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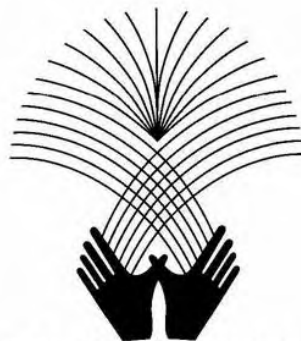


Longleaf Pine: *Making Dollar\$ and Sense*



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USE OF CORROBORATIVE TECHNIQUES TO EVALUATE GROWTH AND NITROGEN FIXATION RATES OF NATIVE LEGUMES

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Abstract: Native herbaceous legumes constitute more than 10 percent of vascular plants in frequently-burned longleaf pine savannas (Hains et al 1999). This sizable group of constituents has been shown to be a key element in the nitrogen cycling of longleaf pine woodlands. As a silvicultural tool, legumes are capable of producing nitrogen input over an extended period of time, they replace nitrogen that is lost from burning and are a source of soil organic matter. The goal of this study is to accumulate useful nitrogen fixation and growth rate data for several species of native legumes. This data can be used to estimate nitrogen inputs of existing groundcover or to make informed decisions regarding which species should be prioritized for reestablishment of native groundcover.

Greenhouse, garden plot and woodland survey methods are all employed in the assessment of several species of legumes. Additionally, corroborative methods of evaluating nitrogen fixation are used within each study: $\delta^{15}\text{N}$, nodule biomass, plant total N, acetylene reduction assays, and transport product analysis. Fixation is evaluated in varying light and water regimes in order to investigate factors that may be limiting to nitrogen fixation for each species.

INTRODUCTION AND BACKGROUND

Longleaf pine reforestation initiatives on private lands in the southeastern U.S. have resulted in the conversion of approximately 700,000 acres of former agricultural, pulpwood plantation and fire suppressed land back into longleaf pine stands since 1998. Recovering understory diversity is a key element of wildlife management, and is necessary for restoring a longleaf-wiregrass ecosystem on nitrogen depleted soils (Markewitz et al 2002). This study seeks to identify those legumes that are important for nitrogen replacement in young planted stands and that show promise in the design of restoration systems.

Historically, frequent fire disturbance due to lightning and Native American ignitions has continuously suppressed hardwood populations and maintained the structure of the system. However, fire causes nutrient losses due to the consumption of litter and volatilization (Carter 2004). The mineralization of phosphorus during periodic fires may serve an important role in the nutrition, growth and reproduction of phosphorus-demanding legumes, facilitating the eventual replacement of nitrogen lost during burning (Hendricks et al 1999, Sprent 1987). Conservative estimates of legume nitrogen inputs from biological N_2 -fixation in the longleaf-wiregrass system range from 7 to 9 kg N ha⁻¹ yr⁻¹ (Hendricks and Boring 1999). Hiers et al (2003) determined two species, *Tephrosia virginiana* and *Centrosema virginianum* to contain 74-92 percent nitrogen derived from the atmosphere.

As a silvicultural tool, legumes are capable of producing nitrogen input over an extended period of time and are a source of soil organic matter. Fixed nitrogen is typically less subject to leaching and is less likely to raise public concern about chemical application (an “organic” source) (Gordon, 1983). However, the use of legumes as a replacement for applied nitrogen is limited by the scientific community’s knowledge of their capabilities as a nitrogen input.

Thus, whether the goal of a land manager is to assess the nitrogen dynamics of an existing stand of longleaf pine with an intact understory or to establish native groundcover beneath planted pine, understanding the life history and nitrogen fixing capabilities of several species of native legumes is imperative. The goal of this study is to accumulate useful nitrogen fixation and growth rate data for several species of native legumes that can be used to estimate nitrogen inputs of existing groundcover or to make informed decisions regarding which species should be prioritized for reestablishment of native groundcover.

OBJECTIVES

The overarching objective of this study is to explore the impact of various degrees of shading on growth and nitrogen fixation rates of legume species found in longleaf-wiregrass savannas. Three approaches will be used to explore these environmental influences.

Experiment 1: Potted Plants, Gainesville, FL

The primary objective is to observe the effects of shading versus full sun over the course of a single growing season on the growth of roots and shoots, nodule development, and nitrogen fixation rates. Next, the experiment seeks to investigate shading effects on nitrogenase activity over time in an individual plant using serial acetylene reduction

assays. The final objective is the corroborate ureide transport product analyses with nodule biomass and acetylene reductions assays.

Experiment 2: Plantation Garden Plots, Ichauway

The primary objective of this experiment is to observe the differences in growth rate and habit between plants grown under three light regimes created by canopy shading over the course of a single growing season. The second objective is to compare nitrogen fixation capabilities of the various species in three light environments using %N, total N, biomass, $\delta^{15}\text{N}$ and N derived from the atmosphere (Ndfa). Finally, retranslocation and soil flux of nitrogen will be studied in select plants by comparing N content and $\delta^{15}\text{N}$ values of live and senescent leaves and root contents.

Experiment 3: Native Longleaf Woodland, Ichauway

A series of surveys of native longleaf woodland, will compare the density, biomass, nitrogen accumulation, and species richness of legumes on wet-mesic versus xeric site types on Ichauway. A second objective of this experiment is to observe the species and treatment differences in $\delta^{15}\text{N}$, plant biomass, and nitrogen transport products in irrigated versus adjacent reference woodland plants in early and late season samples.

METHODS

There are abundant ways of estimating biological nitrogen fixation by legumes *in situ*. The most useful methods are those that approximate amounts or proportions of plant N derived from atmospheric N_2 fixation, distinct from N acquired from the soil. One such method compares the natural abundance of 15-nitrogen isotopes with reference plants and soil values (Table 1). Additional methods of comparing N_2 -fixing and non-fixing plants include analysis of xylem sap constituents, assays of nodule nitrogenase activity and analysis of total nitrogen contents. This study uses each of these methods which together propose a useful depiction of the nitrogen fixing capability of a species.

Analysis of plant tissue for isotopic nitrogen composition has become a common integrator for understanding the nitrogen cycling of a system. The ^{14}N isotope represents 99.6337% of all nitrogen atoms on earth, and 100% of atmospheric nitrogen (N_2). Soil nitrogen usually has 9.2‰ higher ^{15}N content than the atmosphere. The ^{15}N content of a sample is expressed as $\delta^{15}\text{N}$, which represents the isotopic ratio of the sample compared to an atmospheric standard. Thus, those plants that are able to accumulate nitrogen that originated in the atmospheric pool should have $\delta^{15}\text{N}$ values nearer zero than those plants that acquire their nitrogen solely from the soil (Table 1).

Table 1. $\delta^{15}\text{N}$ isotopic profile of burned and unburned xeric longleaf pine ecosystem.

Regime	Sample	$\delta^{15}\text{N}$
Burned	Non-Fixers (reference)	-3.62532
Unburned	Non-Fixers (reference)	-2.99349
Burned	Fixers	-0.95099
-	Atmospheric standard	0.00000
Unburned	Soil, 0-20cm, average	3.80065
Burned	Soil, 0-20cm, average	4.44864

Fixed nitrogen from nodules is transferred through the xylem in the form of transport molecules such as amides, ureides, and amino acids. Ureide is an especially efficient transport molecule that is utilized by warm season legumes. A relatively small amount of photosynthate is required to transfer several fixed nitrogen molecules as ureide due to its low carbon to nitrogen ratio. Ureide-producing plants, such a soybean (*Glycine max*) commonly display high rates of nitrogen fixation. Izaguirre-Mayoral et al (1992) observed significant differences between relative amounts of ureide present in nodulated versus non-nodulated plants, and concluded that the ureide technique can be useful for detecting and quantifying symbiotic N_2 -fixation in native legumes.

The acetylene reduction assay is based on the fact that the nitrogenase enzyme, which reduces atmospheric nitrogen to plant available forms, is also able to catalyze the reduction of acetylene to ethylene. Sample tissue is exposed to acetylene for an incubation period and then a gas sample is analyzed for ethylene concentration. The amount of ethylene present is indicative of the amount of N_2 that would have been reduced by that tissue given the same exposure.

Experiment 1:

One half of the specimens for each species of potted plants was grown at one-half and full sun, respectively. Species included in this experiment are *Chamaecrista nictitans*, *Centrosema virginianum*, *Clitoria mariana*, *Crotalaria rotundifolia*, *Lespedeza hirta*, *Psoralea canescens*, *Orbexilum lupinellum*, *Rhynchosia renformis*, *Tephrosia virginiana*, and *Mimosa quadrivalvis*.

Samples from the first set of specimens, planted in one-gallon pots, will be harvested in spring, summer and fall. At each harvest, dried biomass from above- and belowground and nodules will be compared. A 2-3cm section removed from the base of each stem will be collected for transport product analysis. A second set of specimens was grown in root elongation tubes. Root growth and nodule development was monitored and recorded twice weekly. Once roots reached or exceeded 85cm, the leaf area was determined, and stem and leaves were harvested and dried for further analysis. This experiment was concluded in September, 2004. Finally, a set of specimens was potted for a flow-through acetylene reduction system. These plants have been assayed once monthly to determine their relative nitrogenase activity over time. All plants, from across each of the three potting situations has been measured weekly for leaf addition and stem elongation.

Experiment 2:

Ten by ten foot plots were selected and marked under an even-aged 15 year-old stand of longleaf pine. Four plots were located under each of three overstory coverage levels: open, intermediate, and closed. The light environment in each plot will be more specifically characterized by hemispherical photographs (Battaglia et al 2003). Forty legumes, five each of eight species, and *Solidago sp.* as a reference plant, were randomly planted in rows within the plots. TDR rods were placed one foot inside each of the four corners of each plot, alternating 30 and 90cm rod lengths. Bi-weekly soil moisture readings have been taken from these locations in order to further characterize the microclimate of each plot.

Planted species are *Centrosema virginianum*, *Desmodium ciliare*, *Lespedeza angustifolia*, *Lespedeza hirta*, *Mimosa quadrivalvis*, *Orbexilum lupinellum*, *Psoralea canescens* and *Tephrosia virginiana*.

Entire plants from each plot will be destructively harvested once each in May, September and November. Assessment of plant height and leaf, stem, and root biomass will be conducted. Stem sections will be collected and placed in phosphate buffer solution for transport product analysis. Biomass will be dried, ground, and analyzed for total N and $\delta^{15}\text{N}$.

Experiment 3:

Four irrigated plots located in a xeric site and four in a wet-mesic sight, were surveyed. Within each plot, three transects radiating from the center of the plot marked an equal distance within and adjacent to each irrigated area. Aboveground plant material was collected from legumes and *Rubus argutus*, *Vaccinium myrtifolia*, and *Ruelia sp.* (as reference) along these three transects using the line-intercept method. A 2-3 cm section from the base of each legume stem was harvested into a vial of phosphate buffer solution for transport product analysis. Plants from along each transect represent irrigated and non-irrigated specimens. Aboveground biomass has been dried to constant weight and will be ground for analysis. Total nitrogen and $\delta^{15}\text{N}$ contents of select species will be determined on a composited per-transect basis.

Concurrently, a strip-transect was delineated 25m x 0.5m within each of the eight plots and also extended an equal distance outside of each irrigated area. Species rooted within this strip-transect were counted in order to further describe the species distribution and density in each irrigated plot and adjacent area (Table 2). Using this data in coordination with the data gained from the clipped samples, total aboveground nitrogen in legumes can be described on a land-area basis for dominant each species. Both the line-intercept and strip-transect surveys will be conducted in June and October of 2004.

Table 2. Diversity Analysis of Irrigated vs. Non-irrigated Xeric Plots

Species	Number Per Hectare		Ranking	
	Non-irrigated	Irrigated	Non-irrigated	Irrigated
<i>Tephrosia virginiana</i>	7200	800	1	11
<i>Mimosa quadrivalvis</i>	7000	10400	2	7
<i>Centrosema virginiana</i>	5200	6000	3	6
<i>Crotalaria rotundifolia</i>	5000	16400	4	2
<i>Dalea pinnata</i>	4800	11000	4	4
<i>Clitoria mariana</i>	4400	8600	6	9
<i>Tephrosia spicata</i>	3600	3200	7	8
<i>Psoralea lupinella</i>	3200	-	8	-
<i>Stylosanthes biflora</i>	2800	13000	9	3
<i>Galactia spp.</i>	2600	22000	10	1
<i>Desmodium ciliare</i>	1800	8200	11	5
<i>Lespedeza repens</i>	1800	600	11	12
<i>Cassia nictitans</i>	1000	3000	13	10
<i>Lespedeza angustifolia</i>	800	-	14	-
<i>Crotalaria purshii</i>	-	-	15	-
<i>Lespedeza hirta</i>	-	-	15	-
<i>Rhynchosia renformis</i>	-	3600	15	12
<i>Zornia bracteata</i>	-	200	-	14
<i>Total Legumes Per Hectare</i>	51,200	107,000		

LITERATURE CITED

- Battaglia, M. A., R. J. Mitchell, P. P. Mou, and S. D. Pecot 2003. Light transmittance estimates in a longleaf pine woodland. *Forest Science* 49:752-762.
- Carter, M. C. and C. D. Foster 2004. Prescribed burning and productivity in southern pine forests: a review. *Forest Ecology and Management* 191:93-109.
- Gordon, J. C. 1983. Silvicultural systems and biological nitrogen fixation. In *Biological nitrogen fixation in forest ecosystems: foundations and applications*. J. C. Gordon and C. T. Wheeler (eds). The Hague: Kluwer Academic Publishers Group (1-6).
- Hains, M. J., R. J. Mitchell, B. J. Palik, L. R. Boring and D. H. Gjerstad 1999. Distribution of native legumes (Leguminosae) in frequently burned longleaf pine (Pinaceae)-wiregrass (Poaceae) ecosystems. *American Journal of Botany* 86:1606-1614.
- Hendricks, J. J. and L. R. Boring 1999. N₂-fixation by native herbaceous legumes in burned pine ecosystems of the southeastern United States. *Forest Ecology and Management* 113:167-177.
- Hiers, J. K., R. J. Mitchell, L. R. Boring, J. J. Hendricks and R. Wyatt 2003. Legumes native to longleaf pine savannas exhibit capacity for high N₂-fixation rates and negligible impacts due to timing of fire. *New Phytologist* 157:327-338.
- Izaguirre-Mayoral, M. L., O. Carballo, S. Flores, M. S. Sicardi de Mallorca and T. Oropeza 1992. Quantitative analysis of the symbiotic N₂-fixation, non-structural carbohydrates and chlorophyll content in sixteen native legume species collected in different savanna sites. *Symbiosis* 12:293-312.
- Markewitz, D., F. Sartori and C. Craft 2002. Soil change and carbon storage in longleaf pine stands planted on marginal agricultural lands. *Ecological Applications*. 12:1276-1285.
- Sprent, J. I. 1987. *The ecology of the nitrogen cycle*. Cambridge:Cambridge University Press.