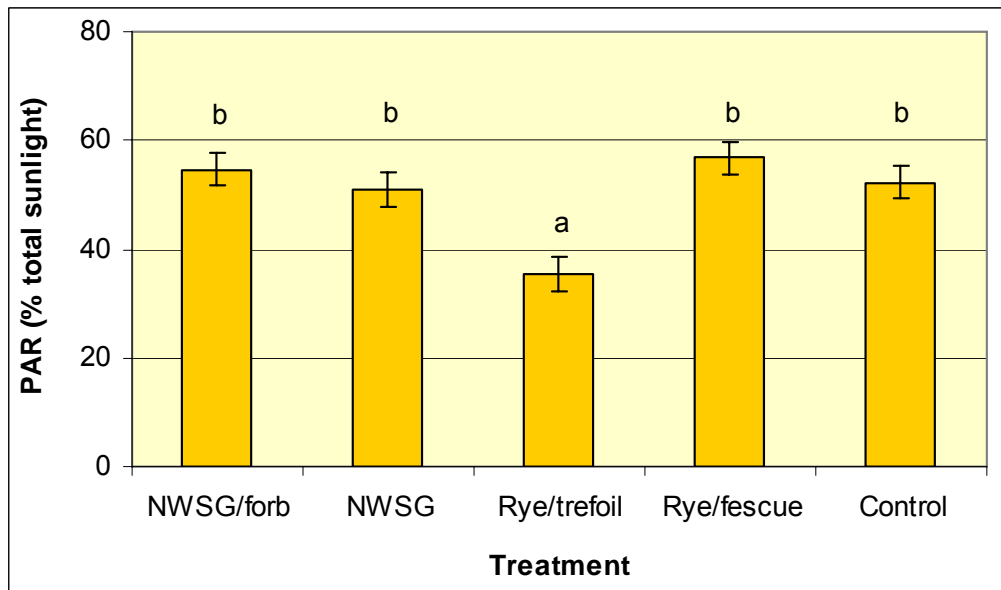


Figure 11: Photosynthetically active radiation readings sampled 5cm above the ground surface within each of the five treatments. Bars represent standard error of means. (Means with the same letters are not significantly different at $\alpha = 0.05$ using Duncan's technique.)

Seedling transpiration rate

No significant differences in transpiration rate of any species occurred between treatments during the two years of this study. There were no correlations between transpiration, soil moisture, soil surface temperature, 6cm soil temperature, or 15cm soil temperature readings during the two year project. Transpiration rates were averaged over each year for each species individually (Table 11). Comparisons were not made between years for the purposes of this study. Sampling was conducted in mostly sunny conditions with normal humidity levels ranging from 60 to 99% over the sampling time in 2006, similar to the humidity conditions during the 2005 sampling period.

Soil respiration rate

Soil respiration rates, sampled around sugar maple seedlings, were not significantly different between treatments or years. Mean respiration rate for the sugar maple seedlings sampled in 2005 was 4.03 mmol CO₂ m⁻²s⁻¹ (SE = 0.33). In 2006 the mean respiration rate was 6.08 mmol CO₂ m⁻²s⁻¹ (SE = 0.41). Several outliers were present in the data and removed for analysis. There was considerable variation in the respiration rates which ranged from 1.04 to 20.03 mmol CO₂ m⁻²s⁻¹ and 1.13 to 20.10 mmol CO₂ m⁻²s⁻¹ in 2005 and 2006, respectively. There were no correlations between respiration, soil moisture, 6cm soil temperature, or 15cm soil temperature during either year of the study.

Table 11: Mean transpiration rates (mmol H₂O m⁻²s⁻¹) and standard error (SE) for each species of seedling during the two year study period.

Species	Mean 05	SE	Mean 06	SE
Yellow-poplar	2.32	0.11	7.93	0.37
Sugar maple	1.50	0.06	2.22	0.13
Northern red oak	1.51	0.12	0.59	0.05
Eastern redbud	1.80	0.08	4.05	0.13
Virginia pine	1.38	0.06	7.43	0.26

C. Microsite and seedling interactions

Herbaceous cover surrounding seedlings

Cover class distribution changed noticeably between the first and second growing season (Figure 12). In the first year, almost 70% of the seedlings were growing within 1-25% cover; this was reduced to 20% in the second growing season. During the first season only 5% of the seedlings were growing in cover above 50%. While in the second season over 30% of the seedlings were growing in cover above 50%. There was an increase in seedlings in 0-1% cover in the second season.

Survival and herbaceous competition

There are significant differences in the overall survival of the planted seedlings within the herbaceous cover classes. Overall, moderate cover demonstrated the highest survival rates, with cover less than 25% or more than 75% significantly reducing the survival of planted seedlings (Figure 13). Northern red oak survival was significantly different between herbaceous cover classes ($p = 0.029$). Survival was highest for red oak when surrounded by 25-50% cover. Survival

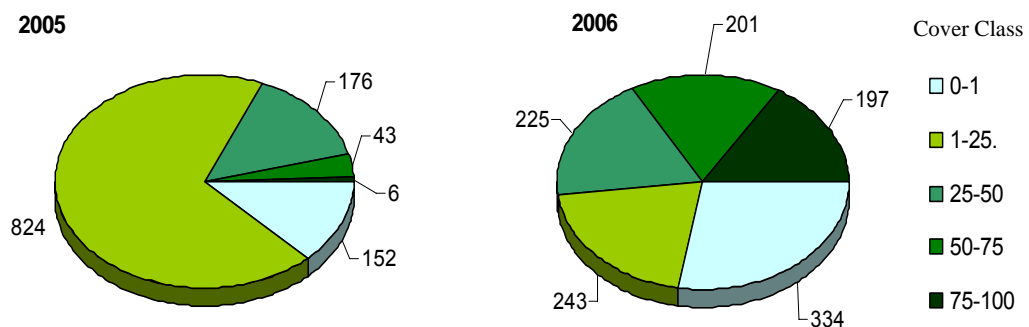


Figure 12: Distribution of herbaceous cover class around each of the 1200 seedlings based on the Braun-Blanquet (1932) cover scale. Data were analyzed from data collected in August 2005 and August 2006. Labels refer to the total number of seedlings in each respective cover class.

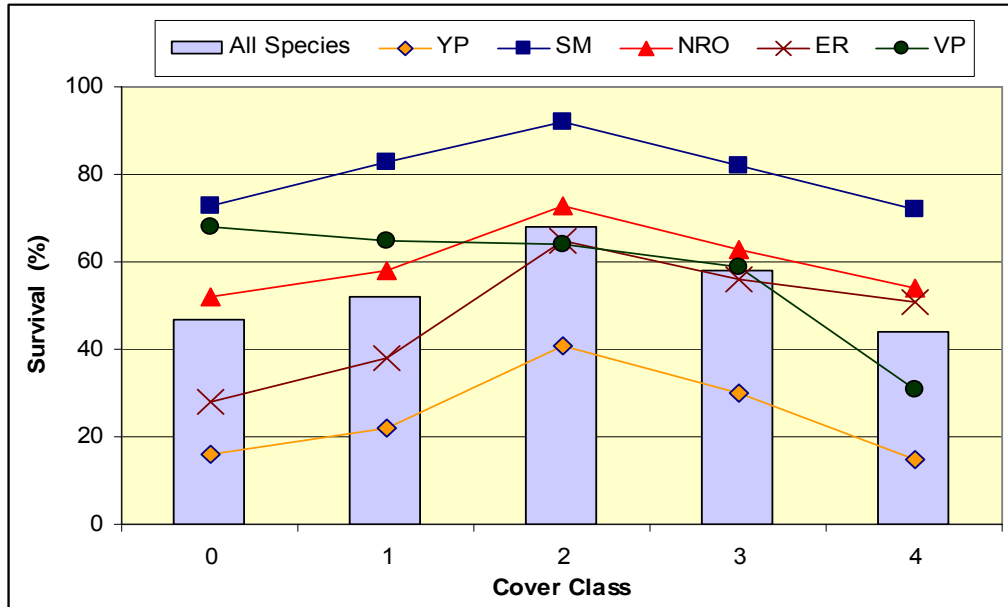


Figure 13: Second year mean survival of all seedlings (bars) and each species (symbols) based on herbaceous cover class. Herbaceous cover class zero is 0-1% cover, class one is 1-25%, class two is 25-50%, class three is 50-75% and class four is 75-100%. YP = yellow-poplar, SM = sugar maple, NRO = northern red oak, ER = eastern redbud, VP = Virginia pine. Standard error of means and post hoc tests of differences in Appendix A table 14.

was lowest when there was 0-1% cover present around this species. Eastern redbud also had significantly different survival rates between cover classes ($p < 0.000$), although this effect differed between treatments (Figure 9). Across treatments, survival of this species was significantly higher in the 25-50% cover than in other cover classes (Figure 13). However, survival of yellow-poplar ($p = 0.428$), sugar maple ($p = 0.429$), and Virginia pine ($p = 0.413$) was not significantly different between the herbaceous cover classes. Except for Virginia pine, the moderate cover classes demonstrated the highest survival rates.

Growth and herbaceous competition

Within species there were no significant differences in RCD between herbaceous cover classes during the first year of the study. During the second growing season there were some cover effects on seedling RCD growth. Root collar

diameter growth was influenced by the amount of cover surrounding each seedling (Figure 14). Northern red oak ($p = 0.023$) and Virginia pine ($p = 0.007$) RCD growth was significantly different between cover classes. Species differences in height growth were not analyzed for this project. There were no significant differences in height growth of the seedlings between treatments or herbaceous cover classes in either year of this study.

Photosynthetically active radiation differences in cover

Measurements of PAR were not recorded during the first growing season. Light interception data indicate there were significant differences between herbaceous cover classes ($p < 0.000$) at the 5 cm level (Figure 15). There were not any significant differences when PAR was measured at 25cm and 1m above the ground between cover classes.

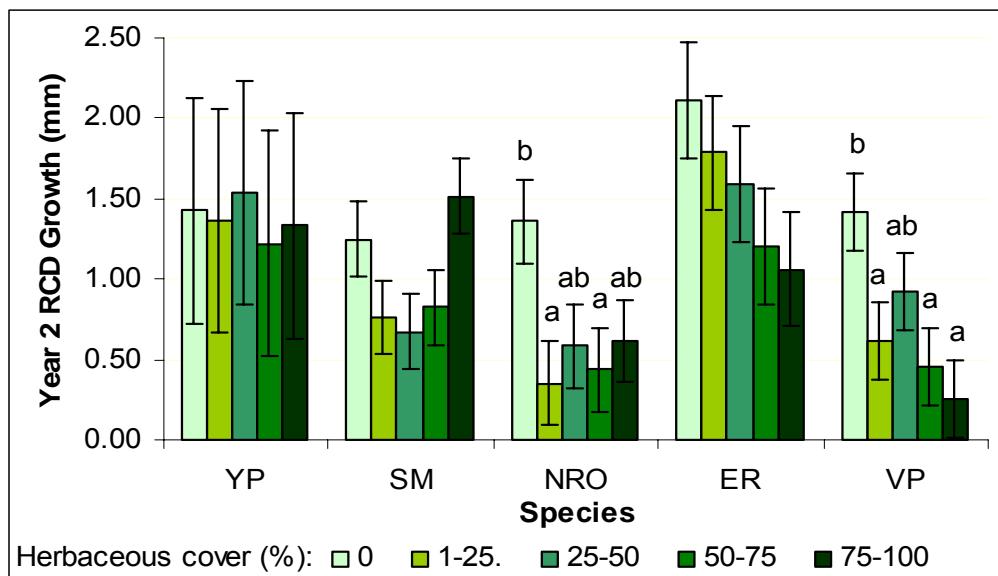


Figure 14: Seeding root collar diameter (RCD) growth during the second growing season for each species within each cover class. Bars represent standard error. Different letters represent significant differences between treatments within a species. YP = yellow-poplar, SM = sugar maple, NRO = northern red oak, ER = eastern redbud, VP = Virginia pine.

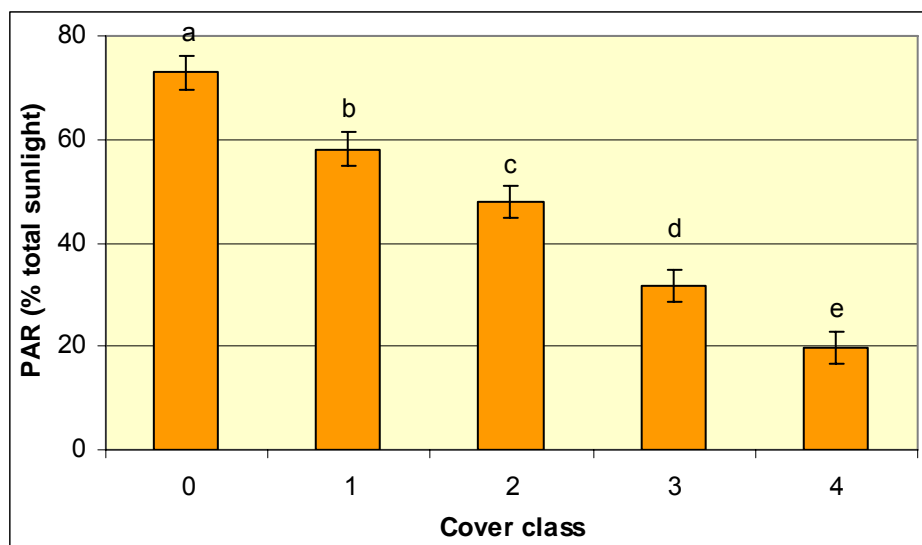


Figure 15: PAR sampled at 5cm above the ground surface within herbaceous cover class. Herbaceous cover class zero is 0-1% cover, class one is 1-25%, class two is 25-50%, class three is 50-75% and class four is 75-100%. Bars represent standard error. (Means with the same letters are not significantly different at $\alpha = 0.05$ using Duncan's technique.)

Seedling transpiration rate

Comparisons of transpiration rate within cover classes were not made between years of this study, each year was analyzed separately. Cover class differences were detected for sugar maple and northern red oak seedlings, but all others were statistically similar. Transpiration rate was not significantly different between cover classes during the 2005 season for sugar maple seedlings ($p = 0.956$). There were no seedlings growing within cover classes three and four, while 72% of those sampled were growing in cover class one. During the 2006 season, the transpiration rate for sugar maple was significantly higher in the 50-75% cover class ($p < 0.000$) (Figure 16). Mean transpiration rate for northern red oak was significantly different between cover classes in both years of the study (Figure 17). During the first year, red oak transpiration rate was highest when surrounded by 1-25% cover. However, during the second year, the mean transpiration rate for red oak seedlings growing in 0-1% cover was significantly higher than those surrounded by 1-25% cover ($p = 0.001$).

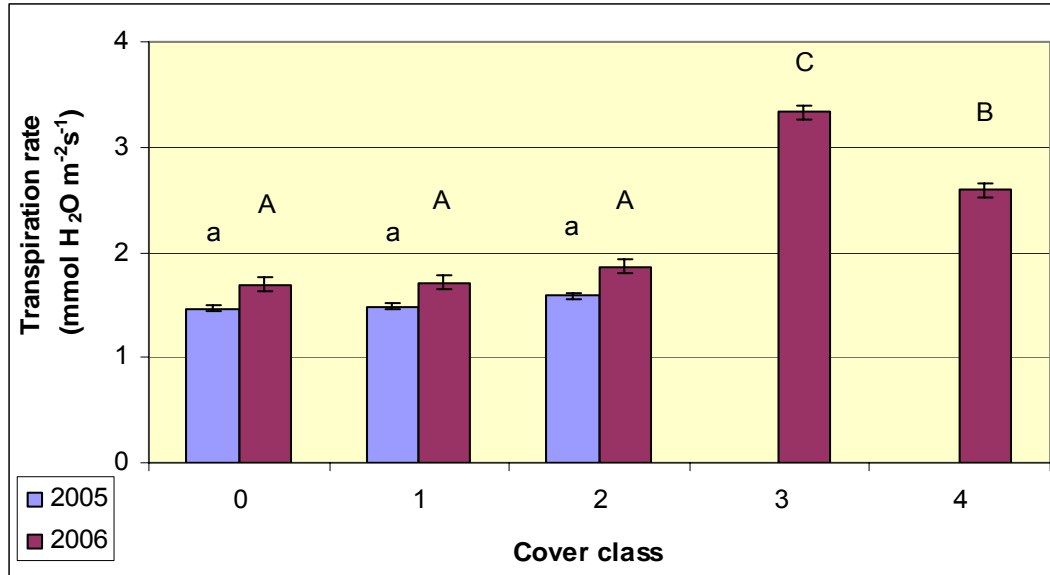


Figure 16: Mean sugar maple leaf transpiration rates for 2005 and 2006. There were no seedlings growing within cover classes three and four during 2005. Cover class zero = 0-1% cover, one = 1-25%, two = 25-50%, three = 50-75% and four = 75-100%. Bars represent standard error. (Means with the same letters, within each year, are not significantly different at $\alpha = 0.05$ using Duncan's technique.)

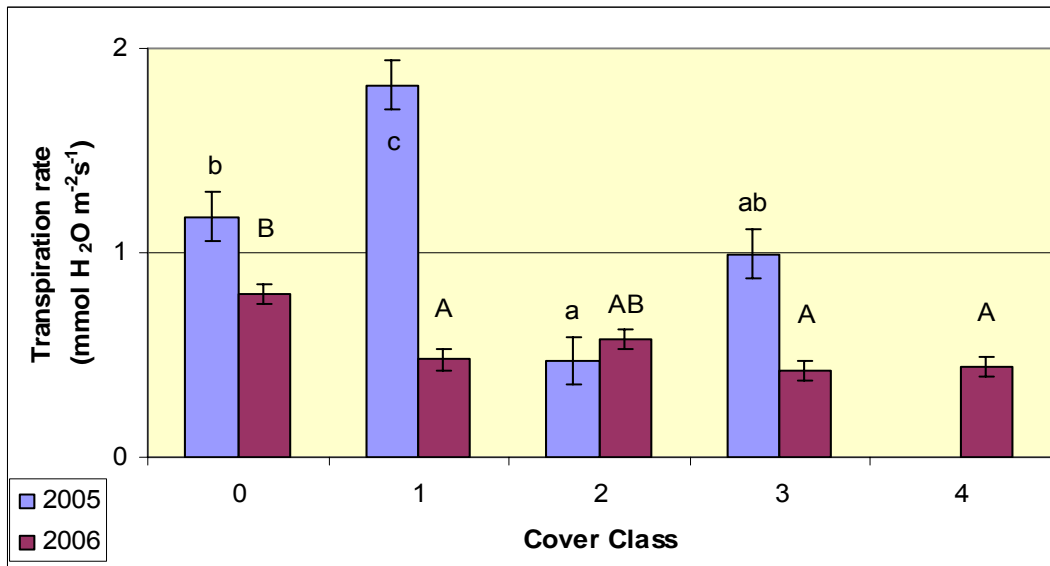


Figure 17: Mean northern red oak leaf transpiration rates in 2005 and 2006. There were no seedlings growing within cover class four during 2005. Cover class zero = 0-1% cover, one = 1-25%, two = 25-50%, three = 50-75% and four = 75-100%. Bars represent standard error. (Means with the same letters, within each year, are not significantly different at $\alpha = 0.05$ using Duncan's technique.)

Soil respiration rate

Soil respiration rates, sampled around sugar maple seedlings, were not significantly different between herbaceous cover classes or years. Although there were no significant differences in soil efflux rate between cover classes ($p = 0.507$), there was a general trend demonstrating increased soil respiration within higher amounts of cover in both year one and year two (Table 12).

Correlation matrixes were formed to determine if any correlation exists between soil respiration rate and the predictors which included soil temperature and moisture. No correlations were present, and soil temperature and moisture were removed as covariates in the regression model. There was a significant Pearson's Correlation between respiration rate and cover (correlation = 0.157, $p = 0.027$) in the second year of the study.

Regression analysis was conducted on first year data. The model included treatment, cover class, RCD growth, height growth, and seedling transpiration rate as selection variables. Statistically, this regression model was significant ($F = 5.246$, $p = 0.004$), however, this model was only able to explain 6.8% of the variation in respiration rates. The second year data was also statistically significant ($F = 4.327$, $p = 0.045$), however, this was able to explain only 6.6% of the variation in soil respiration rate. Thus, none of the variables measured in this study were able to explain a significant amount of variation in soil respiration rates.

Table 12: Mean soil respiration rate ($\text{mmol CO}_2 \text{ m}^{-2}\text{s}^{-1}$) within each herbaceous cover class around sugar maple seedlings. Means and standard error (SE) for each of the two growing seasons. Cover class zero = 0-1% cover, one = 1-25%, two = 25-50%, three = 50-75% and four = 75-100%.

Herbaceous cover class	Mean 2005	SE	Mean 2006	SE
0	2.47	0.25	5.43	0.75
1	5.71	0.87	6.58	1.11
2	6.50	1.79	6.00	0.81
3	NA		7.91	1.62
4	NA		7.99	1.29

4. Discussion

A. General site observations

Soil

Soil characteristics can play a significant role in the success of reforestation of drastically disturbed landscapes. Appalachian minesoils have been studied for many years and the ability of these soils to be used as a growth medium for tree restoration has been well documented. Sites in Virginia, similar to the site in this study, have a pH of unoxidized overburden materials between 6.5 and 8.0 (Roberts *et al.*, 1988). The pH of the soil occurring on this site averaged 7.6. Soil pH is directly related to the overburden parent materials. On this site, much of the overburden materials are unweathered, unoxidized sandstone, siltstone, and shale coming from deeply buried parent materials (Haering *et al.* 2005, Jones *et al.* 2005). This site was amended with lime when hydroseeding operations occurred which may also help explain the relatively neutral pH on this site.

The soil elemental concentrations vary considerably from those found on natural soils in Anderson County (Ammons *et al.*, 2000), however, neither nutrient deficiency nor toxicity were likely to be limiting to growth on these sites. During the two years of the study, increases in total Cu, Fe, Na, and Zn levels were apparent. There was no evidence suggesting that the increase of these elements had any effect on seedling development. My results show that the soil elemental concentrations were significantly higher than those of other minesoils (Showalter *et al.*, 2006) in the southern Appalachians. Chemical weathering of the overburden is the primary contributing factor to the changes of soil chemical properties (Haigh, 2000). Although high, concentrations of Cu (Paschke and Redente, 2002), Zn (Paschke *et al.*, 2000), Mn (Paschke *et al.*, 2005) and Fe (Kabata-Pendias and Pendias, 2001), the micronutrients sampled in this project, were far less than toxic levels that have been observed in other reclamation

research plantings. Mine sites in western Virginia had soil nutrient concentrations far below those found on Patterson Mountain (Salzberg and Burger, 2006). The phosphorus and calcium concentrations were ten times higher and magnesium was two times higher than the minesoils in western Virginia. Phosphorus concentrations were also ten times higher than two year old minesoils in southeastern British Columbia (Fyles *et al.*, 1985). Spoil weathering accelerated by freeze-thaw events and vegetation development has been attributed to increased phosphorus levels on newly reclaimed sites (Fyles *et al.*, 1985). High concentrations of total phosphorus and pH levels ranging from 6.9 to 7.9 indicate that there should be a sufficient amount of plant available phosphorus in the soil (Mullen *et al.*, 2005). Potassium levels were slightly higher than those reported on the Powell River Project in Virginia where fertilizer treatments were applied annually (Salzberg and Burger, 2006). Potassium is found in very high levels in fresh mine spoils but is also subject to long-term leaching losses (Daniels and Zipper, 1999). The increased iron concentrations occurring on this site are a direct result of weathering and oxidation of the mine soils in the southeastern US (Daniels and Zipper, 1988). As these soils become enriched in iron-oxides, adsorption of water soluble phosphorus is then "fixed" into unavailable forms over time.

Although nitrogen was not measured, it has been shown to be the most critical nutrient for plant establishment (Fyles *et al.*, 1985). However, early site fertilization and the establishment of legume species, such as birdsfoot trefoil, should alleviate any nitrogen deficiencies. The measured soil nutrient concentrations present on this site were not likely to have any adverse impacts on the growth and survival of the seedlings or herbaceous ground cover.

Vegetative cover

Several herbaceous species were not seeded, but were observed growing within the study plot boundaries in August 2005. The species detected include coltsfoot (*Tussilago farfar*), an invasive exotic on the noxious weed list in Alabama,

Connecticut, and Oregon (Miller, 2003). Tennessee ranked coltsfoot as a significant threat to native communities in 2004 (Tennessee Exotic Pest Plant Council, 2004). Another invasive exotic, Japanese stiltgrass (*Microstegium vimineum* [Trin.] A. Camus), was found on this site. This species is well adapted to grow in low light conditions, and will flourish on disturbed sites (Horton and Nuefeld, 1998). However, the ability of this exotic to displace native species is unknown. Although an annual, it produces significant amounts of seed which proliferate for long periods of time and is extremely difficult to manage once established (Drake *et al.*, 2003). Lambsquarters (*Chenopodium album*) an annual native forb commonly found in areas with full sun exposure was also identified within the study area. Although native, this species is considered an invasive weed which can grow to 1 meter in height (Tennessee Exotic Pest Plant Council, 2004). Lastly, American pokeweed (*Phytolacca americana*), a perennial native forb that is considered invasive in many states in the southeast with poisonous tap root and berry (Tennessee Exotic Pest Plant Council, 2004), was also found. This forb can reach 3 meters in height, can provide food for some bird species, and has been used medicinally by natives.

Several non-invasive herbs were also found within the plot boundaries including, Canada lettuce (*Lactuca canadensis*), an annual warm season forb species native to the United States that can reach 3 meters in height, and annual/common ragweed (*Ambrosia artemisiifolia*), an annual native forb which can grow to over 1 meter in height but is shallowly rooted in the soil. As time progressed, observations determined that the naturally colonizing invasive and exotic species comprised an increasingly smaller component of the overall vegetation due to the establishment of the seeded cover crops.

Several native tree species were volunteering into the site including sycamore, black locust, yellow poplar, and red maple. These are all species common to the surrounding forest and most likely are being disseminated by wind or wildlife activity occurring on the site. Research has demonstrated the difficulties

associated with restoring all aspects of species diversity (Martin *et al.*, 2005), but increases in diversity can occur naturally by the invasion of native plants.

Survival of outplanted tree seedlings

Overall, northern red oak, sugar maple, and Virginia pine survival was within the 60% required in the permit for bond release by the Office of Surface Mining. Yellow-poplar demonstrated only 24% survival on this site over the two year study period. In forest systems yellow-poplar is considered a pioneer species but planting yellow-poplar seedlings on these steep reclaimed sites is not ideal as survival rates below 30% have been observed on several southern Appalachians strip mines (Griffith, 1991), and Zeleznik and Skousen (1996) found yellow-poplar survival to be 21% on alkaline surface mines in Ohio. Angel *et al.* (2006) showed significantly increased survivability as compaction was minimized. Surface compaction may have had an effect on the seedlings planted, although this was not measured and the site was presumed to be reclaimed using minimal compaction techniques. Hay *et al.* (1987) reported survival rates of yellow-poplar above 70% on native soils present in east Tennessee. They suggest that yellow-poplar is very site-sensitive and requires deep, moist, well-drained, acidic soil for optimal survival. Although these minesoils are deep and well drained, these sites were not acidic and have very different physical properties from the nearby native soils, such as high rock fragment content. Yellow-poplar prefers moist conditions; the wetting and corresponding rapid desiccation characteristic of minesoils in this region can detrimentally impact seedling establishment. My observations of the site and other nearby sites indicate that yellow-poplar is readily able to volunteer onto surface mined areas naturally when a nearby seed source is present. This observation corresponds to the findings of Carter and Ungar (2002). Volunteers may have the best opportunity to colonize suitable sites, while planting crew personnel may struggle to locate the most favorable microsites.

Survival of sugar maple seedlings was 80%, the highest overall survival during the study period. This study area, a northerly facing strip mine, is suited to the survival of this species as demonstrated by Burger and Torbert (1999). Ashby (1987) suggests that newly reclaimed minesoils are relatively low in available nitrogen and thus appropriate for sugar maple establishment due to their low N requirements.

With the second best survival, northern red oak demonstrated its ability to be used in reclamation plantings in east Tennessee. Northern red oak has been studied extensively on reclaimed mined sites, mainly due to its potential for production of valuable timber in the future. This species has demonstrated good survivability in Maryland (Bagley and Shaffer, 1992), Virginia (Burger *et al.*, 2005b), Indiana (Andersen *et al.*, 1989), and Kentucky (Torbert and Burger, 1992).

Overall, eastern redbud survival was below 50%. Redbud has been identified as a poor species for planting on compacted mine soils (Bagley and Shaffer, 1992). Redbud has the ability to grow well in full sun and the high wildlife value of this species makes it advantageous for planting on drastically disturbed sites.

Although Virginia pine averaged less than 60% survival on this site, on other sites in South Carolina survival averaged 70% suggesting that it can be a good candidate for reclamation plantings on mine sites (McMinn and Crane, 1984). As a vigorous pioneer species well suited to growing in open sun conditions, planting these seedlings on mine sites will help to shade and protect other more valuable timber species. Burger and Zipper (2002) suggest planting Virginia pine on poor sites.

There were significant differences in survival between blocks during this study. Blocks were considered a random effect variable in analysis. Each planting block differed in elevation, but not aspect. The blocks were within several hundred meters of one another. Calcium and potassium levels also differed between blocks; however, there was no correlation with survival or any other seedling responses. The differences in survival between blocks varied for every

species and were not consistent. There were no significant differences between blocks with respect to soil moisture, soil temperature, amount of herbaceous cover, or soil pH. The reasons for these block differences in survival are not understood at this time.

Across this site, several factors were identified as having a measurable impact on survival rates: the initial size of the seedling, erosion channel development, winter mortality, deer browsing, and transplanting stress.

Seedling size at the time of planting differed greatly between species, and to a lesser extent between individuals within a species. While many researchers have suggested that increased seedling size corresponds to greater survival after out-planting, some studies have found no correlation between size and survival rates for many species (Boerner and Brinkman, 1996). We found a significant positive relationship between initial seedling size and survival rate at the end of the second growing season in four of the five species studied. In contrast to Deirauf and Garner (1996), yellow-poplar was the only species studied that did not have greater survival rates for larger seedlings. Because mortality was so high for this species, the ability to detect such a relationship was greatly reduced. Research on northern red oak bareroot seedlings has similarly demonstrated that planting larger, more vigorous seedlings can ensure success after outplanting (Jacobs *et al.* 2006, Kormanik *et al.* 1995). Vogel *et al.* (1984) and Schlarbaum *et al.* (1997) advocate planting larger red oak seedlings to avoid smothering by herbaceous vegetation. I found the largest seedlings did not demonstrate the highest survival rates. This suggests the need to follow species specific grading standards (Clark *et al.* 2000, Ezell and Moorhead 2004) in order to provide the highest quality seedlings, not necessarily the largest seedlings, for use in disturbed land reclamation plantings.

Observing the biotic and abiotic factors influential to the survival of the seedlings was important to explain the causes of mortality for each individual. Of the mortality causes that could be identified, the main influence on the survival of the seedlings was winter mortality, similar to the findings of Berkowitz *et al.* (1995).

Other studies indicate that winter mortality rates can differ significantly by species, planting site, and year (Fenner, 1987). Andersen *et al.* (1989) demonstrated that frost heaving was a significant cause of winter mortality on fine-textured soils with minimal vegetative cover, similar to the soils on my site during the first winter of this study.

The torrential rains that occur as a result of summer thunderstorm events during the early stages of site development can drastically impact the ability of the reclaimed area to resist erosion (Nicolau and Asensio, 2000). The development of erosion channels can pose a serious threat to outplanted seedlings on steep slopes soon after surface reclamation plantings are completed. Sheet, rill and gully erosion expose the roots of the newly planted seedlings (Muncy, 1985) as was observed in this study. Erosion gullies developed throughout the study area as well as throughout the reclaimed mine bench. These channels stretch from top to bottom of the reclaimed slope and ranged from a few centimeters to one meter in depth. In this project, survival was not directly related to seedling position in relation to erosion channels. However, if seedlings were growing within a developing channel, the observed rate of mortality was high. These rills and gullies accounted for a total of eight percent mortality of the seedlings planted. Many factors including soil compaction and vegetative protection dictate the extent and duration of site instability. I found that the study area was relatively stable during the second growing season as the herbaceous cover and seedlings took hold and losses from erosion decreased. Deep rooted tree seedlings reduce erosion and aid in soil development (Haigh and Gentcheva-Kostadinova, 2000).

Deer browsing was another detrimental impact on the seedlings. During the first season, the herbaceous cover of the site was minimal and the seedlings were the only vegetation present in certain areas. Many of the seedlings were browsed upon by the whitetail deer that are common to the area. Although mortality attributed directly to browsing was relatively low, the removal of shoot biomass hinders seedling growth. The deer would browse as much as half of the

seedling's above ground biomass. Deer browsing reduces vigor and growth as the seedlings consume valuable reserves to replace lost biomass (Morgan, 1987). Research speculates that the high nitrogen availability on newly fertilized reclaimed sites helps seedlings develop more desirable succulent foliage (Davidson, 1970). My project did not investigate available nitrogen levels or the exact timing of the browse events. However, the high incidence of pine browse recorded in the spring of the second season suggests that most browsing occurred during the first winter, which could explain the higher level of mortality for Virginia pine seedlings.

The incidence of seedling flushing was used to determine if the seedlings were killed due to transplanting stress. If seedlings were unable to produce leaves during the first season, it is reasonable to assume that they were killed by the stresses of transplanting from the nursery. Jacobs *et al.* (2005) and Haase and Rose (1994) have presented data suggesting that the water deficit experienced by newly planted seedlings is a major contributor to transplanting stress. Initial establishment is cited as the critical stage for many seedlings, especially oak species (Ashby, 1995).

B. Evaluation of seedling and treatment interactions

Herbaceous treatment cover

Initially, it seemed that the type of grass cover was highly variable between treatments. However, the five different ground cover treatments were not significantly different at the end of the study period. The hydroseeded mix that was applied on the site seemed to overpower the species that were seeded within each treatment. The native warm season grass treatments that were hand broadcast have yet to dominate the cover of these treatments. This seed was applied at the recommended rates of pure live seed per hectare (Ashby *et al.* 1989, Burger and Torbert 1999) from a reliable seed source. However, it is recommended that NWSG be planted using a seed drill or at least disked into the

soil 1-3 centimeters (Bartholomew *et al.*, 2001). Also, the timing of the treatment application could not have been less ideal. Precipitation during the spring of the first season had been near normal, and the seeding was done within the approximate seeding window. However, after seeding was completed, rainfall was very minimal. Overall precipitation was 70% of normal after the seeding was completed and very little rainfall occurred during the month of June. After two growing seasons, the NWSG treatments looked very similar to the surrounding herbaceous vegetation. This was demonstrated by the lack of differences in cover type from the control plot. However, we did find signs of the NWSG growing underneath the dense mat of clover and birdsfoot trefoil during our biomass sampling. Barnes and Washburn (2000) showed that many of the NWSG seedlings were suppressed by intense weed competition until the third growing season after planting. This suggests that the NWSG treatment differences were present and developing during the study period, although these differences were not quantified by the sampling methods used.

Differences were observed between the control and the two non-native grass treatments. The annual rye and birdsfoot trefoil treatment was able to rapidly establish on this site. Annual rye did emerge later in the first growing season and the birdsfoot trefoil demonstrated an ability to establish well in the second season. The annual rye/birdsfoot trefoil treatment demonstrated the highest forb component; however, this was not a significant factor in the growth or survival of the seedlings. Although I was not able to detect any statistical differences between the perennial rye and creeping red fescue treatment and other treatments, general observations show that these grasses were establishing well within the site. During the second season the grasses were small and well dispersed in these treatments. Similar to the NWSG treatments, the aggressive red clover, ladino clover (*Trifolium repens* L.), and sweet white clover (*Melilotus alba* L.) created a dense mat of vegetation that may have hindered the establishment of the seeded grasses.

Herbaceous treatment biomass

There were no differences in the herbaceous biomass of the treatments. On my site, the overall herbaceous cover was 70%; almost two-thirds consisted of the forb component. The oven dry weights were similar to the findings of Andersen *et al.* (1989) during the second year of their study when chemical control methods were employed and total cover of the site was about 45%. They did find that without any herbaceous plant control, biomass weights were almost 5 times higher during that same time period and total cover was 100%. Similar numbers were found on revegetated sites in Kentucky by Vogel *et al.* (1984) for tall fescue plantings. The lower biomass on my study site may be due to the larger component of forbs as compared with the above mentioned studies.

Andersen *et al.* (1989) suggest that the tall fescue, which made up all of their sampled biomass, may weigh more than lower growing species such as birdsfoot trefoil per unit area. Separation of the tall sweet white clover from the other forbs and grasses was thought to be important to determine the composition of the area; however no statistical significance was determined. The tall clover covered the entire study area, as it does in the reclaimed area surrounding the study plots. This clover reached five feet in height and was very thick earlier in the growing season, however, at the time of harvesting, the clover had flowered and was dropping seeds and lost many leaves, thus total biomass of this species was not collected during sampling. An early sampling date may have provided a more accurate measure of clover biomass on these plots.

Seedling survival within herbaceous treatments

Survival was not significantly different between herbaceous treatments, but a trend of greater seedling survival in the NWSG treatments was apparent.

Yellow-poplar was the only species that had higher survival rates in the non-native treatments. A microsite interaction may better explain the performance of this species for reclaimed mine land plantings. Sugar maple survival tended to be highest in the native grass treatments, but overall, the treatments did not have

a significant impact on the survival of this species, demonstrating its ability to successfully colonize drastically disturbed sites. Sugar maple seedlings have been reported to survive successfully in open field plantings (Godman *et al.*, 1990). Few studies have focused on the competitive effects NWSG have on sugar maple seedlings. Although not significant, red oak seedlings growing in the rye/fescue treatment plots did tend to have the lowest survival of the five treatments, corresponding to the findings of Vogel *et al.* (1984). They showed significantly lower northern red oak survival in fescue plantings, suggesting that heat stress may have created unfavorable conditions in this cover type. However, I found no differences in soil temperature between cover types during this study. Virginia pine was the only species to survive significantly better on the two NWSG treatments than the other herbaceous treatments. Bagley and Shaffer (1992) and Burger and Torbert (1999) suggest this species prefers dryer areas with shorter grasses; however, neither soil moisture nor height of the NWSG treatments were different in this study. The NWSG treatments may offer less competition because of their clumping effect and can provide the tree-compatible ground cover adequate for the successful establishment of this species. When looking at all five treatments, the lowest survival was shown when seedlings were grown in the rye/birdsfoot trefoil treatment. This treatment had the highest amount of herbaceous cover and may have provided areas more appealing to browsing deer.

Seedling growth rate

Results of a previous study (Ashby *et al.*, 1989) supported the use of warm-season grasses for revegetation of mined sites, but warned that their size and competitive ability may limit tree growth. Root collar diameter increased over time, although differences between treatments were not detected during this study. However, my results indicate that height growth is not the most appropriate means for determining success during the early stages of site development due to dieback and herbivory of certain species. Studies suggest

that non-natives commonly found on reclaimed sites will retard early height growth and likely prevent the future establishment of a diverse forest ecosystem (Andrews *et al.* 1998, Holl 2002, Torbert and Burger 2000); however this is a species specific response. Andersen *et al.* (1989) demonstrated that poor height growth of northern red oak and other species planted on mine sites in Indiana was due to dieback caused by competition with fescue (*Festuca arundinacea*) and red clover (*Trifolium pratense*). Vogel and Curtis (1978) showed that height growth was restricted for Virginia pine seedlings growing in competition with seeded lespedeza; however, they also found that other tree species grew taller in the presence of legumes due to the enhanced nitrogen availability. The growth of each species responded differently and should continue to be monitored as the NWSG treatments develop on this site.

C. Evaluation of seedling and microsite interactions

Soil moisture

The mean soil moisture was 13.4% on this site, similar to what Carter and Ungar (2002) have reported for mined lands where soil moisture levels were commonly below 15%. However, the moisture readings varied greatly across the site. Mound areas generally demonstrated lower soil moisture levels, while high moisture readings in seep areas created highly variable moisture readings on this site. Variation in soil moisture readings across established forests has also been reported (Buckley *et al.* 2003, Kolka and Smidt, 2004). Similar to my results, Kolb and Steiner (1990) showed that soil moisture readings were not consistently different between the cover of grass and non-grass areas over their two-year study period. However, in the second year of my study, although not significant, a trend did exist demonstrating that soil moisture levels were lower as the amount of herbaceous cover increased. Similarly, Davis *et al.* (1999) demonstrated that herbaceous vegetation could substantially reduce soil water

content. Survival of the seedlings was not correlated to the amount of soil moisture during the project.

Soil temperature

Temperature was not affected by the differences in treatments or herbaceous cover classes on the surface. Considerable variation existed within the surface, 6cm, and 15cm readings across the site. Environmental factors may have contributed to this variation, but no patterns were detectable during this study. Temperature variation related to depth was also not consistent with any pattern. The maximum surface soil temperature measured was 50.0° C; however, this temperature was recorded before 2 pm which suggests that surface temperatures may have been higher on certain days. Helgerson (1990) demonstrated that soil surface temperatures over 52° C were lethal to young seedlings due to cambial damage at the soil surface. The dark soils present on much of the site could easily exceed that temperature threshold and be a significant reason for seedling mortality. Repeated exposure to high temperatures would increase the chances that the seedling would suffer heat damage. As the seedlings and ground cover grow, slight shading of the soil immediately near the stem could reduce surface temperatures and prevent heat damage.

Photosynthetically active radiation

Sampling PAR results in an instantaneous measure of the level of light exposure to the seedling in the presence of herbaceous competition (Barwatt, 2004). The effects of light levels on the growth and survival of the species studied on this site is questionable. Although PAR did decline with an increase in cover, this was only present at the 5 cm level at the time the measurements were made. The seedlings of all five species had foliage above the 5 cm level during the second growing season. However, the white sweet clover had lost its leaves and was not detectable by the Ceptometer at the 25 cm and 1 m levels at the time of

sampling. Therefore, it is possible that seedlings were affected by differences in light level at different times during the growing season, and measurements of PAR several times during the growing season is recommended for future studies on similar sites. Soil surface temperatures were not correlated with PAR. Competition for water and nutrients could more likely explain the reduced growth of seedlings when growing in greater amounts of cover.

Seedling transpiration rate

Considerable variation existed in the transpiration rates of each of the species sampled. The transpiration rates of each species were considerably higher during the second growing season. However, this increased transpiration rate was not correlated to the size of the seedling. Environmental factors including evapotranspiration and soil moisture content weigh heavily on the ability of a seedling to transpire (Eitzinger *et al.*, 2002). The amount of ground cover did have an influence on the transpiration rate of certain species.

Yellow-poplar

Together, planting stress and winter mortality accounted for 43% of yellow-poplar mortality when outplanted in this mined area. This suggests that although yellow-poplar is considered an early-successional species, they were particularly sensitive to stresses associated with exposure on this site.

Yellow-poplar survival was favored under moderate cover conditions. Similarly, Bagley and Shaffer (1992) reported that yellow-poplar does not withstand vegetative competition on reclaimed mine sites in Maryland. Burger *et al.* (2005b) reported that, although not significant, yellow-poplar survival was best when ground cover was controlled to less than 50%. Others have reported that yellow-poplar seedlings do not respond well to competition (Bagley and Shaffer 1992, Kolb and Steiner 1990). These trees are highly sensitive to microsite soil nutrient and moisture conditions (Buckner and McCracken, 1978). However, for surviving trees, we found that yellow-poplar seedling RCD growth was not

dependant upon the amount of cover surrounding the seedlings in this study suggesting that established trees may be less affected by competition. During the second year of the study, yellow-poplar tended to increase root collar diameter and height growth more than in the first year, regardless of competition.

Sugar maple

Sugar maple, a mid to late-successional species, is shown to be very tolerant of competition in forest settings (Godman *et al.*, 1990). This species had greatest survival rates when surrounded by moderate amounts of ground cover similar to the results of Burger *et al.* (2005b) in Virginia. This species responded poorly in heavy grass cover on reclaimed mined sites in Maryland (Bagley and Shaffer, 1992). Burger *et al.* (2005b) found that sugar maple seedlings tend to exhibit stem dieback in heavy ground cover. Godman *et al.* (1990) suggest that this is due to their inability to compete with herbaceous vegetation for moisture and nutrients. However, my data suggests that ground cover on these plots did not limit moisture availability, and may have instead had a positive effect on water relations. Sugar maple seedlings did have higher transpiration rates in the second year when surrounded by higher amounts of ground cover, but transpiration rates were not correlated to seedling size, soil moisture, or soil temperature. Water relations have been shown to influence transpiration rates more than other factors (Hinckley *et al.*, 1978). Welander and Ottosson (1999) suggest that seedling and leaf age may also influence transpiration rates greatly; however the mechanisms responsible are unknown. Bradbury and Malcolm (1977) suggest soil nutrient concentrations can drastically impact the water relations of seedlings thus altering seedling transpiration rates.

In this experiment, we were not able to detect differences in root function, as none of the sampled variables were able to explain the variation in soil respiration rates around the sugar maple seedlings. Similar to my data, other research has demonstrated the difficulties in predicting soil respiration responses in the complex eastern deciduous forest (Edwards and Norby, 1999). Many

processes are at work in the below ground system of a newly revegetated mine site. My findings support research showing soil respiration rates on mine spoil in British Columbia tended to increase with age (Fyles *et al.*, 1985). As the seedlings develop, their root respiration rates should continue to increase as will soil microorganism and herbaceous root respiration (Ponder, 2005). Even near seedlings with no herbaceous vegetation present, respiration rates were three to five times higher than efflux rates for sugar maple seedlings found on agricultural soils in Oak Ridge, Tennessee by Edwards and Norby (1999). They concluded that the respiration rates measured were a combination of the vegetative root systems and its associated rhizosphere which can differ drastically between sites. Tang *et al.* (2005) add that heterotrophic respiration from free-living microbes is an essential component to total soil efflux rates. Soil respiration measurements were not able to depict the processes involved in the restoration of site function during this study.

Edwards and Riggs (2003) suggest that the point-in-time measurements that I took should have been sufficient for making comparisons of treatment responses. They determined that mineral soil nitrogen, phosphorus, and organic matter availability were not influential to the respiration rate differences of their study. Raich and Tufekciogul (2000) concluded that soil respiration rates are controlled primarily by climatic and substrate factors, with vegetation having a secondary effect. Additionally, Edwards (1975), Edwards and Riggs (2003), and Garret and Cox (1972) showed that soil temperature and moisture significantly affected soil respiration rates. However, my research was unable to determine any correlation between soil respiration, temperature, and moisture. Singh and Gupta (1977) suggest that soil temperature, when soil moisture is low, has little effect on respiration measurements. Edwards and Riggs (2003) did determine that areas with greater grass cover tended to have greater soil respiration rates. However, my research shows that respiration was not significantly higher in areas with more cover present during the study period. Ponder (2005) suggested that even after four years since disturbance activities, soil respiration

components had not fully recovered. High rates of respiration indicate that site function is being restored which can indicate the success of reforestation projects. Additional investigation into the soil respiration rates on reclaimed minesoils may help to determine the exact processes at work.

Northern red oak

Ashby (1987) proposes that both the absence and abundance of herbaceous cover are considered limiting factors to the survivability of oaks on mined lands. Considered a midseral species, northern red oak seedlings are thought to be relatively tolerant of competition. This species had greater survival rates when surrounded by moderate amounts of groundcover, as also demonstrated by many other projects (Andersen *et al.* 1989, Brynes *et al.* 1984, Buckley *et al.* 1998, Burger *et al.* 2005b, Franklin and Buckley 2006), while high amounts of ground cover limited growth. Beckage and Clark (2003) demonstrated that the relative height growth of red oak seedlings was insensitive to competition and performed consistently across all microenvironments. In contrast, Andersen *et al.* (1989) and Brynes *et al.* (1984) concluded that red oak shoot dieback was a result of heavy ground cover around seedlings. Kolb and Steiner (1990) found that in the presence of herbaceous competition, red oak seedlings allocated significantly more resources to root growth than shoot elongation. Root collar diameter may therefore provide a better measure of total growth of young northern red oak seedlings (Ezell and Moorhead 2004, Duryea 1984). In this study, RCD growth was significantly greater in the absence of cover. In the second year of this experiment, red oak seedlings had higher transpiration rates when surrounded by less ground cover, suggesting that dense ground cover may compete with oak for available water. A lack of correlation between soil moisture, seedling growth, and transpiration suggest that other processes may play a role in seedling transpiration rates. Studies on bottomland oak transpiration rates were unable to identify trends during the first two years of outplanting (McCurry, 2006). Although my study was unable to pinpoint the

reasons red oak seedlings were able to colonize specific microsites, research suggests that the complex interactions between soil conditions, water use, and climate may be responsible (Hull and Wood, 1984).

Eastern redbud

Redbud demonstrated significantly better survival when surrounded by moderate amounts of herbaceous cover. This species survived best when cover surrounding the seedling was between 25 and 50%, similar to the other species studied in this project. For this species, the type of ground cover as well as the amount of cover influenced survival. The RCD growth of redbud decreased with increasing ground cover, suggesting they are relatively intolerant of competition as reported by Bagley and Shaffer (1992).

Virginia pine

Both survival and RCD growth of Virginia pine seedlings decreased with increasing ground cover, suggesting they are relatively intolerant of competition as reported by Bagley and Shaffer (1992), but tolerant of exposed conditions on this mine site. Virginia pine performed poorly in all cover classes for height growth. This is attributed to the high rates of browse that occurred on the stem leader, which may not have a lasting effect. McMinn and Crane (1984) suggest that Virginia pine will become a fast growing species during the fifth growing season.

5. Conclusions

Soil chemical properties did not differ at the scale of the treatment plots within the study area, but may have differed at the microsite scale. On this site, significant variation in soil moisture levels may have affected seedling and herbaceous establishment.

Three of the species studied, sugar maple, red oak, and Virginia pine will meet OSM revegetation standards of 60% survival on reclaimed mine sites after two seasons without modification of current reclamation practices. This study shows that planting these three species of seedlings 1.8 meters apart was adequate to ensure that stocking rates are met for bond release. Planting yellow-poplar 1-0 seedlings may not be beneficial because this species survived poorly and is readily able to invade from the surrounding forest. When planted in mixed stands Virginia pine is a very desirable browse for deer, which can help reduce the detrimental effects that herbivory has on the more valuable oak species.

My results suggest that reducing stresses associated with bareroot transplanting for yellow-poplar, northern red oak, and eastern redbud may result in increased survival rates. Methods should be investigated and implemented for reducing winter mortality rates, as this was the single largest identified factor in seedling mortality for all species.

The hypothesis that NWSG treatments allow for greater growth and survival of certain tree seedlings is partially supported by my results. My data suggests that seeding native warm season grasses will benefit the survival of outplanted seedlings used for forest reclamation projects on these mine sites as survival was generally highest in the NWSG treatments. However, there was no difference in seedling growth between treatments in this study. The exact mechanisms which are creating beneficial growing conditions for the outplanted seedlings were not identified during this project.

Higher rates of survival can be obtained by altering the environment surrounding seedlings. The hypothesis that a microsite can elicit a different response from

each species is supported by the findings of this project. The quantification of the microsite conditions required for seedling survival and growth has shed light on the importance of microsite associations. Soil respiration rates, soil moisture, and soil temperature, and seedling transpiration rates were unable to provide additional explanation for microsite seedling relations. My research findings emphasize that the absence or abundance of herbaceous cover surrounding each seedling was a consistent and important factor affecting tree survival. When forestry is the post mining land use, seeding to obtain moderate amounts of cover is most desirable. All tree species had better survival when growing in moderate amounts of herbaceous ground cover, while root collar diameter growth generally decreased with increasing cover. When seedlings are planted simultaneously with moderate amounts of herbaceous cover, optimal survival and growth rates can be obtained.

Management implications and recommendations

Efforts must continue to help reduce surface compaction and development of erosion rills and gullies on reclaimed sites for successful forest reclamation. My findings suggest native warm season grasses can benefit seedling survival, but are slow to establish and should be planted with low growing forb species to comply with the reduced ground cover regulations for forest reclamation in Tennessee. I suggest focusing planting on high value timber species such as oak, maple, hickory, walnut, and pine, as my observations of pre-SMCRA reclaimed mine areas along the Cumberland Plateau indicate that certain crop species may have difficulties invading on disturbed sites. Although average Virginia pine survival was slightly below 60% in this study, my research demonstrates that alteration of herbaceous cover type and ground cover density can improve survival when planted on drastically disturbed sites. I would recommend planting larger eastern redbud seedlings on minesoils exhibiting reduced surface compaction to ensure successful establishment. For all species,

I recommend obtaining seedlings with larger root collar diameters for planting on reclaimed surface mined areas in order to ensure high survival rates.

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Appendix A

Table 13: Overall seedling survival and each tree species survival within each of the five herbaceous treatments. Means in percent survival and standard error (se) of means presented. (Means with different letters are significantly different at $\alpha = 0.05$ using Duncan's technique.) YP = yellow-poplar, SM = sugar maple, NRO = northern red oak, ER = eastern redbud, VP = Virginia pine.

Species	p	Treatment				
		NWSG/forb	NWSG	Rye/trefoil	Rye/fescue	Control
All Spp	0.102	58	59	50	50	51
se		3.2	3.2	3.2	3.2	3.2
YP	0.552	17	21	29	29	20
se		5.9	5.9	6.6	6.6	6.2
SM	0.383	85	84	84	77	71
se		5.6	5.6	5.6	6.5	6.5
NRO	0.411	57	68	56	51	65
se		6.8	6.7	7.0	7.1	6.8
ER	0.635	54	50	44	39	46
se		6.8	7.5	5.2	7.4	6.8
VP	0.002	72B	73B	42A	54AB	48A
se		6.4	6.2	7.2	6.8	8.0

Table 14: Second year mean survival and standard error (se) of all seedlings and each species based on herbaceous cover class. (Means with different letters are significantly different at $\alpha = 0.05$ using Duncan's technique.) Herbaceous cover class zero is 0-1% cover, class one is 1-25%, class two is 25-50%, class three is 50-75% and class four is 75-100%. YP = yellow-poplar, SM = sugar maple, NRO = northern red oak, ER = eastern redbud, VP = Virginia pine.

Species	p	Cover				
		0	1	2	3	4
All Spp	0.000	47A	52AB	68C	58B	44A
se		2.7	3.2	3.1	3.5	3.5
YP	0.038	16A	22AB	41B	30AB	15A
se		4.6	6.0	8.2	7.6	5.9
SM	0.097	73	83	92	82	72
se		6.0	6.5	4.0	6.4	6.9
NRO	0.192	52	58	73	63	54
se		5.7	6.7	6.7	6.8	9.6
ER	0.000	28A	38AB	65C	56BC	51BC
se		5.7	7.2	6.5	7.7	8.1
VP	0.001	68B	65B	64B	59B	31A
se		5.7	6.4	7.8	8.8	6.8

Vita

John Rizza was born in Connecticut on April 29, 1981. He graduated from Salisbury School in 1999. From there he ventured to the Rocky Mountains of Colorado attending Colorado State University in Fort Collins. He earned the Bachelors of Science degree in Natural Resources Management with a minor in Forestry in December, 2003. After graduation, and to fulfill another life goal, he relocated to northern California where he worked as a Consulting Forester earning his Certified Arborist license in 2004. Deciding to further his education and knowledge of natural resources, John accepted a position as a Graduate Research Assistant at the University of Tennessee in Knoxville, TN. While at the University, he was awarded the University Chancellors Citation for Extraordinary Professional Promise. In May, 2007 he successfully earned the Masters of Science degree in Forestry. John is relocating to western Montana after graduation to continue his dream of working in the natural resource field.