ASSOCIATION OF ROBINIA PSEUDOACACIA AND RHIZOBIUM: POTENTIAL NITROGEN ACCRETION[1]

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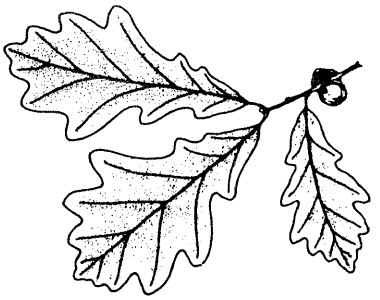

ABSTRACT.--Robinia pseudoacacia L., black locust, is a fast growing leguminous tree capable of forming symbiotic associations with the dinitrogen-bacterium Rhizobium. Black locust has been used for soil stabilization, intercropping, mined land reclamation, and short-rotation intensively-cultured biomass plantations. This study determined the dinitrogen-fixing capabilities of black locust nodules to assess both the variation and the potential for nitrogen accretion. The nodules were collected from three sites representative of the adaptive range of the tree. The sites were located in Indiana and typical of 1) marginal farmland, 2) reclaimed surface mined land, and 3) abandoned poor old field. Dinitrogen-fixing capabilities of 300 nodules per planting (5 trees per planting, 60 nodules per tree) were measured in situ by the acetylene reduction activity (ARA) assay. The ARA rates for the farmland, reclaimed mined land, and old field averaged 1.122, 0.421, and 2.133 nmoles of C_2H_4/mg nodule f.w./hr, respectively. If improvements in the mean ARA rates are made, either through improved silvicultural practices or by selective bacterial inoculation, the potential rates of nitrogen accretion are estimated to be five times the present rate.

INTRODUCTION

During the 1940's and 1950's, Robinia pseudoacacia L., black locust, was planted extensively in the United States for soil erosion control and reforestation (Boyce and Merz 1959; Keresztesi 1980). The rapid growth and extensive root system (Fowells 1965) inabled black locust to effectively stabilize the disturbed soils of surface mine spoils. Yet, due to its susceptibility to the locust borer (Megacyllene robiniae Forster), commercial use of black locust in the United States waned during the 60's and the 70's.

Interest in black locust once again is on the rise. Over 875 thousand hectares of Eurasia is covered by black locust, even though black locust is native to the Appalachian Mountains of the United States. In the U.S., the rapid growth, high caloric value, and vigorous coppicing ability have made it a choice for short rotation intensive culture biomass plantations (Carpenter and Eigel 1979; Pope 1980). Also, the rise in the cost of nitrogenous fertilizers has stimulated interest in the potential uses of woody species, both leguminous and nonleguminous, which form symbiotic dinitrogen-fixing nodules (Carpenter and Hensley 1979; Haines 1978; Haines et al. 1978; Gordon and Dawson 1979; Gordon 1983).

The contribution of nitrogen to forest ecosystems via black locust symbiotic dinitrogen fixation may play a significant role in natural plant succession. Previous estimates of the level of N input by symbiotic N_2-fixation are 30 kg N/ha/yr (Boring and Swank 1984), 35 kg N/ha/yr (Ike and Stone 1958), and 70 kg N/ha/yr (Haines 1978). Shramm (1966) stated that successful plant colonizers on mining wastes in Pennsylvania formed

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[1]A paper presented at the Fifth Central Hardwood Forest Conference held at University of Illinois, Urbana-Champaign, IL on April 15-17, 1985.

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either a dinitrogen-fixing symbiosis or an ectomycorrhizal symbiosis. Black locust appears to be one of these successful colonizers as evidenced by its frequency on disturbed sites. In the southern Appalachians, black locust comprises 30-40% of the woody biomass within three years after clearcutting or allowing old fields to go fallow (Boring and Swank 1984).

The purposes of this study were to 1) determine the variability of the dinitrogen-fixing capabilities of black locust nodules in the Central Hardwood Region, 2) examine the distribution of N_2-fixation rates at each of the study sites, and 3) compare soil chemical properties "within" and "outside" the selected black locust stands.

MATERIAL AND METHODS

Site Descriptions

Black locust roots were collected from three extremes in soil-site conditions which were representative of the adaptive range of black locust. The plantings were located in Indiana on sites typical of 1) marginal farmland, 2) reclaimed surface mined land, and 3) abandoned old field. The 36 year old farmland planting was within the Morgan-Monroe State Forest on moderately drained Wellston silt loam (Ultic Hapludalfs - fine-silty, mixed, mesic) with 6-12% slopes and north to northeast aspect; the site index (base age 50 yrs) was 27 to 30 m for yellow poplar (Liriodendron tulipifera L.). The reclaimed surface mined site was located in Sullivan County on property owned by AMAX Coal Company. The 18 year old planting occurred on graded cast overburden with 10-30% slope and a south to southwest aspect. No information was available on site index. The old field site, located at the Southern Indiana Purdue Agricultural Center (SIPAC), was planted in 1960 on an eroded phase of Zanesville silt loam (Typic Fragiudalfs - fine silty, mixed, mesic) of 3-5% slope, and southwest aspect in Dubois County; the site index for yellow poplar was 18 to 20 m.

Experimental Material

The root systems of five healthy R. pseudoacacia trees on each site were excavated by hand until root nodules were located. At each site, sampling occurred on two clear days in July 1983 during the midday hours (between 10 am and 3 pm). Roots with intact nodules were severed, washed with distilled water to remove excess soil, and immediately assayed for acetylene reduction activity (ARA). The ARA assay, an indirect assessment of the dinitrogen-fixing capability, was performed in situ using a modification of the technique described by Boring and Swank (1984). Sixty nodules were assayed per tree at each site. Each individual nodule was excised at the point of attachment with the root and placed in a 125 ml incubation bottle. The bottle was sealed with a moistened serum stopper and 10 ml of air was removed from the bottle. The extracted air was replaced with ten ml of acetylene (C_2H_2) gas. The bottles were buried in the soil to a depth of 5 cm and covered with litter to maintain a stable ambient temperature through the one hour incubation time. After incubation, a 10 ml gas sample from the bottle was transferred to a pre-evacuated blood sampling tube (Vacutainer Brand, Benton, Dickinson, & Co., Rutherford, NJ). Each nodule was transferred to a 5 ml test tube, stored in insulated coolers in the field, and transferred to cold storage (approx. 4 C) in the laboratory until fresh weight and length of the individual nodules were determined.

The ethylene (C_2H_4) content of the gas sample corresponding to each nodule was analyzed with a Varian gas chromatograph Model 3700 with a flame ionization detector and are reported as nmoles of ethylene (C_2H_4) produced per mg nodule fresh weight per hour of incubation. The following specifics applied: 1) injected sample volume of 250 ul, 2) injector/oven/detector temperatures set at 90/50/110 C, 3) a glass column packed with Porapak R, 100-120 mesh, and 4) nitrogen as the carrier gas at a flow rate of 30 ml/min.

The mean nodular biomass per hectare for each planting was determined from 25, 8 cm diameter, soil core samples taken to a depth of 20 cm. Nodules were removed within 7 days of collection and fresh weights were determined.

For each site, five soil samples were analyzed for texture, pH, soluble salts, % organic matter, % total N, P, K, NH_4^+-N, NO_3^--N, and soil moisture by the Soil Testing Laboratory of Purdue University.

In addition, 5 soil samples were collected from the top 20 cm on adjacent plots at each location which were outside the black locust plantings but on the same soil type. At both the marginal farmland and the old field sites, the adjacent plant communities were mixed hardwoods. At the reclaimed mined land site the adjacent community consisted of assorted grasses. Chemical analyses were identical to those reported for the black locust planting with the exception of the soil moisture.

Analysis of variance of a nested (hierarchical) design (Anderson and McLean 1974) was used to analyze the effect on mean ARA rates, mean nodule weight, mean nodule length, and mean nodular biomass per hectare. Nodules showing ARA rates equal to zero were not included in the analysis. The nodule weights, lengths, and ARA rates were found to be correlated with the variances so a logarithmic transformation was performed on the data set prior to analysis of variance. To determine the degree of correlation between the ARA rate and either the nodule weight or length, a simple linear regression was conducted. Student t-test was used to compare the results of the soil tests from "within" and "outside" the plantings.

RESULTS AND DISCUSSION

The mean and maximum acetylene reduction rates, the mean weight and length of individual nodules,

and the mean nodular biomass per hectare is given for each site (Table 1). Mean ARA rate was significantly affected by site and tree within site (Table 2). The distribution of the ARA rates at each site were both positively skewed and leptokurtic, i.e., the distributions were assymmetric to the left and highly peaked. Site was not observed to have a significant effect on mean nodular biomass (Table 3), whereas tree within site had a significant effect on both the weight (Table 4) and length (Table 5) of individual nodules. It should be noted that the effect of site is confounded with the age of the planting.

If the data is pooled for all sites, the ARA rates were not found to be correlated with either nodule weight (R^2 = .00683) or nodule length (R^2 = .00003).

The results of the soil analysis of locations within and outside, yet adjacent to, the plantings are given in Table 6. Only at the farmland site was there a significant difference in percent total N and percent organic matter of the soil samples collected "within" and "outside" the planting. The NO_3^- level was significantly higher for "within" the planting at the farmland and the reclaimed mined land and, though not significant, tended to be higher for the old field as well. The opposite trend occurred for the NH_4^+ levels at all three sites. Texture and pH were not significantly different between the "within" and "outside" soils at any of the sites.

Planting nitrogen-fixing plants, either trees or herbs, offers several advantages for the improvement of site quality over nitrogen fertilization alone. Not only can nitrogen be added to the ecosystem in a steady rate over time, but there are also reduced losses of N due to volatilization or leaching so common to nitrogenous fertilizer application (Gordon 1983). Also, the addition of organic matter can ameliorate both chemical and physical properties of severely disturbed soils such as reclaimed mine spoils (Pritchett 1979).

TABLE 2.--Analysis of variance table for ARA rates.

Source of variation	df	Mean square	F
Site	2	17.21	4.46[a]
Tree within site	12	3.86	6.36[b]
Error	659	0.61	

[a]significant at P < 0.05
[b]significant at P < 0.001

TABLE 3.--Analysis of variance table for nodular biomass.

Source of variation	df	Mean square	F
Site	2	19.72	1.2
Error	72	16.49	

TABLE 4.--Analysis of variance table for nodul fresh weight.

Source of variation	df	Mean square	F
Site	2	4.31	1.71
Tree within site	12	2.51	13.32[a]
Error	659	0.19	

[a]significant at P < 0.001

TABLE 1.--Acetylene reduction activity (ARA) rates, mean fresh weight and lengths of individual nodules, and nodular biomass per hectare for three black locust plantings in Indiana.

Planting	ARA Rate (nmoles C_2H_4/mg/hr)		Individual Nodule Fresh Weight (mg)	Individual Nodule Length (mm)	Nodular Biomas per Hectare (kg nodule/ha)
	Mean ± SE[a]	Maximum	Mean ± SE[a]	Mean ± SE[a]	Mean ± SE[a]
Marginal Farmland (Morgan-Monroe S.F.)	1.122 ± 0.184	32.015	25.53 ± 2.04	5.10 ± 0.16	44.6 ± 8.4
Old Field (SIPAC)	2.133 ± 0.221	23.804	32.58 ± 2.49	4.91 ± 0.20	37.0 ± 9.0
Reclaimed Mined Land (AMAX Chinook)	0.421 ± 0.045	3.814	17.59 ± 1.66	3.86 ± 0.19	27.0 ± 7.1

[a]SE equals standard error

TABLE 5.--Analysis of variance table for nodule length.

Source of variation	df	Mean square	F
Site	2	1.37	2.23
Tree within site	12	0.61	12.25[a]
Error	659	0.05	

[a]significant at $P < 0.001$

TABLE 6.--Soil characteristics within and outside three black locust plantings in Indiana.

Location	Texture	pH	Sol Salts[a]	% OM[b]	P[c]	K[c]	% TN[d]	NH_4^+[c]	NO_3^-[c]	% Mois[e]
Farmland										
within	silt loam	5.2	9[f]	3.10[f]	2	115	0.15[f]	8.58	16.32[f]	17.26
outside	silt loam	5.2	0[f]	2.51[f]	3	117	0.12[f]	12.37	1.56[f]	---
Old Field										
within	silt loam	4.4	4	3.23	8[f]	90	0.14	6.02	12.48	16.88
outside	silt loam	4.7	5	3.24	4[f]	88	0.14	7.68	9.86	---
Mined Land										
within	loam	7.7	22	2.13	3	105[f]	0.12	2.90	6.18[f]	7.27
outside	loam	7.9	13	2.58	3	82[f]	0.13	3.15	3.04[f]	---

[a]electrical conductivity of soluble salts measured in mohs/cm $\times 10^{-5}$

[b]percent organic matter

[c]phosphorus, potassium, ammonium, and nitrate levels measured in parts per million (ppm)

[d]percent total nitrogen

[e]soil moisture measured gravimetrically

[f]significant difference based on Student's t-test ($P < 0.05$)

The percent total nitrogen and percent soil organic matter were only significantly higher "within" the planting as compared to the "outside" location at the farmland site. The difference represents 672 kg N/ha f s (based on 2.24×10^6 kg soil/ha f s). The farmland planting is the oldest of the three plantings which suggests that time may be required before the increase in N can be observed. Although an increase in N-cycling may be more important than the actual increase in percent total N, this value represents an average annual N accretion rate of 19 kg N/ha f s/yr.

Nitrogen fixed in the nodule is quickly assimilated into plant tissue and enters the soil system after degradation of plant material (Postgate 1982). At the old field and reclaimed mined land site, the fixed N may be primarily incorporated in plant biomass. The radial growth rates, measured by mean diameter increment, was highest at the mined site followed by the old field, then farmland.[3] It is possible that more N is required to sustain the tree growth in the younger stands so soil N did not rise appreciably over the reference location. The differential nutrient uptake of the plants "within" the planting compared to those "outside" in the reference location must also be considered when interpreting the soil data.

[3]Unpublished data collected by the authors.

To estimate the N-input via N_2 fixation, a mathematical summation of the mean N_2 fixation rates and nodular biomass values for several sampling times throughout a growing season is necessary (La Rue and Patterson 1981). Our fixation rates were assessed at only one point in time so meaningful estimates for the annual rate of N added cannot be calculated. The ARA rates were based on excised nodules which was necessary given the field nature of the study. Postgate (1972) stated that disturbances such as excision often adversely affects the net ARA rates. Also, although our earlier investigations did not indicate a significant reduction in ARA rates from 30 to 60 minutes of incubation, previous authors have (Boring and Swank 1984). Finally, sampling occurred at the beginning of a drought in late summer of 1983. For the above reasons, the ARA rates presented in this paper, between 0.001-32.015 nmoles C_2H_4/mg nodule f.w./hr, may actually be lower than those occurring in nature. Nonetheless, the ARA rates and nodular biomass values (27.0-44.6 kg/ha) reported here are consistent with those obtained by Boring and Swank (1984) for black locust stands in North Carolina. They reported ARA values between 0.25-1.00 ul C_2H_4/mg nodule d.w./hr (= 2.5-9.9 nmoles C_2H_4/mg nodule f.w./hr) and nodular biomass values between 0.83-3.59 g/m^2 (= 8.3-35.9 kg/ha). Recognizing a slightly shorter growing season in Indiana verses North Carolina, it is still reasonable to assume that rough estimates of N-input at the stands examined in this study are comparable to the reported estimate of 30 kg N/ha/yr (Boring and Swank 1984).

There was considerable variation between the ARA rates of individual nodules. Since efforts were made to reduce diurnal and seasonal variation by sampling only during midday hours of one season, this variation was postulated as dependent on the genetic variability of the host (black locust), the microsite differences surrounding the nodule, and/or the variability of the bacterial symbionts.

Tree within site did show a significant effect on ARA rates as well as nodule characteristics. Yet for any given tree on any site there was a wide distribution of different nodular ARA rates. Thus, the genetic constitution or vigor of the tree cannot explain all the variation observed. Microsite characteristics, such as aeration, nutrient availability, or moisture content, have been shown to affect the rate of N_2 fixation (Postgate 1982; Zimmerman et al. 1982). Also, it has been repeatedly demonstrated that different strains of Rhizobium respond differently in symbiotic effectiveness, e.g., N_2 fixation rates, nodule size, and nodule shape (Thornton and Davey 1983; Materon and Hagedorn 1983; Nelson and Child 1981; Munevar and Wollum 1982).

Can the net annual N input rate via N_2 fixation be increased for stands of black locust? Data collected from legume crops suggests the answer is a definite yes (Graham et al. 1982; Nambiar et al. 1982; Hedve 1982). Hypothetically, it is possible to raise the mean N_2 fixation rate either by increasing favorable nodular microsites via effective soil management, by genetically improving

the host, or by inoculating with superior strains of Rhizobium.

In this study we observed a wide distribution of ARA rates of individual nodules at each site. For the purpose of comparison, if a subgroup of nodules is designated as those nodules exhibiting ARA rates in the top 3 percent for each site, the mean ARA rates for these subgroups were 11.528, 12.904, and 2.306 nmoles C_2H_4/mg/hr for the marginal farmland, old field, and mined land, respectively. These values are 10, 6, and 5 times greater than the overall mean ARA rate the respective sites. Clearly, the goal of any management plan would be to increase the number and likelihood of nodules within this subgroup.

Ham (1980) suggests that the most economical way to provide the added N needed to maximize yields is to use more efficient strains of Rhizobium. Because many inoculants fail in field trials due to competition with indigenous strains of bacteria (Hedve 1982; Trinick 1982), we propose the best source of effective strains is from the natural sites which most closely resemble the target sites. Assuming that the bacterial strain is a major determiner of the N_2 fixation rate for a given nodule, then selection of bacterial symbionts from the subgroup of nodules showing high ARA rates offers the greatest potential for obtaining superior strains. Further research is required to determine if "custom-tailoring" the bacterial inoculant to a specific site will produce the potential increase in N_2 fixation. Also, this method should be coordinated with efforts to establish optimal soil conditions which enhance favorable nodular microsites.

SUMMARY

The results reveal that there was considerable variation in the dinitrogen fixation rates between nodules at any of the three sites and between sites. The soils within black locust plantings were shown to have increased levels of total % N and soil organic matter at one site and increased NO_3^- levels at all three locations when compared to reference soils. It is suggested that the amount of N added could be further increased if overall N_2 fixation rates are increased by effective soil management or by inoculation with selective rhizobia. The estimated potential increase in N_2 fixation rate is over five times the present values. This may prove to be a very real alternative, or supplement, to fertilization especially on sites of initial low fertility such as reclaimed mined lands.

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