EFFECTS OF MANAGEMENT AND SOIL FERTILIZATION ON TRIGGERING WIREGRASS REPRODUCTION

By

LISA L. SHAW

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To my son Matthew; always strive to achieve your goals, do your best and soar on wings like eagles.

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CHAPTER 1
INTRODUCTION

Longleaf Pine-Wiregrass Savannas

Longleaf pine (*Pinus palustris* Mill.) savannas are fire-maintained habitats found in the southeastern Coastal Plain of the United States (Fig. 1-1). These savannahs once stretched from southern Virginia to central Florida, and west to eastern Texas (Frost, 1993). The ecological persistence of these areas is a product of long-term interactions among fire, climate, soil type, and key plant traits. One of the major understory components in this ecosystem is wiregrass (*Aristida stricta* Michx.). This is a key species for maintaining healthy fire behavior in the habitat.

Figure 1-1. Pine Wiregrass Savanna at Twin Rivers State Forest (Hamilton County). (FNAI, 2010).

Prior to European settlement, longleaf pine-wiregrass communities covered nearly 25 million ha (Myers and Ewel, 1990; Gilliam, 2006). Changes in land-use and the exclusion of fires have caused large losses of longleaf pine stands (Boring et al., 2004).

As a result, only 770,000 ha of natural longleaf pine remain and only a fraction of that has intact understory vegetation (Fig. 1-2) (Ware et al., 1993). Reduced to less than 3% of its original range, longleaf pine-wiregrass represents one of the world's most endangered ecosystems (Frost,1993; Simberloff, 1993), and it presents one of the most critical challenges to conservation biology in the southeastern United States (Frost, 1993). In addressing land-use changes from longleaf pine, Gilliam and Platt (2006) developed interesting relationships with the decline of longleaf pine forests and population growth and the increase of other pine species plantations (Fig. 1-3).

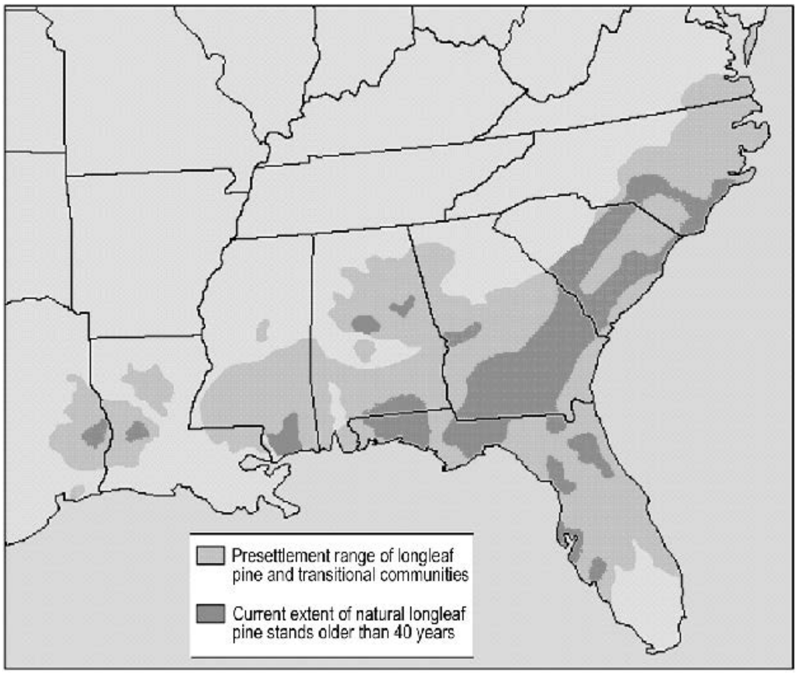

Figure 1-2. Proposed presettlement and current range of longleaf pine forests in the southeast U.S. (Trani-Griep, 2013).

Wiregrass Understory

Wiregrass in the longleaf pine ecosystem exists along the southern portion of the Coastal Plain (from southern South Carolina to Florida and west to Mississippi). The wiregrass taxonomic designation is somewhat unclear, where Peet (1993) reports that *A. stricta* Michx. (Poaceae) is confined to central North Carolina and parts of South Carolina, while *A. beyrichiana* Trin. & Rupr. coverage occurs from southern South Carolina to Florida. The most observable difference between the two is that *A. beyrichiana* has woolly bearding or hairs present at the leaf base and surrounding the collar (Peet, 1993). Even so, the Institute of Systematic Botany, University of South Florida (host site for the Atlas of Florida Vascular Plants) uses the *A. stricta* designation for speciation. The Florida water management districts often use the *A. stri*cta designation, as well. The Atlas of Florida Vascular Plants assigns *A. beyrichiana*, *A. stricta* var. beyrichaiana, and *Chaetaria stricta* as synonyms. For clarity purposes, 'wiregrass’ will be the term used in the remainder of this report.

Wiregrass is often associated with open canopy pine forests and woodlands composed of varying densities of longleaf pine and occasionally slash pine (*Pinus elliottii*). It can also occur in open, wet, treeless savannas and pitcher-plant depression meadows (Clewell, 1989). Wiregrass survives well in unfertile, sandy and sandy loam soils, such as pine flatwood soils or Spodosols (Uchytil, 1992), but clay hills (Ultisols) and other more fertile soil types can also host wiregrass if succession-suppressive fire is allowed. Peet (2006) developed a classification of longleaf pine vegetation using geography, soil moisture, and soil texture, and described six broad categories of longleaf community types. In order of increasing soil moisture, these are: 1) Xeric sand barrens and uplands; 2) Subxeric sandy uplands; 3) Silty uplands; 4) Clayey and rocky uplands; 5) Flatwoods; 6) Savannas, seeps, and prairies.

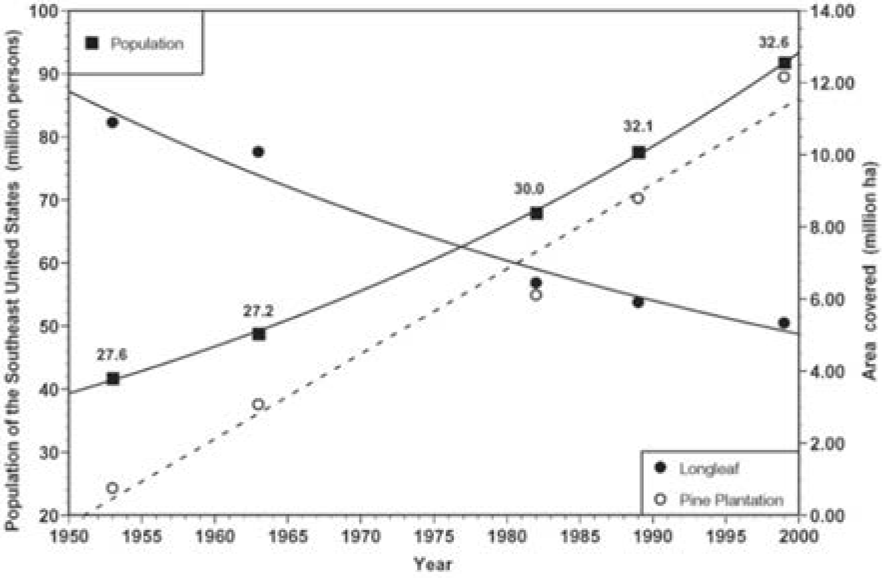

Figure 1-3. Changes in human population (exponential; r^2=0.9995) in the southeast US, compared to *Pinus palustris* strands (exponential; r^2=0.970), and pine plantations (linear; r^2=0.970). Numbers above the population symbols represent southeastern proportion of entire US population. Figure by Gilliam and Platt (2006), using data from Ware et al. (1993) and Wear and Greis (2002).

Fire exclusion in this ecosystem typically results in a successional change from herbaceous understory to shrubs and hardwoods. With greater moisture and fertility (such as might exist in Ultisols) succession can happen rapidly (4 to 8 years), as reported by Tall Timbers Research Station, FL (Christensen, 1988). Wiregrass can tolerate some shade under hardwoods, but within 20 to 40 years, the stand may be completely eliminated (Clewell, 1989).

A more typical place to find a longleaf-wiregrass community is in the Florida sandhills. The Florida sandhills have well-drained, infertile, sandy soils that are susceptible to nutrient leaching, which contributes to their infertile condition. Sandhill habitat is open, with a low understory. The open canopy created by longleaf pine and smaller oak species allows ample of sunlight to reach the forest floor, permitting a variety of grasses and other herbaceous plants to grow. The understory is predominately wiregrass but it may also include a diverse array of other herbaceous plants and grasses, such as little bluestem (*Schizachyrium scoparium* (Michx.)), broomsedge bluestem (*Andropogon virginicus* L.), hairawn muhly (*Muhlenbergia capillaris* (Lam.) Trin), indiangrass (*Sorghastrum spp*.), oblongleaf twinflower (*Dyschoriste oblongifolia (*Michx.) Kuntze), narrowleaf silkgrass (*Pityopsis graminifolia* (Michx.) Nutt.), pineland silkgrass (*Pityopsis aspera* (Shuttlw. ex Small)), scaleleaf aster (*Symphyotrichum adnatum* (Nutt.) G.L. Nesom), bracken fern (*Pteridium aquilinum* (L.) Kuhn), goldenrod (*Solidago spp.*), squarehead (*Tetragonotheca helianthoides* L.), soft greeneyes (*Berlandiera pumila* (Michx. Nutt.), yellow jessamine (*Gelsemium sempervirens* (L.) W.T. Aiton), and rice button aster (*Symphyotrichum dumosum* (L.) G.L. Nesom) (FNAI, 2010). The ability for these pine savannahs to provide a rich plant understory, make them among the most species-rich plant communities outside of the tropics (Peet and Allard, 1993).

To maintain the sub-climax longleaf-wiregrass community, routine fires are required. Lightning induced fire has been a natural occurrence in the longleaf pine ecosystem, taking place every two to eight years (Outcalt et al., 1999). Fire is carried by lower pine needles, wiregrass, and other understory plants to create an open savannah. The bare mineral soil allows for wiregrass and other understory seed establishment, soil nutrient cycling, and promotes new shoot growth from existing vegetation. Fires move slowly in a longleaf-wiregrass habitat, until being extinguished by rain or lack of fuel.

These fires are mostly low-intensity surface blazes, where the fire remains far from the crown of existing trees, so the heat rarely kills them.

Between 1880 and 1920 the lumbering industry in the south was thriving, and by 1930, nearly all old-growth longleaf had been harvested (Outcalt, 2000). A misguided attempt was made to protect the remaining forests by implementing a policy of fire suppression. However, without fire, wiregrass was being replaced with thick litter layers of pine needles and hardwood species. A thick litter layer may suppress wiregrass seed germination. Additionally, fire suppression often results in a more heat-intensive fire when it eventually occurs that can damage or kill fire resistant tree and understory species. As the health and abundance of the ecosystem continued to decline, ecologically sound restoration and management practices became crucial for recovering pine savannas. In 1993, USDA Forest Service Southern Research Station initiated a Longleaf Pine Ecosystem Restoration research program (Kush et.al., 1996). Prior, in 1991, Tall Timbers Research Station and Land Conservancy's conference focused on the longleaf pine ecosystem, restoration, and management (Hamrick, 1993).

Wiregrass Restoration Efforts

In well maintained longleaf savannahs, wiregrass accounts for up to 90% of the understory cover (Christensen, 1977). It is considered a keystone species in this pine community because of its structural dominance (Glitzenstein et al., 1995) and its function as a high-quality fire fuel source (Outcalt et al., 1999). It also contributes to soil organic matter, which improves soil structure, moisture, and nutrient holding capacity (Outcalt, 1994). Its presence promotes biological diversity, including animal species that depend on it for food and shelter (Outcalt et al., 1999; Hardin and White, 1989). Recent efforts to restore characteristic structure and function to longleaf systems include the reintroduction of wiregrass as a primary understory species.

The deterioration and loss of longleaf-wiregrass habitats necessitates establishing maintenance and restoration projects. Much focus has been on the regeneration and establishment of wiregrass (*Aristida* sp.) as a means to reestablish ecological function and structure in the ecosystem (Mulligan et al., 2002). However, cultivating Florida native grasses can be challenging. Many grass species, including wiregrass, have poor seed production and viability (Pfaff and Maura, 2001). Rates of germination (seeds planted in the field) are unpredictable and frequently fall below 10% (Outcalt, 1994; Mulligan et al., 2002). In addition, native seedlings, such as wiregrass, have difficulty competing with the abundant number of weed species dominating disturbed lands. Wiregrass evolved within fire ecology, and therefore it requires fire to produce ample, viable seeds (Outcalt, 1994).

Many longleaf-wiregrass restoration projects are underway in Florida. Public agencies and private conservation groups are investigating ways to use wiregrass to revegetate native habitat. The Northwest Florida Water Management District (NWFWMD) initiated a Florida Panhandle land purchase and restoration program in 1997, in order to restore pine plantation land to longleaf-wiregrass habitat along the Econfina Creek and surrounding sandhills, located in Bay County, Florida. Over 40,000 acres of land in the Econfina Basin have been purchased for restoration and 8,000 acres have been converted back to their natural state. The NWFWMD restores 800-1,000 acres of pine plantation to longleaf pine savannas each year (NWFWMD, 2008).

Even though water quality is the prime objective of NWFWMD in purchasing the land, restoring native habitat is an important objective, as well. A typical restoration effort includes clear-cutting nuisance species, such as slash and sand pines, in spring and preparing the land for longleaf pine plugs at a density ranging from 500 to 1,250 plugs ha^{-1} (Vaughan, 2001). Any re-growth by sand pines or other hardwood seedlings are cut and/or treated with herbicide. Prescribed burning is then used for maintenance on a biannual rotation. The NWFWMD has been using mycorrhizal-inoculated longleaf pine saplings to ensure successful establishment, but the supply of wiregrass seed and successful plant establishment has been erratic. Land managers at the Apalachicola Bluffs and Ravines Preserve have also determined wiregrass re-establishment is more of a challenge than pine re-establishment in longleaf-wiregrass ecosystems (Vaughan, 2001).

Wiregrass Propagation

A dependable supply of wiregrass seed is needed to meet the growing restoration demands in Florida. A decade ago, cultural methods that maximize viable seed production and stand longevity needed to be developed (USDA NRCS PCM, 1999) and little has changed since then. Wiregrass seeds are usually ready to collect from mid November to mid December. Seed yields on dry day collections (relative humidity < 75%) are twice as great as those on humid days (Pittman, 2000). Seeds should be held at ambient temperatures for 5 months but used within 8 months to improve seed germination (Hermann, 1999). Seed viability drops precipitously, thereafter. Even with careful collection strategies, seeds collected from nursery plants often demonstrate low viability (Pittman, 2000).

Although wiregrass requires fire to produce viable seed, burning is not always feasible. Limits on when and where to burn and land that is located in more urban areas, are some of the challenges one might face when relying on controlled burns for inducing wiregrass seed production. Other means of vegetation removal, such as clipping, may be a practical alternative in some cases. Parrott (1967) reported that wiregrass will flower when clipped. Pfaff et al. (2001) reported that clipping wiregrass was just as effective at stimulating the production of reproductive culms as burning. Vegetation removal, via clipping, most likely affects plant signaling in some manner. However, the mechanism has not been reported at the time of this publication.

Direct seeding is the most economical method for restoring wiregrass to the landscape. However, few, if any, commercial seed sources exist, as most propagators gather their seed from off-site locations (Bissett, 2006). There are many challenges in commercially producing wiregrass seed. Besides having a fire requirement, wiregrass can have low seed production and viability. Secondly, seeds are light and chaffy, which makes harvesting difficult with conventional equipment. Thirdly, wiregrass seed often lack seedling vigor and they do not compete well against weed species.

Wiregrass plugs are often established from field collected seed. It is an expensive source of planting material. At Andrews Nursery, Chiefland, Florida, Pittman and Karrfalt (2000) developed the following wiregrass propagation procedure for Florida Water Management Districts, as an alternative to natural recruitment. Bareroot production proved to be less efficient, than plugs. In either case, seeds were sown after the last frost, in a coarse, soilless mixture containing controlled release fertilizer. Seeds were first mixed with medium- to coarse-grade vermiculite (150 g seed per 0.12 m^3

vermiculite) and the mixture spread upon the growing tray surface. Once watered, the trays were placed outside and covered with a layer of shade cloth to protect the seed from washing out of the trays.

The seeds germinate and grow through the shade cloth. The shade cloth is removed after the seedlings have grown about 25 mm (1 in) tall. Plugs are ready for transplanting in approximately 3 months, although they may remain at the nursery for 12 months or more. After approximately 5 months, most plants begin flowering. Seedling plugs can be kept refrigerated for up to a month. Seedling production costs ($170 per 1,000 plants) are less than 15% of the cost of vegetatively propagated plants collected in the wild (Pittman and Karrfalt, 1998).

Wiregrass restoration via plugs is labor intensive and costly. For example, it cost $7,400/ha (approximately $3,000/acre) for the Apalachicola Bluff and Ravines Preserve (ABRP) restoration project (Vaughan, 2001). Plug can also introduce weeds to the restoration site. We found broomsedge (*Andropogon viginicus* L) contamination of over 50% of wiregrass plugs developed for a NWFWMD restoration project in 2011. Without the proper training to identify plug contamination, the weed species get planted along with the wiregrass, where selective grass weed control is difficult. At ABRP, it was found that direct seeding was a successful alternative for restoring wiregrass in cleared and leveled slash plantation sites, and it was more cost effective than planting seedlings or plugs (Hattenbach et al., 1998).

At two Florida restoration sites (Reedy Creek Mitigation Site and Florida Gas Transmission Anclote Mitigation Site), it was found that wiregrass establishment success depended mostly on maintaining soil moisture after seeding and controlling exotic aggressive grasses (Bissett, 2006). Wiregrass plant density also increased from 3% coverage during the first year to 42.9% coverage after burning. The authors suggested that providing 1 to 2 years rest between burns would likely increase the survival chances of natural wiregrass recruits. Field planted wiregrass plugs near 6 months old were believed to have reached a developmental stage that was key for survival in the field (Mulligan and Kirkman, 2002). Burning the planted plugs after a two year establishment period was not harmful (Aschenbach et al., 2010).

Wiregrass Nutrient Management

Longleaf-wiregrass savannas are commonly found on infertile sands, as typified by the Leon series (Aeric Haplaquods), and excessively drained soils, such as Lakeland series (Typic Quartzipsamments). This requires plant adaptations for successful survival. Most of the wiregrass plant biomass is located in root tissues (75 to 80% belowground, with 55 to 60% in the top 5 cm of soil) (Parrott, 1967). Therefore, wiregrass has a dense, but shallow root system. Most roots are within 20 to 45 cm of the soil surface (Uchytil, 1992). The fine roots capture more nutrients per unit soil volume than coarse roots, and therefore are less dependent upon arbuscular mycorrhizae fungi (AMF) for nutrient and water capture. Even so, mycorrhizal filaments are much smaller than roots and have a greater surface area, which allows their hyphal networks to explore many meters within a single gram of soil (Leake et al., 2004).

Arbuscular Mycorrhizae Fungi

Mycorrhizae are symbiotic associations between fungi and plant roots. More than 90% of all plant families (80% of species) form mycorrhizal associations (Solomon, 2010). Mycorrhizae have 2 broad classifications: ectomycorrhizae and endomycorrhizae, which are based on the location of the fungal hyphae in relation to the roots; ecto meaning outside the root and endo meaning inside.

Mycorrhizae are placed within the Glomeromycota phylum of the Fungi kingdom. Within glomeromycota there are endomycorrhizae and ectomycorrhizae. There are several types of endomycorrhizae, including Arbuscular, Ericoid (including 2 subgroups; Arbutoid and Monotropoid), and Orchidaceous. Arbuscular mycorrhizal fungi (AMF) form arbuscules and vesicles that penetrate plant root cortical cells. In comparison, ectomycorrhizae will completely surround a root with a fungal sheath. Many common mushrooms (ascomycetes and basidomycetes) are examples of ectomycorrhizae. Ectomycorrhizae display varying degrees of host specificity, with some fungal species being able to colonize many plant species, along with some tree species hosting many different mycorrhizal species. Even so, most ectomycorrhizal plant associations are with one or two mycorrhizal species within a given area of root. Ectendomycorrhizae exhibits characteristics of both ectomycorrhiza and endomycorrhiza).

Members of the Family Pinaceae, including, *P. palustruis,* have predominantly ectomycorrhizal fungal (EMF) associations (Malloch and Malloch, 1981; Harley and Harley, 1987; Brundrett et al., 1990). However, pine forests are known to host both, endo- and ectomycorrhizae. In comparison, AMF are commonly associated with grasses, row crops, vegetables, and shrubs. Since most AMF tend not to be host-specific (van der Heijden, 2004), it is likely that wiregrass supports mycorrhizal associations, due to local AMF abundance within the pine savannah habitat. There is little information available in the literature to either support or dispute such an assertion. Even so, Mullahey and Speed (1991) reported AMF root colonization on wiregrass.

Arbuscular mycorrhizal fungi facilitate the capture and uptake of nutrients from the soil, especially N and P (Hodge and Fitter, 2010) and produce enzymes involved in the extraction of minerals from the soil particles and chelate nutrients. In low-nutrient soils many plants are unable to grow without this association (Schultz et al., 2001). The amount of available soil P often determines a plant’s dependency on mycorrhizal fungi (Schultz et al., 2001). Areas that have been disturbed by compaction, weeds, erosion, removal of topsoil, mixing, and land clearing, often lack sufficient mycorrhizal populations to promote plant establishment and growth (Amaranthus and Perry, 1994; Amaranthus et al., 1996; Page-Dumroese 1997)

The interaction between plants and AMF has been regarded as mutualistic, (Smith and Read, 1997). However, the costs and benefits of the plant/ AMF symbiosis is not always beneficial (Powell et al., 1982; Jensen, 1984; Haas et al., 1987; Raju et al., 1990; Streitwolf-Engel et al., 1997; van der Heijden et al., 1998a; Klironomos 2000). Whether plants benefit from the association depends on a number of factors, including the genotypes of the interacting organisms, and their environmental conditions. (Klironomos, 2003).

Plants and AMF communities are locally adapted and therefore, local plants have more positive responses to local AMF then to non-native AMF (Klironomos, 2003). When addressing native plants grown in soils with native AMF, the number of negative and positive responses was approximately equal (Klironomos, 2003). In areas where remnant native (as compared to nursery grown) plants are present, and soil aggregation is good, with adequate moisture and nutrients, then native mycorrhizal populations may be relatively high (Wilson et al., 2009) and commercial inoculants may not be necessary. However, at phosphorus deficient sites, AMF often benefits plants (Anderson et al., 1994). Several studies (container and field) demonstrate the benefits of AMF (van der Heijden, 2004; Amaranthus and Steinfeld, 2003; Van Auken, 1998).

Arbuscular mycorrhizae fungi have been propagated for commercial use. The most commonly found commercial inoculants include one or more of the following: *Glomus intraradices* N.C. Schenck & G.S. Sm., *G. mosseae* (T.H. Nicolson & Gerd.) Gerd. & Trappe*, G. aggregatum* N.C. Schenck & G.S. Sm. emend. Koske*, G. fasciculatum* (Thaxt.) Gerd. & Trappe emend. C. Walker & Koske*, G. deserticola* Trappe, Bloss & J.A. Menge*, G. etunicatum* W.N. Becker & Gerd.*, G. brasilianum* Spain & J. Miranda*, G. clarum* T.H. Nicolson & N.C. Schenck*, G. monosporum* Gerd. & Trappe*, and Gigaspora margarita* W.N. Becker & I.R. Hall. These commercial inoculants are added to seed during seeding. In perennial grasslands, van der Heijden (2004) inoculated seeds of four plant species (two grasses: *Bromus erectus* Huds. and *Brachypodium pinnatum* (L.) P. Beauv. and two forbs: *Prunella vulgaris* L. and *Trifolium pratense* L.) that were planted into old grassland field plots. Several AMF taxa were used. The AMF inoculated plots produced larger seedlings that took up more phosphorus. Varying amounts of phosphorus were obtained by seedlings inoculated with different AMF taxa. The AMF also promoted seedling growth.

Amaranthus and Steinfeld (2003) grew *Agrostis pallens* (dune bent grass) in containers to compare AMF with slow-release fertilizers. They found that the inoculated grass seedlings had 100% survival after two months, while the other treatments (untreated, treated with slow-release fertilizer, or treated with slow-release fertlizer + AMF) resulted in lower survival rates. The lowest survival rate (17.2%) was with the untreated plants. Furthermore, after one year, seedlings inoculated with mycorrhizae had greater shoot and root biomass and higher nutrient content. Sparse colonization occurred on plants receiving fertilizer-only treatments. Seedlings inoculated with mycorrhizae in the nursery also had high nutrient concentrations, as compared to other treatments. Foliar concentrations of P, K, Ca, and S were greater in inoculated plants. In comparison, only nitrogen uptake was greatest in the slow-release fertilized treatments.

Van Auken (1998) examined AMF effects on dry mass production of two native Texas grasses, *Aristida longiseta* Steud. and *Nassella leucotricha* (Trin. & Rupr.) Pohl, when comparing with and without fertilizer (fertilizer treatment =0.2 g N pot^{-1} as NH_4NO_3, 0.15 g P pot^{-1} as N_2HPO_4, 0.1 g K pot^{-1} as KCl and 0.04 g S pot^{-1} as $MgSO_4$). Each pot was packed with 1,400 grams of dry Patrick series soil. Fertilizer reduced the mycorrhizal infection in *A. longiseta* and *N. leucotricha* by 62% and 29%, respectively). In comparison, less fertile soil, particularly low phosphorus, tends to stimulate root infection (Hetrick et al., 1990; Miller and Allen, 1992; Graham and Eissenstat, 1994).

Fire effects on soil fertility and AMF

Nitrogen is often the most limiting plant essential nutrient in temperate forests, and its availability is low in fire dominated ecosystems, such as longleaf pines. The nature of recurring fire disturbance in these ecosystems contributes to their low fertility, in part through C and N volatilization losses during fires (Hainds et al., 1999; Wilson et al., 2002). Fire dynamics and ecosystem responses are complex, and can include short-term pulses of available N and P in the soil (Wilson et al., 2002). Otherwise, these nutrients are often in an organic, where mineralization controls the amount of N and P availability to plants. Burning can occur almost any time of the year but late spring through summer tends to result in the greatest seed head formation (Fig. 1-4).

Although N is volatilized during landscape fires, Boring et al. (2004) found that losses during a dormant season burn can be balanced by other inputs (legume biological dinitrogen fixation) providing addition N storage in soil and biomass. This makes legumes an important understory component of the longleaf-wiregrass community. Other plant essential nutrients, such as Ca, K and Mg are released in the ash, and may be important for stimulating production of viable wiregrass seed.

Fires in longleaf-wiregrass landscapes often result in slight increases in soil P (Anderson and Menges, 1997; Boring et al., 2004), that also translates in increased tissue N and P (Lavoie et al., 2010). Greater Mg and B were also found in wiregrass tissue, following a burn. Soil and tissue nutrient differences generally dissipated quickly (3 months), with the exception of N effects remaining somewhat longer. Further studies (Wilson et al., 1999; Carter and Foster, 2004; LaJeunesse et al., 2006) reported fire enhanced soil nutrient availability lasting for more than one season, but others reported that the effects were short-lived or nonexistent (Boring et al., 2004; Anderson and Menges, 1997).

A favorable response from grasses following fire is most likely due to their ability to store nutrients in belowground structures and their capabilities to resprout following fire (LaJeunesse et al., 2006). Mycorrhizal colonization can be reduced by the burning of pine savannas. The nutrient flush of inorganic nutrients after burning can inhibited mycorrhizal colonization in tropical grasslands (Janos, 1980). Klopatek et al. (1988) attributed reduced colonization of plants in a burned pinyon– juniper woodland (Arizona), to loss of mycorrhizal inoculum in upper soil layers (litter and duff) due to burning. Similarly, Dhillion et al. (1988) and Dhillion and Anderson (1993) reported lower percentages of AMF colonization of plant roots from burned sand prairie sites in Illinois than of plant roots from unburned sites. However, they also suggested that root growth surpassed the rates of AM colonization to a greater extent on burned sites than unburned sites (AMF dilution effect). In comparison, there was an increase in mycorrhizal activity from burned Kansas grasslands compared to unburned grasslands (Bentivenga and Hetrick, 1991). It is unclear as to the cause of AMF behavioral differences reported here. Differences may be related to changes in root growth rate, available natural inoculum, and/or the fire effects on other soil processes and chemistry.

Figure 1-4. Controlled burning of wiregrass plots at Hobbs Field, Econfina Tract, Bay County, July 2007.

Commercial fertilizer use

As with agricultural crops, the 4Rs of nutrient stewardship is a valuable framework to follow for vegetation restoration. The International Plant Nutrition Institute, along with other global institutions, has helped to develop and promote the 4R mission. Fertilizer 4Rs consists of 1) right source, 2) right rate, 3) right time, and 4) right place. Nutrient sources, via mycorrhizae, ash from fires, or slow-release fertilizer products have been addressed in the last section. Attention will now turn to conventional commercial fertilizer rates and timing.

Little is known about the effect soil fertility has on supporting viable wiregrass seed production in the field or commercially. In some studies wiregrass seedling growth was promoted by a combination of site preparations and fertilizer amendments (Outcalt et al., 1999). While in other studies, early succession species benefited more from fertilization applications than did wiregrass (Kalmbacher and Martin, 1996). Different fertilizer timings, such as applications in the second and third growing season (Outcalt et al., 1999) and applying it in close proximity to the wiregrass plants will reduce the likelihood of stimulating competing vegetation growth, while promoting wiregrass growth.

Pfaff et al. (2001) found single applications of no response of wiregrass to P and K applications. Although their soil K was low, soil P at that site was over 100 mg kg^{-1}. Following burning in July, a single application of 56 kg ha^{-1} N or N+ K resulted in no significant increase in seed production, and a double rate of N and K the following year responded similarly. Transplanted wiregrass seedlings fertilized with a low but undisclosed amount of 12–12–12 (N-P_2O_5-K_2O liquid fertilizer) prior to transplanting, exhibited good (67% after 1 and 2 years) survival rates (Aschenbach et al., 2010). However, survival rates declined when planted with other species. This suggests resource competition or direct competition with other plant species (Aschenbach et al., 2010; Milligan and Kirkman, 2002). Fertilization (three annual applications of 0, 40, 80, or 120 kg N ha^{-1}; 0, 25 kg P ha^{-1}; 0 or 100 kg K ha^{-1}) at Florida flatwood sites, promoted the proliferation of goldenrod, dogfennel and beaked panicum (early successional species) while decreasing wiregrass densities (Kalmbacher and Martin, 1996).

Jenkins et al. (2004) investigated seed germination and establishment of 5 Florida native grasses: *Paspalum setaceum* Michx.*, Panicum anceps* Michx.*, Paspalum distichum* L.*, Eustachys petraea* (Sw.) Desv.*, and Eragrostis refracta* (muhl. Scribn., either direct seeded or planted as seedlings that were treated with irrigation, fertilizer, weed control, and mowing. This study found weedy species to dominate coverage and interfere with native cover establishment. Therefore, the authors concluded that reducing the seedbank of competing species would have been more beneficial than fertilizing 900 kg ha^{-1} of 12-8-8 (108 kg N ha^{-1}, 31 kg P ha^{-1}, 60 kg K ha^{-1}) in the spring and fall), which did not result in greater native grass herbaceous cover. Similarly, an annual fertilizer application of 2,722 kg ha^{-1} of a 5–10–15 (136 kg N ha^{-1}, 120 kg P ha^{-1}, 340 kg K ha^{-1}) did not benefit wiregrass seedling survival or growth and reduced survivorship of 3 week old seedlings (Mulligan and Kirkman, 2002). However, they report that extremely dry conditions during the study period may have masked results, due to competition for water directed growth and survival patterns. Even so, excessively high fertilizer applications will not likely achieve the intended response of promoting wiregrass growth, when there are competing weeds. Better use of moderate fertilizer rates might benefit wiregrass growth and reproduction, if the soil fertility is inherently low. No reports were identified that addressed soil fertility effects on wiregrass reproduction.

Triggering Wiregrass Reproduction

A study was conducted to determine if wiregrass reproduction (via reproductive culm production) could be triggered in a natural habitat, through changes in soil fertility and above-ground management.

The objectives were:

- Determine if increased soil nitrogen and phosphorus improved wiregrass growth and reproductive culm (head) production.
- Determine if clipping (mowing) improved wiregrass seed head production, as compared to burning.
- Determine if there were AMF associations on wiregrass roots and if management affected AMF numbers.

Little information exists about the establishment of native longleaf pine understory species (Jenkins et al., 2004). Results from this study provide a greater understanding of wiregrass response to nutrients (N and P), physical factors (burning and clipping), and mycorrhizal associations. The information can be used to increase commercial propagation material and perhaps seed production, under urban land management (i.e., fire restrictions).

CHAPTER 2
WIREGRASS FIELD STUDY MATERIALS AND METHODS

Study Area

This study was conducted in the spring/summer of 2007 and 2008 on Northwest Florida Water Management District Land located at 30.350o N, 85.561o W, in T2S, R13W, Section 5 and T1S, R13W, Section 32 (Powerline). The sites were within the 16,592 ha (41,000 acres) Econfina tract, located in the Sandhill Lakes region of Bay County, Florida, USA. These lands are part of a land acquisition and restoration program that began with property purchased along Econfina Creek in 1992.

Site topography is mainly flat. Soils are sandy, nutrient-poor, and well-drained. The soils at both locations are a Foxworth sand; Thermic, coated Typic Quartzipsamments, with a seasonal high water table at 3.5 to 6 feet below the surface from June through October, featuring low to very low available water capacity (Soil survey staff, 2013).

The dominant undisturbed vegetation is sandhill (FNAI, 1990); also referred to as high pine (Myers and Ewel 1990), consisting of widely-spaced longleaf pines (Pinus palustris), with an open to moderately dense mid-story of hardwoods, most commonly turkey oak (*Quercus laevis* Walter), but occasionally other woody species, such as sand live oak (*Q. geminata* Small) and Florida rosemary (*Ceratiola ericoides* Michx.). Groundcover on the well-maintained sandhills consist mainly of wiregrass (*Aristida stricta* Michx.) and a diversity of other grasses.

The climate is characterized by hot, humid summers and moderate winters. The 30-year average temperature and precipitation is 20.4 C and 155 cm, respectively (1981 to 2010, National Climate Data Center, 2013). For 2007, annual average temperature and precipitation were 21.4 C and 108 cm, respectively. Mean temperature was nearly

5% greater and precipitation 30% below the 30-year average value. In comparison, 2008 annual average temperature and precipitation were near average at 20.2 C and 152 cm, respectively.

The longleaf pine savannas at the Econfina tract were converted to slash pine plantations and logged until 1989 (Earley, 2004). However, the tract is being restored as longleaf pine savannas with wiregrass. Two sandhill sites (approximately 445 m apart) having dense wiregrass understory and little overstory, were selected for this study. Land surrounding the sites was set aside by NWFWMD, as a wiregrass seed collection site soon after longleaf restoration began in the late 1980s. The experiment was repeated, 1) Hobbs Field in Year 1 and 2) Powerline Field in Year 2.

Treatments

The experimental plots were set up in a split plot design, with three different management treatments as the main plots (1.5 m x 6 m each) and four fertilizer treatments as the subplots (1.5 m x 1.5 m). Main plot treatments were 1) undisturbed =Control, 2) controlled burned = Burn, 3) mowing = Clip. Treatments were initiated in July 2007 for Year 1 and June 2008 for Year 2.

1) Control: Vegetation remained undisturbed
2) Burn: A drip torch ignited the main plots for the burn treatment. Water was used to extinguish any remaining fire, as needed.
3) Clip: A gas-powered, 0.53 m wide cut, push mower was used to cut vegetation to a 10 cm stubble height.

The subplots randomly received one of four fertilization treatments:

1) -N -P
2) +N –P

3) -N +P

4) +N +P

All fertilizers were applied soon after (on the same day) main plot treatments were established. Nitrogen treatments were applied as NH_4NO_3 (34-0-0) at 56 kg N ha^{-1}. Phosphorus treatments were applied as triple super phosphate (0-46-0) at 56 kg P_2O_5 ha^{-1}. Potassium was applied as KCl (0-0-60) at 22 kg K_2O ha^{-1} to all plots in Year 2 (none in Year 1). Treatments in both years were replicated four times.

Field Sampling and Data Collection

Year 1 (2007) site was located at what will be designated as Hobbs Field and in Year 2 (2008) the site was located 400 m north of Hobbs Field, at what will be designated as Powerline Field. Prior to treatment applications, 12 soil samples (0 to 15 cm depth) were collected at Hobbs Field on July 12, 2007. The 12 samples were collected from random points within the study area. They were collected using a soil probe (0 to 15 cm depth). The twelve soil samples were mixed into three composite samples. After the growing season, each subplot was sampled twice (2 composited subsamples), using a slide hammer and a 5 x 10 cm aluminum liner. Samples were collected in December 11, 2007 and May 28, 2008. The May sampling was to determine if there was residual N or P in the treatment plots. In Year 2, the Powerline field pretreatment soils were collected with a soil probe (18 composited samples per block) on June 13, 2008. After the growing season, each subplot was sampled twice (two composited subsamples) with a slide hammer and a 5 x 10 cm aluminum liner, on October 30, 2008.

Year 1 plant data was collected December 2007 and Year 2 data was collected October 2008. On-site measurements included plant density (crown number, total crown area, diameter per crown). Above-ground wiregrass biomass was determined by collecting clippings from plants (approximately10 cm stubble height) within a 0.25 m^2 (PVC square) from all treatment plots. Following collection, samples were placed in paper bags and returned to the lab. The plant material was used for determining dry mass production, percent dry mass, and tissue elemental content.

Laboratory Analyses

Above-ground biomass

In the laboratory, culms and spikes (heads) were separated from the above-ground biomass. All tissue was weighed, then oven-dried to constant mass (48 h at 70 C) and reweighed. Above-ground biomass yield and reproductive attempt (head mass/aboveground mass) were determined. Plants were analyzed for mineral nutrient content (P, Ca, Mg, N, K, S, Zn, B, Mn, Fe, and Cu) at a commercial laboratory, (Waters Agricultural Laboratory, Camilla, GA), via inductively coupled plasma atomic emission spectroscopy (ICP-AES). Tissue for nutrient analysis was collected from several plants within each plot.

Soil nutrients

Soil samples were air-dried and sent to a commercial laboratory (Waters Agricultural Lab, Camille GA) for Mehlich-3 extractable inorganic nutrients (Ca, K, Mg, P, S, B, Zn, Mn, Fe, Cu) via ICP-AES, total Kjeldahl N (TKN), water pH (1:1 v:v), and organic matter (loss on ignition or LOI).

Arbuscular mycorrhizal assessment

The second year (at the Powerline field) data were collected in July 2008. Within each subplot, portions of above and below ground biomass were collected for the mycorrhizal colonization assay. This assessment was only conducted in the second year.

1. Root samples. The intact plant samples were returned to the lab and the root portion rinsed with tap water to remove excess soil particles. Fibrous roots were then excised from the plant, using scissors. A portion of the excised roots were cut into 10-cm segments, then 20 root samples from each subplot were wrapped in 16 micron mesh and placed within a Tissue Path Cassette IV in preparation for clearing and staining (Fig. 2-1).

2. Clearing roots. Clearing and staining are essential for detecting and identifying AMF colonization in roots. The following procedures for clearing and staining came from sections Brundrett et al. (1996). Heating instructions were modified by Dr. Sharma at the University of Florida Institute of Food and Agricultural Science (UF/IFAS) North Florida Research and Education Center.

Figure 2-1. Wiregrass root segments being prepared for staining and observation.

Clearing and staining involved staining without the use of phenol. Steps in the techniques are: KOH 10% w/v used to clear the roots. Root samples were completely submerged in KOH solution. The samples were heated for 4 minutes in a microwave oven (at 1 minute intervals) (typically heated to 90ºC for 20-60 minutes) until the roots had softened. The KOH solution was removed and the cleared roots rinsed with tap water 3 times. A post-clearing bleaching rinse with 25% bleach for 1 minute removed phenolic compounds left in cleared roots (Bevege, 1968; Kormanik, 1982). The roots were again washed with tap water 3 times and then acidified with a 1% HCl for 5 min. and drained (not rinsed).

3. Staining roots. Cleared roots were stained with 0.05% Trypan Blue (800ml glycerin, 800 ml lactic acid, 800 ml distilled water, and 1.2 g Trypan Blue) (Bevege, 1968; Kormanik, 1982) and left to soak overnight. The stain was drained from the samples, then immersed in deionized water to de-stain.

4. Examination of stained roots. Stained roots segments were mounted on semi-permanent slides with a Poly-vinyl alcohol based (PVLG) mount, and observed under a dissecting and compound microscope. In order to determine if plants were producing mycorrhizal structures indicative of mycorrhizal activity, arbuscles and vesicles were identified. Hyphal occurrence was noted but not measured.

Statistical analyses

Statistical analyses were conducted using Proc Mixed in SAS 9.2 software (SAS Institute, Cary, NC). All treatments were significant at $P<0.05$, unless otherwise indicated. Biomass data were log transformed prior to analysis and results back-transformed for the figures. Treatments were compared with the Tukey-Kramer test.

CHAPTER 3
WIREGRASS FIELD STUDY RESULTS AND DISCUSSION

Plant Productivity Results

Seed head biomass

As wiregrass seed is quite small and it was exceedingly difficult to separate seeds from chaff, it was decided that seed heads (entire reproductive culm) would be used to represent potential reproductive success in this study. Locations were analyzed separately. Seed head biomass was affected by both, management and fertilization. The unchallenged (control) plots resulted in the least amount of seed heads at both locations (Fig. 3-1).

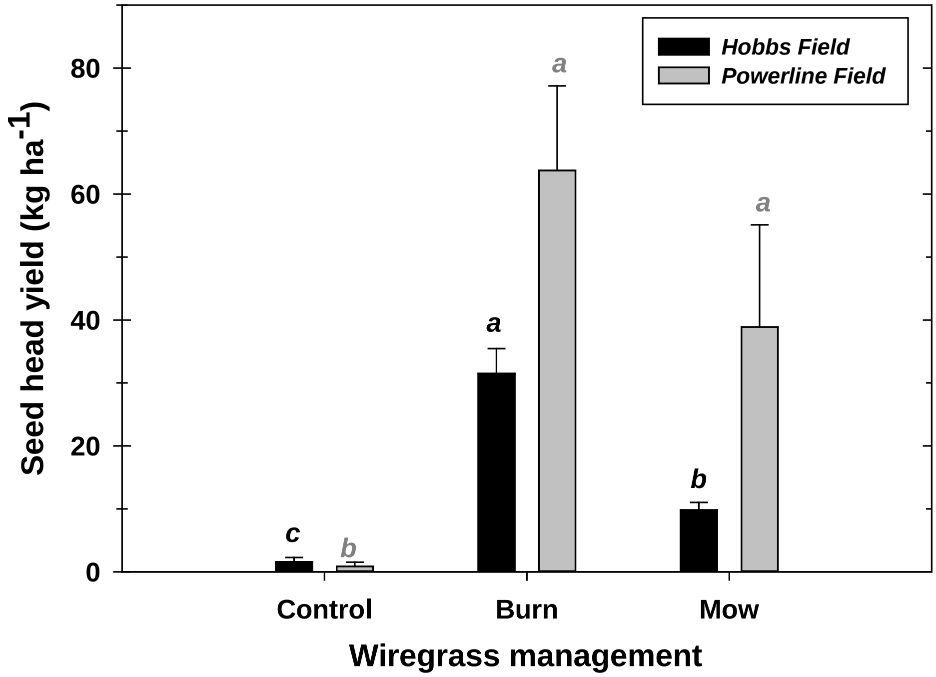

Figure 3-1. Plant management effect on seed head dry mass at Hobbs Field (2007) and Powerline Field (2008) (n=16). Bars=means ± standard error. Different letters of the same font color are significantly different ($P<0.05$), using Tukey-Kramer test.

Seed head production was greater (*P*<0.05) when 56 kg N ha^{-1} (50 lbs ac^{-1}) was used at the Powerline field in 2008. The same can be said about the Hobbs field in 2007, but at a lower level of significance (*P*<0.10) (Fig. 3-2). Phosphorus did not affect seed head yield at either location.

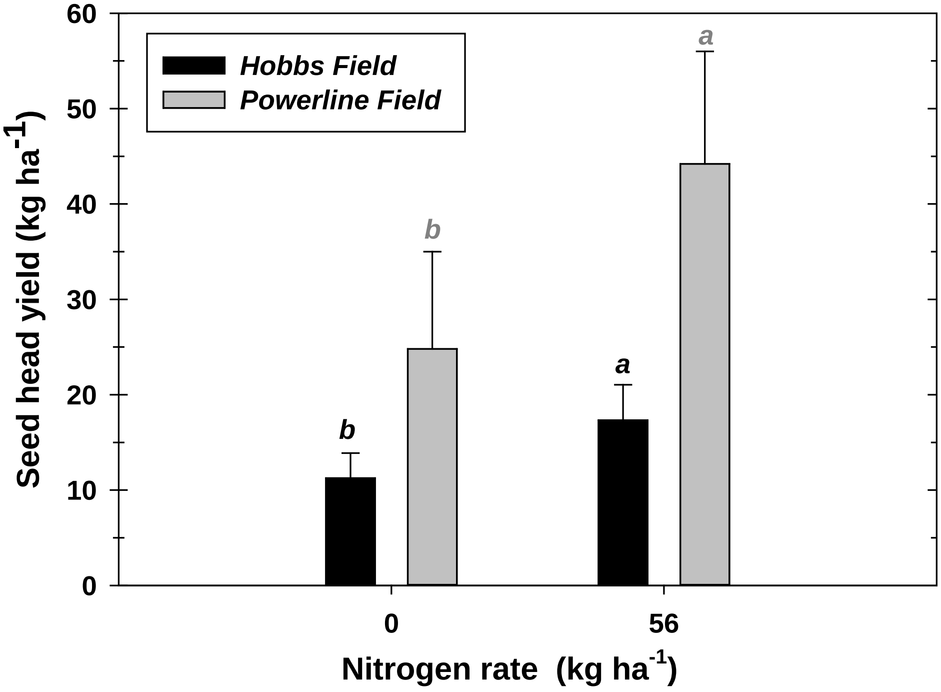

Figure 3-2. Seed head dry mass yield at Hobbs Field (2007) and Powerline Field (2008), as affected by N fertilization (n=24). Bars=means ± standard error. Different letters of the black font color are significantly different (P<0.10), using Tukey-Kramer test, while the gray font color letters are significantly different (P<0.05).

Vegetative biomass

At the end of the growing season, all vegetative biomass was collected from each subplot by using hand clippers. No effort was made to distinguish between old and new biomass. The undisturbed (control) plots resulted in the greatest shoot biomass at the end of the season (Fig. 3-3).

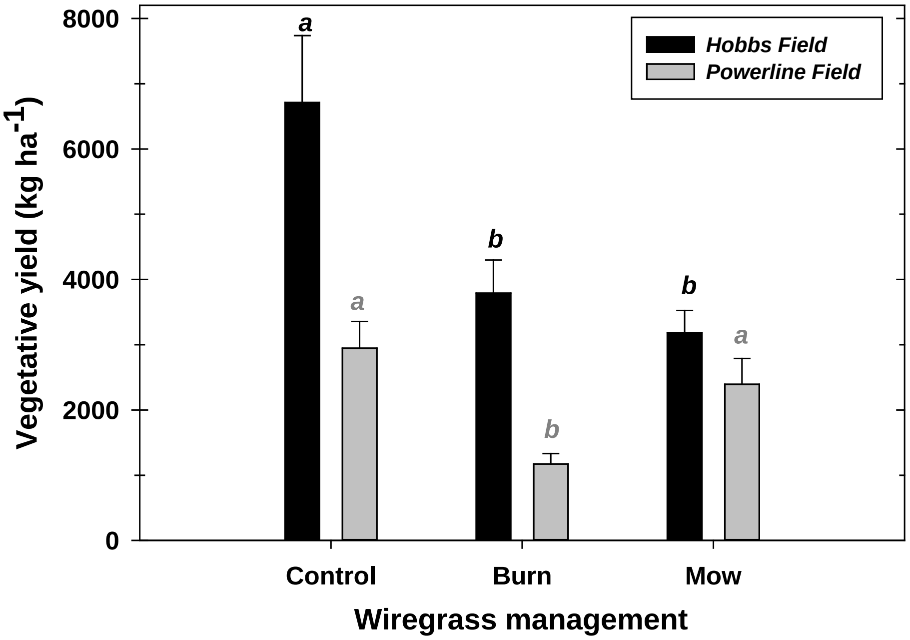

Figure 3-3. Plant management effect on vegetative dry mass at Hobbs Field (2007) and Powerline Field (2008) (n=16). Bars=means ± standard error. Different letters of the same font color are significantly different (P<0.05), using Tukey-Kramer test.

Above-ground, vegetative biomass responded to a 56 kg ha^{-1} N application by increasing biomass at the Hobbs field in 2007. The same response (although weaker) occurred at the Powerline field in 2008, at $P<0.10$ (Fig. 3-4). Fertilization with 56 kg P_2O_5 ha^{-1} did not affect wiregrass vegetative production at either location, which was similar to the seed head yield response.

Percent vegetative dry matter

The percent dry mass of the vegetative above-ground biomass was about 10% greater (73% vs 65%) for control plots, compared to challenged plots in the Hobbs field (2007). The control plots in the Powerline field were 82% dry mass and mowing was only 6% less, while burning was 10% less (Fig. 3-5).

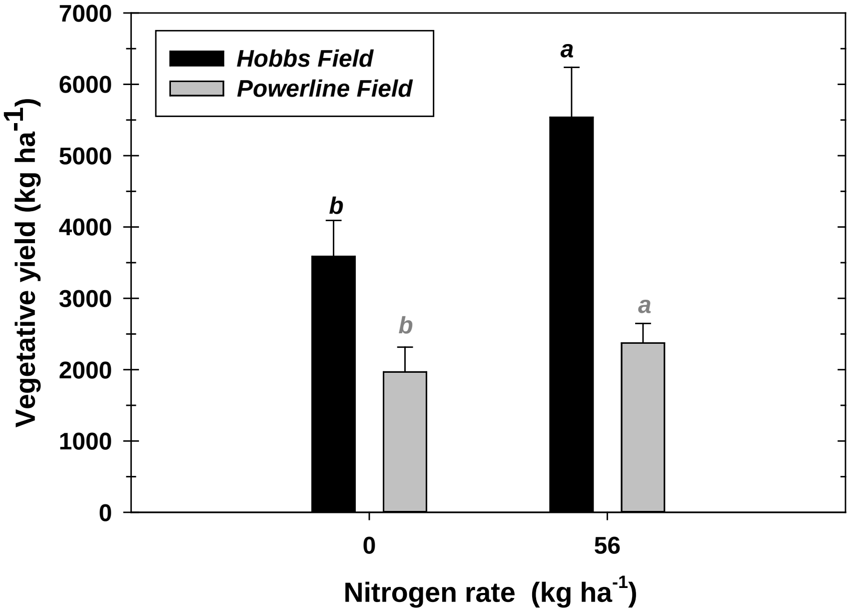

Figure 3-4. Above-ground, vegetative dry mass yield at Hobbs Field (2007) and Powerline Field (2008), as affected by N fertilization (n=24). Bars=means ± standard error. Different letters of the black font color are significantly different ($P<0.05$), using Tukey-Kramer test, while the gray font color letters are significantly different at $P<0.10$.

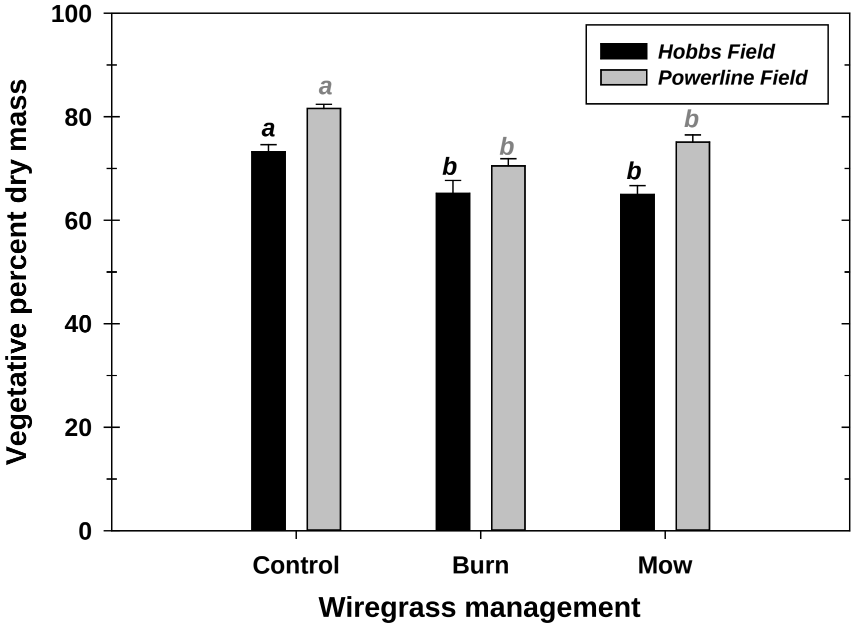

Figure 3-5. Plant management effect on vegetative percent dry mass at Hobbs Field (2007) and Powerline Field (2008) (n=16). Bars=means ± standard error. Different letters of the same font color are significantly different ($P<0.05$), using Tukey-Kramer test.

Tissue Composition Results

Reproductive culms

Tissue from seed heads were analyzed only from the Powerline field (2008). Sample size limited the number of samples that could be analyzed so box plots were used to represent fertilizer effects on seed head nutrient composition. Data included only a single control management treatment, while the remaining treatments were from burned and mowed plots. Applying N had no appreciable effect on seed head N concentration (Fig. 3-6). In comparison, the P fertilization trended towards greater seed head P concentrations (Fig. 3-7).

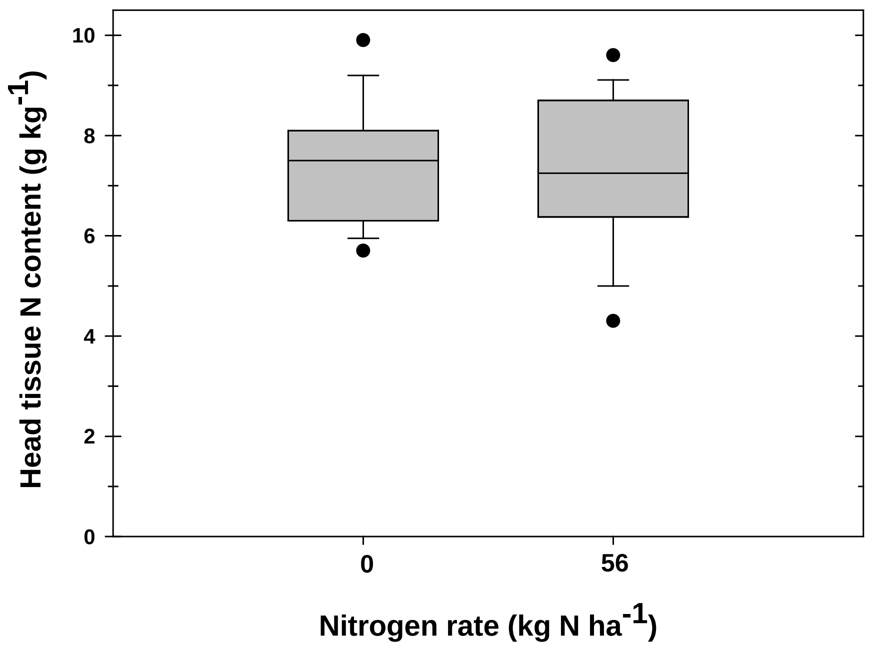

Figure 3-6. Box plots of N fertilization effect on seed head N concentration from Powerline Field (2008). Boxes=25th to 75th percentiles and horizontal lines=median values. The error bars=10th and 90th percentiles and closed symbols=outliers.

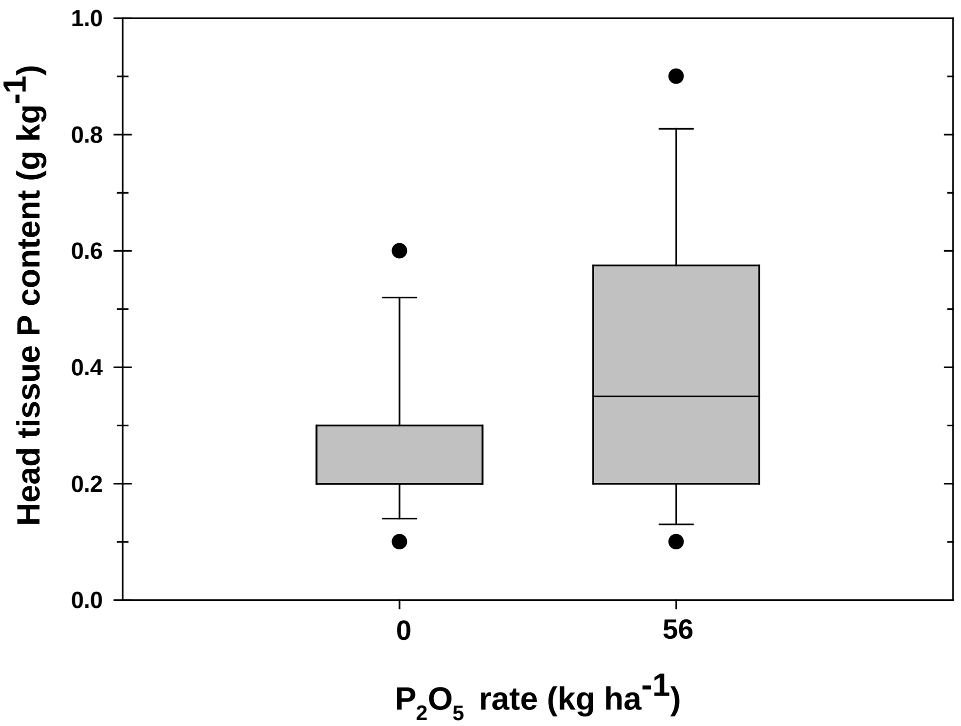

Figure 3-7. Box plots of P fertilization effect on seed head P concentration from Powerline Field (2008). Boxes=25th to 75th percentiles and horizontal lines=median values. The error bars=10th and 90th percentiles and closed symbols=outliers.

Above-ground vegetation.

As with the seed heads in 2008, the above-ground vegetation was analyzed for tissue N from the Powerline field (2008). There were no treatment effects on tissue N. Total Kjeldahl N (TKN) and concentrations averaged 6.3 g kg^{-1} across management and N fertilization treatments. Tissue was not analyzed from the Hobbs field (2007).

Unlike N, there were both, management and fertilization effects on tissue P concentrations for both locations. The vegetation tissue P content was greatest from burned plots and mowed plots for Hobbs field (2007), but at Powerline field (2008), the mowed plots were intermediate between burned and control plots (Fig. 3-8).

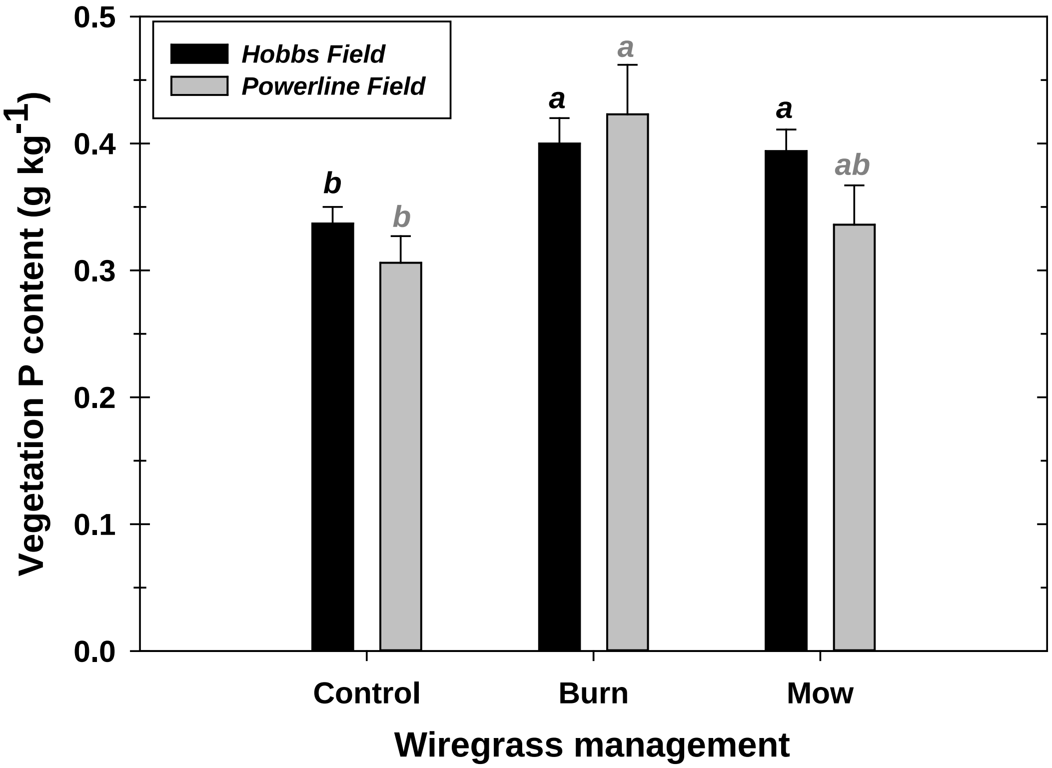

Figure 3-8. Plant management effect on tissue P concentration at Hobbs Field (2007) and Powerline Field (2008) (n=16). Bars=means ± standard error. Different letters of the same font color are significantly different (P<0.05), using Tukey-Kramer test.

Nitrogen fertilizer had no effect on tissue P concentration (0.37 h kg^{-1} in 2007 and 0.36 g kg^{-1} in 2008). In contrast, P fertilization did affect tissue P concentrations, where the plots receiving P resulted in greater tissue P upon harvest (Fig. 3-9).

It is interesting to note that vegetative tissue K concentrations from burned plots were greater than from other management treatments at the Hobbs field (2007), and it was similarly higher with the mowed plots at the Powerline field (2008) (Fig. 3-10). Plots receiving N had greater tissue K concentrations from both locations, although to a lesser degree of significance ($P<0.10$) at the Powerline field (2008) (Fig. 3-11). Phosphorus treatments also increased K tissue content (0.25 vs 0.29 g K kg^{-1}) for 0 vs 56 kg P_2O_5 ha^{-1} application rates respectively, but only at the Hobbs field (2007).

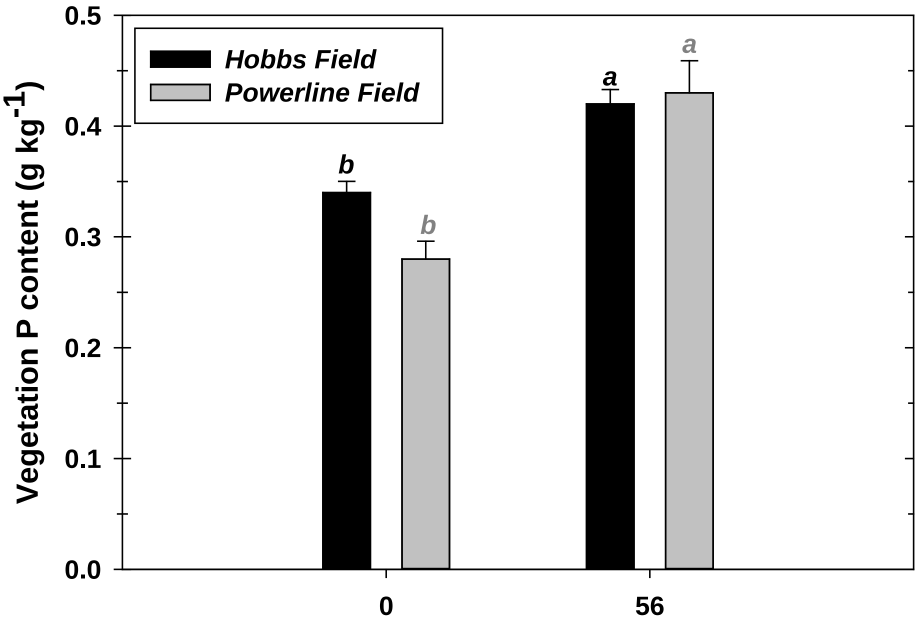

Figure 3-9. Tissue P concentration at Hobbs Field (2007) and Powerline Field (2008), as affected by P fertilization (n=24). Bars=means ± standard error. Different letters of the same font color are significantly different (P<0.05), using Tukey-Kramer test.

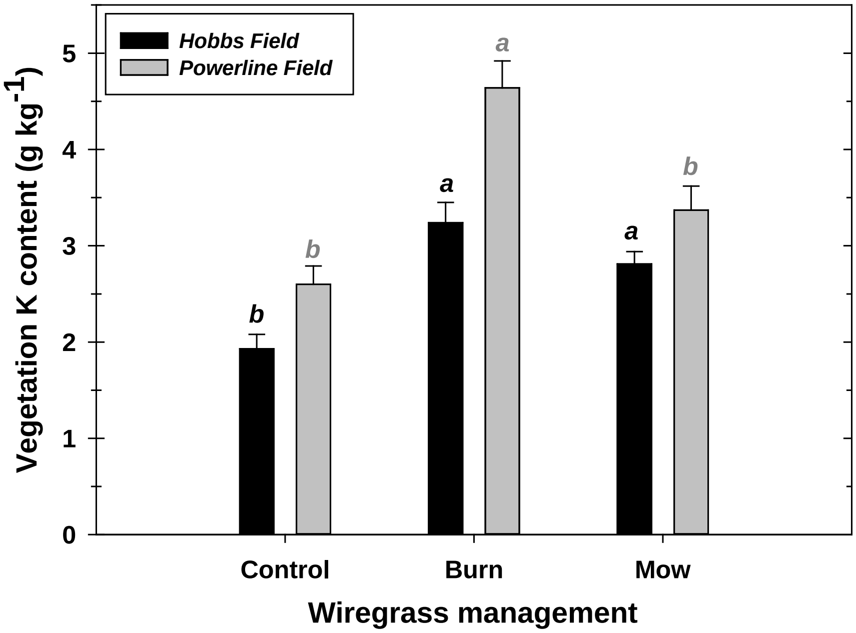

Figure 3-10. Plant management effect on tissue K concentration at Hobbs Field (2007) and Powerline Field (2008) (n=16). Bars=means ± standard error. Different letters of the same font color are significantly different (P<0.05), using Tukey-Kramer test.

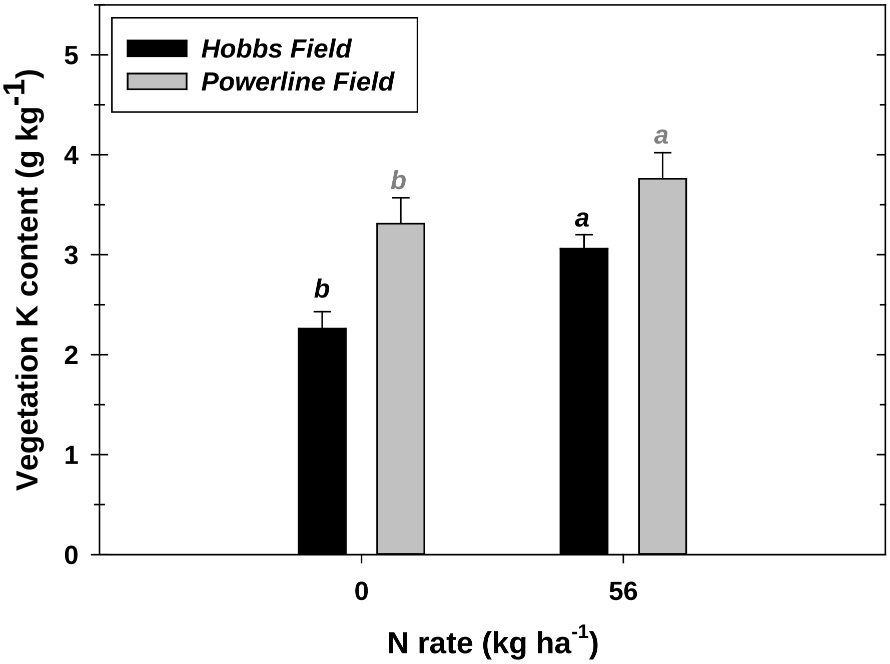

Figure 3-11. Tissue K concentrations at Hobbs Field (2007) and Powerline Field (2008), as affected by N fertilization (n=24). Bars=means ± standard error. Different letters of the black font color are significantly different ($P<0.05$), using Tukey-Kramer test, while the gray font color letters are significantly different at $P<0.10$.

Soil Fertility Results

Surface soils were minimally affected by single, initial fertilizer applications at each of the sites. There were no management or fertilizer application effects on any of the measured essential plant nutrients except for soil P and K. Soil TKN (470 mg kg^{-1}) and NO_3-N (1.11 mg kg^{-1}) that were measured at the end of the growing season at the Powerline field (2008), were not affected by management or surprisingly, N application rate. There was a minimally significant Management X N rate interaction ($p = 0.0433$), but means were not different, as per the Tukey-.Kramer test. The soils from the Hobbs field (2007) were not tested for soil TKN or NO_3-N.

Unlike soil N, the soil P fertility increased with P applications. In fact, plots at Hobbs field that received P, continued to show elevated soil test P the following spring

(Fig. 3-12). Although the soil P remained elevated with P fertilization, the values declined from the fall sampling at harvest (Dec, 2007) through the following spring (May, 2008). Elevated soil test P was also observed at the Powerline field (2008), where soil test P was 9.3 ± 0.3 mg kg^{-1} and 16.9 ± 0.8 mg kg^{-1} ($p < 0.001$) for the unfertilized and fertilized plots, respectively.

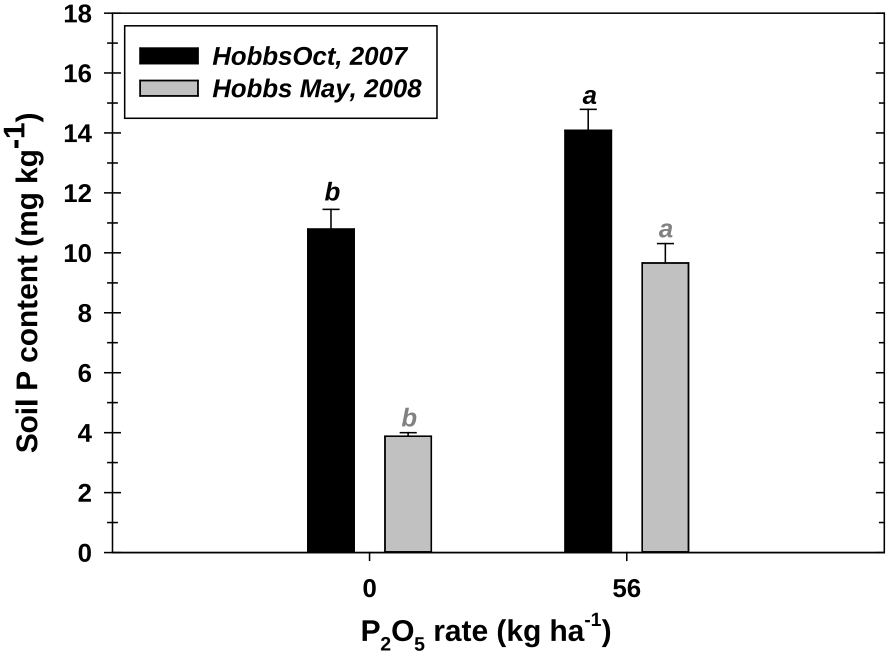

Figure 3-12. Soil test P concentrations at Hobbs Field (Dec, 2007) and the following May (2008), as affected by P fertilization (n=24). Bars=means ± standard error. Different letters of the black font color are significantly different (P<0.10), using Tukey-Kramer test, while the gray font color letters are significantly different at P<0.05).

Soil management did not affect soil K fertility but N and P applications increased soil K compared to no fertilizer application. In 2007 fall sampling, there was a significant N X P interaction (Fig. 3-13); however, by the following spring, surface soil K was similarly low among all treatments (9.7 mg kg^{-1}). Soils sampled from the Powerline field in Oct, 2008 showed a similar soil K response to N and P fertilization, and overall soil K values trended higher at the Powerline field (Fig. 3-14). Other nutrients (Mg, Ca, S, Zn, Mn, Fe, and Cu) showed little to no response from management or fertilizer treatments at either location.

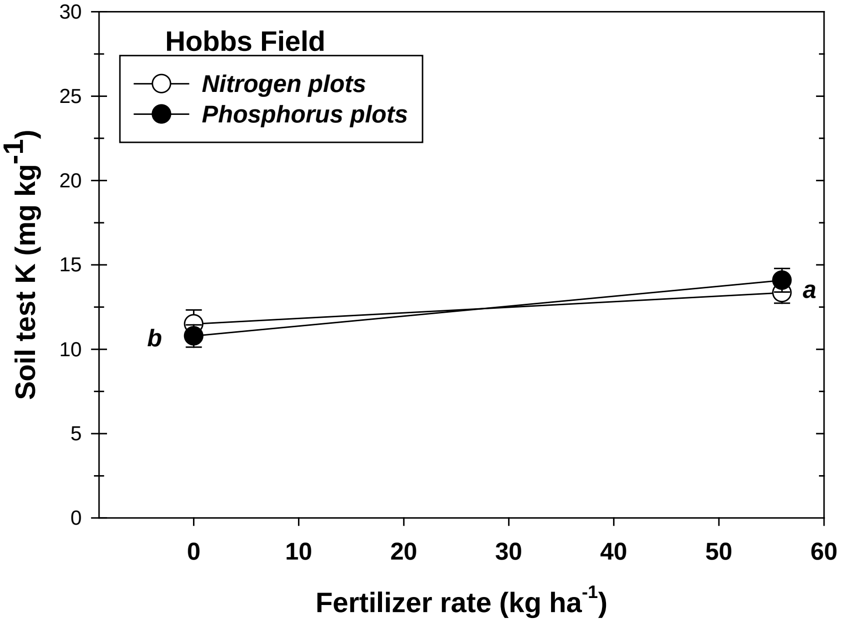

Figure 3-13. Soil K test at Hobbs Field (2007), as affected by N and P fertilization (n=24). Bars=means ± standard error. Different letters are significantly different ($P<0.05$), using Tukey-Kramer test.

Root AMF Results

Root segments from Powerline field (collected in Sept., 2008) were processed, in order to determine if plants were producing mycorrhizal structures (arbuscles and storage vesicles) indicative of mycorrhizal activity. Hyphae were not used to indicate if the plants within a plot were actively using the mycorrhizal associations.

Arbuscular mycorrhizae fungi are important to plants at sites deficient in phosphorus (Anderson et al., 1994). Mehlich-3 soil P was low, near 10 mg kg^{-1} in unfertilized plots, which was not expected to impede AMF colonization.

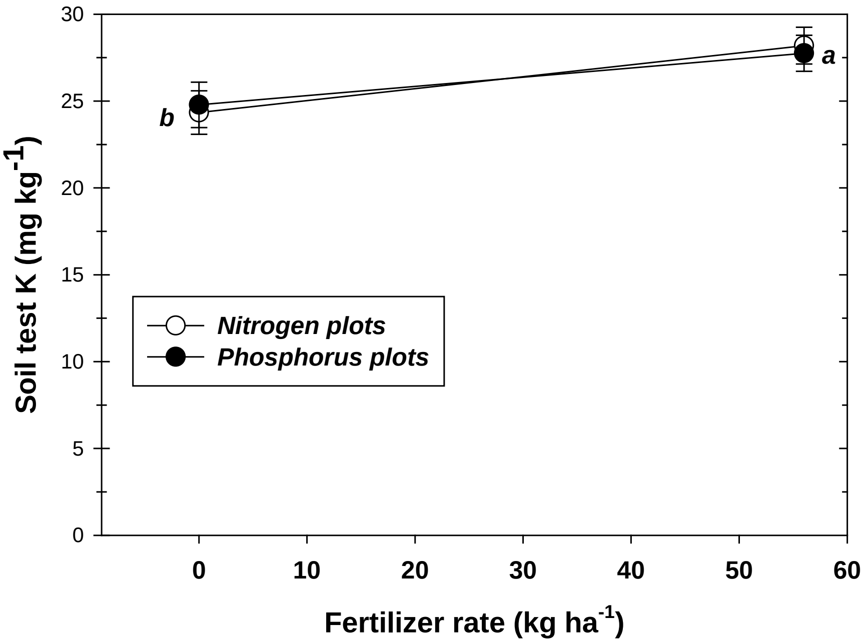

Figure 3-14. Soil K test at Powerline Field (2008), as affected by N and P fertilization (n=24). Bars=means ± standard error. Different letters are significantly different ($P<0.05$), using Tukey-Kramer test.

No arbuscles were found in the stained root segments but vesicles were identified. All infection rates of vesicles across management and fertilization treatments were ≤ 40%. Percent vesicles were lowest in the unchallenged (control) plots (Fig. 3-15).

Discussion

Management and N fertilization

At one time, it was thought that wiregrass did not flower, but it was found to flower after burning (Fig 3-16), defoliation or minor soil disturbances (Clewell, 1989). Presently, growing season burns are often used to effectively stimulate wiregrass flowering. The plots where vegetation was left intact resulted in the greatest shoot biomass (this included plant growth from previous seasons) at the end of the test season. This is likely because much of the previous season's biomass was included in the sampling. The greater percent dry mass from control plots also indicated that the control treatment included dead and dying biomass from previous seasons' growth.

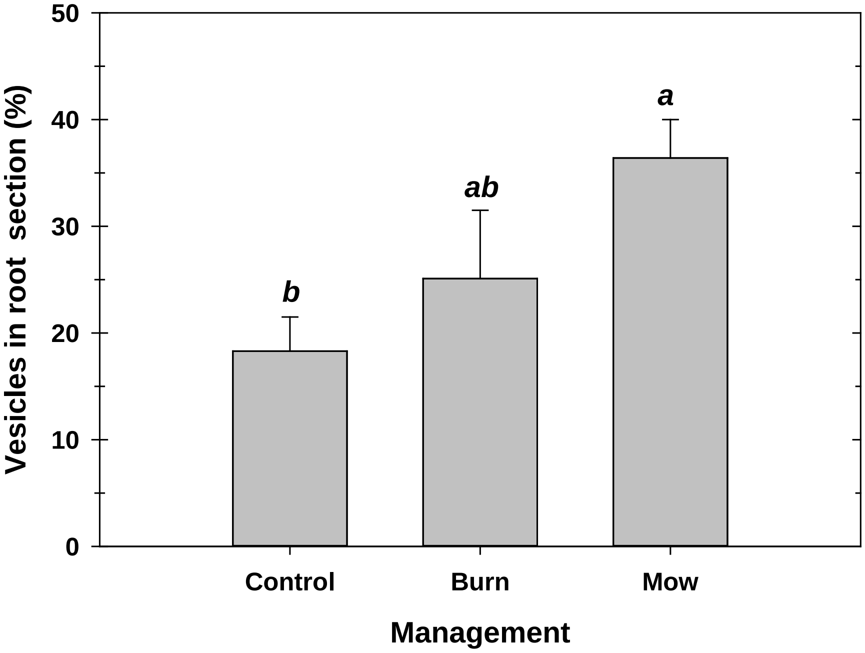

Figure 3-15. Plant management effect on mycorrhizal vesicle identification in root sections collected from plants at Powerline Field (2008) (n=16). Bars=means ± standard error. Different letters are significantly different ($P<0.05$), using Tukey-Kramer test.

Figure 3-16. Wiregrass with reproductive culm (seed head) from Hobbs Field burn plot.

Results from this study show that clipping (mowing) and a moderate (56 kg ha^{-1}) N application resulted in similar reproductive culm mass as burning. Others have reported similar results (Outcalt et al., 1999; Brejda, 1995). However, Pfaff et al. (2001) found no significant differences when burning with N or N+K fertilization, but they indeed found that mowing produced similar seed production as burning. Soil P in their study was over 100 kg ha^{-1}. Since excessive soil P can limit production by interfering with Zn and Fe uptake, there may have been overriding limiting nutrition at the Pfaff et al. (2001) that limited plant response to N fertilization.

Information gathered from this and other studies may help seed producers develop wiregrass cultural practices under conditions that limit the use of fire to trigger flowering. It must be noted that this study did not assess treatment effects on seed mass, vigor, or germination. Further work is needed to test these important parameters, but Pfaff et al. (2001) found numerically greater (although not significant) increase in seed number with N+K fertilizer applications.

Fertilizing with N in this study increased above-ground vegetative yield, as well. However, previous reports show conflicting results for N fertilization response. Kalmbacher and Martin (1996) reported that N applications tended to eliminate wiregrass from native Florida flatwoods; while Outcalt et al. (1999) found that applications of slow-release N significantly increased the growth rate of wiregrass transplants established in cultivated plots. Developing larger plants more rapidly, is important to nursery producers and perhaps to land restoration managers. Since the plants had a strong response to fertilizer N, it can be concluded that they were N-limited. In addition, there was no indication of residual N in the surface soil of the

Powerline field at the end of the growing season. Any residual N may have been taken up by the vegetation or leached deeper into the soil profile. Since many species benefit from additional N applications, there may be some concern that in a natural setting, a wiregrass N application at establishment may also fertilize the weeds, thereby allowing the weeds to more successively compete for light, water, and nutrients.

Nitrogen and potassium interactions

Results from Kalmbacher and Martin (1983) found high levels of K within wiregrass seed head but reported fertilizing with K did not significantly affect seed production. However, they hypothesized that N fertilization may facilitate K uptake and promote flowering and seed production. The +N scenario, in our study did not affect seed head K content but it led to an increase in biomass K. Further research is needed to better understand the interaction of N fertilization on K uptake.

Phosphorus fertilization

Interestingly, the P fertilization treatment did not affect wiregrass productivity (reproductive culms or above-ground vegetation), even though Mehlich-3 soil P at the study initiation was near or below 10 mg kg^{-1}. Wiregrass tissue P concentrations were approximately 10 times less than what is typically reported as critically low for many of the locally (Florida) grown improved forage grasses, such as bermudagrass and bahiagrass. Applying P at 56 kg P_2O_5 ha^{-1} increased tissue P and soil P values. However, since the plants did not respond to P applications with increased production, it may be concluded that these plants were not P-limited. Higher N application rates, if resulting in even greater production might lead to a P limitation, but it is not known at what rate of N (if any) might result in a greater demand for soil P.

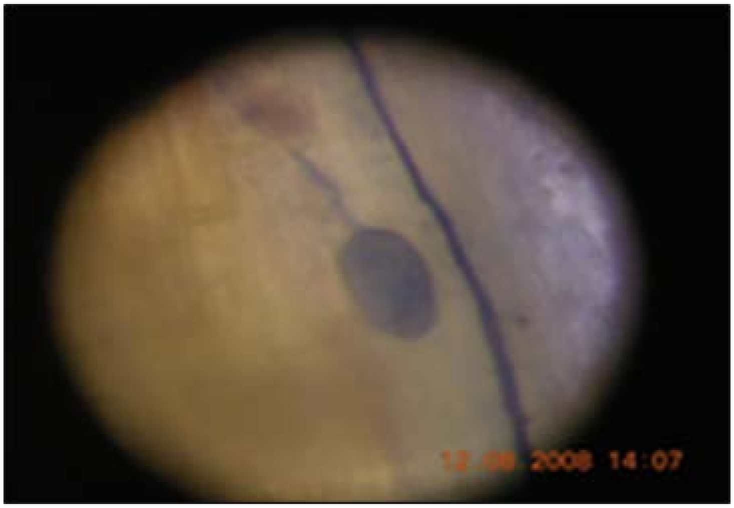

Figure 3-17. Mycorrhizal vesicle found in wiregrass sample from 2008.

Arbuscular mycorrhizal fungus (AMF) association

Across all management and fertilization treatments, there was less than 40% vesicle occurrence (Fig. 3-17). This low level is consistent with results reported by others (Anderson and Menges, 1997; Fitter, 1991). In fact, Anderson and Menges (1997) found no detectible mycorrhizal associations with wiregrass growing in Hyperthermic, uncoated Typic Quartzipsamments (Astatual series) soils. Our data also indicate that wiregrass, despite a low concentration of soil P, does not have a well-developed mycorrhizal association.

Phosphorus is a nutrient for which mycorrhizae have demonstrated improved availability to the host plant. Investment in a mycorrhizal system may not be advantageous if it does not increase availability of nutrients most limiting to growth. If soil P is not limiting, then there may not be a need for a strong AMF association. Fitter (1991) suggested that because of the cost to the plant in maintaining a mycorrhizal system, the association is a viable option only when the limiting nutrient will increase photosynthesis. Under conditions of low availability of several inorganic nutrients, plants may adopt varied strategies to enhance uptake of nutrients. These could include increased root fineness, root/shoot ratio, or number or length of root hairs (Lamont, 1982; Hetrick, 1991). Strong dependency on mycorrhizae may be limited to species with little plasticity of root or those with relatively high P requirements.

Conclusions

The major loss of longleaf-wiregrass habitat necessitates establishing protocols for maintaining and restoring these areas, including a focus on wiregrass establishment. One of the challenges to successful restoration is to determine wiregrass cultivation practices, as this species tends to have poor seed production and viability. While burning wiregrass remains the most natural and simplest way to stimulate reproductive vigor in this plant, data from this study found that when burning is not an option, clipping (mowing at 10 cm stubble height) and applying N fertilizer can result in seed head production similar to burning. A single N application (56 kg ha^{-1}) in early summer, increased seed head and above-ground, vegetative yield of mature plants. Using techniques such as these may not be feasible on large-scale restoration projects, but it might in nursery production or other, more urban settings, where burning is restricted.

Seed head and above-ground, vegetative productivity was not affected by P fertilization (56 kg ha^{-1} P_2O_5 as TSP), even though soil test P (Mehlich-3) was often below 10 mg kg^{-1}. Vegetative tissue K concentrations increased from burning treatments, or when N fertilizer was applied at both locations. As some suspect that wiregrass requires ample K nutrition to ensure seed production, a better understanding of the relationships among N, P, and K in wiregrass cultivation may aid with increasing seed production. Reproductive success in this study was limited to measuring reproductive culms (seed heads). Future work should include seed harvesting, along with viability and germination testing, to ensure that treatment benefits translate to true reproductive success.

Considering that the study soils were low in P, it was hypothesized that wiregrass would have a high rate of mycorrhizal colonization. However, the plants did not have well-developed mycorrhizal associations. The low level of mycotrophy observed in this study may be due to other limiting nutrients or perhaps this species does not form strong associations with AMF under the observed growing conditions. Future research is needed to determine which species of mycorrhizae may enhance wiregrass growth and development.

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