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An Interim Report on Influences of Inoculation with Nitrogen-Fixing Symbionts on Reforestation Efforts in Haiti

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Summary

A major concern in the AOP is how to most efficiently grow tree species which have the potential to fix nitrogen. The study described here measured nursery growth and outplanting results when tree species were inoculated with N-fixing symbionts in the nursery. Inoculation with Niftal Rhizobium was tested on calliandra, piyon, taverno, and two akaysa species. A locally-collected inoculum also was compared to Niftal inoculum on Acacia auriculiformis, and A. auriculiformis was fertilized with phosphorus to see if colonization could be improved. Two kazwarina species were tested for Frankia inoculation effects.

Niftal-inoculated seedlings generally were larger than non-inoculated seedlings, but no statistically significant differences were found. In contrast, inoculated kazwarina had more nodules, greater root collar diameter, and were taller than non-inoculated seedlings. Inoculated kazwarina seedlings also survived better after three months. No outplanting differences due to inoculation were seen for four of the leguminous species, but outplanted A. auriculiformis seedlings generally grew better when inoculated.

The two kazwarina species must be inoculated with Frankia inoculum in the nursery to get good growth and survival. Although the legume species had growth differences which were not significant in the nursery, they may be after a year in the field. Inoculation should be continued at least until data from that portion of the study are collected and analyzed.

Contents

Introduction

Many of the tree species produced in the AOP are able to fix nitrogen when their roots are colonized with the appropriate symbiotic microorganisms. In order to ensure colonization, tree seeds are inoculated at planting with the appropriate micro-symbiont. This practice was started without rigorous testing in Haiti of the benefits of nursery inoculation with either inoculum obtained from the Nitrogen Fixation by Tropical Agricultural Legumes (Niftal) Project in Hawaii or from indigenous inoculum.

The primary objective of the study described here was to define the gains, if any, resulting from inoculating tree species with N-fixing symbionts in the nursery. Additionally, since a major annual cost is buying the imported inoculum, a locally-collected inoculum was compared to Niftal inoculum on <u>Acacia auriculiformis</u>. Finally, <u>A. auriculiformis</u> was fertilized with phosphorus to see if colonization could be improved.

This interim report covers results from the first phase of the study. Morphological measurements were made and analyzed and those data are presented below. Additional work on the harvested seedlings will include shoot and root biomass and nutrient analysis. Representative seedlings from each of these treatment combinations were outplanted so development can be monitored. Three-month measurements are presented here, with final measurements of those seedlings to be made in October 1989.

Methods

Dr. Bob Mitchell of Auburn University began this study during a short-term assignment in March 1988. Leguminous species tested for effects of inoculation with Niftal *Rhizobium* were *Acacia auriculiformis* (or akasya in Creole), *A. magium* (akasya), *Calliandra calothyrsus* (calliandra), *Gliricidia sepium* (piyon or lila etranje), and *Lysiloma latisiliqua* (taverno). Two other species, *Casuarina equisetifolia* and *C. glauca* (both kazwarina), were tested for *Frankia* inoculation effects.

Leguminous species were inoculated by the gum-arabic method, which mixes seed and *Rhizobium* with an adhesive, causing the two to stick together. *Casuarina* species were inoculated by the salt-shaker method, which places a powder made from finely-ground *Frankia* nodules in the cell below the seed. An additional inoculation treatment (+local) was imposed on *A. auriculiformis*. Nodules, roots, and rhizosphere soil were collected from under established *A. auriculiformis* trees growing on Operation Double Harvest property, dried in the sun, and ground to a powder. The gum-arabic method was used to inoculate seeds using this locally-produced inoculum.

All species were sown at the rate of 60 cells per case in Winstrips filled with peat moss. Inoculated (+Niftal or +Frankia) and non-inoculated (0) treatments were replicated four times for each species. All seedlings were grown in the Operation Double Harvest nursery at Cazeau, Haiti. Irrigation and fertilization regimes generally were the same as those received by the rest of the nursery. The exceptions to this were replicates of +Niftal *A. auriculiformis* and +Frankia *C. equisetifolia* and *C. glauca*. These replicates received a one-time application of triple-super phosphate (TSP) soon after emergence. This application killed most of the *Casuarina*, but the *Acacia* survived it. Those seedlings were cared for from that point on as an additional Niftal-inoculated replicate (+Niftal+TSP).

Seedlings were harvested when approximately seven months old, in October 1988. Data collected at harvest were shoot height, root collar diameter, and number of root nodules per seedling. Ten seedlings were harvested from each treatment combination, and ten seedlings were outplanted on ODH land to test inoculation effects on field growth and survival. Differences between or among inoculation treatments were detected for each species using two-way analysis of variance.

Results

Table 1 shows mean values for each variable measured on nursery seedlings. As can be seen, +Niftal seedlings were larger than non-inoculated seedlings. Seedlings of *C. calothyrsus* are the exception to this trend, but number of nodules per seedling indicates non-inoculated seedlings of this species were colonized more heavily than the +Niftal seedlings. However, increases with inoculation proved to be only trends for the leguminous species; no statistically significant differences were found, as representative ANOVA's indicate (Table 2). If the value in the last column of Table 2 is greater than 0.05, the corresponding treatment had no statistically significant effect on the measurement. Grouping seedlings based on numbers of nodules (not shown) had no effect on statistical differences.

These same patterns can be seen in initial measurements of outplanted seedlings (Table 3). Trees in Table 1 were measured in the laboratory, while those in Table 3 were measured after outplanting. The values in Table 1 are slightly larger than those for the same measurement in Table 3 because Table 3's seedlings were measured slightly above the root collar, making those values smaller. However, except for G. sepium seedlings, size differences between +Niftal and non-inoculated seedlings are the same for the two sets of seedlings.

In contrast to the legumes, the two Casuarina species had differences in one or more variables which were significant (Table 2). Inoculated C. equisetifolia had 97% more nodules, 92% greater root collar diameter, and were 77% taller than non-inoculated seedlings. Inoculated C. glauca had 70% more nodules, 27% greater root collar diameter, and were 52% taller (Tables 1 and 3).

Effects of +local and +Niftal+TSP treatments on nursery growth of A. auriculiformis are shown in Table 4. The measured variables did not vary among the four inoculation treatments when subjected to analysis of variance (not shown).

Effects of inoculation on three month's growth in the field are shown in Tables 5 and 6. The negative growth for G. sepium reflects browsing which occurred on that species (Table 5). Some browsing also occurred on Casuarina, and probably explains the unexpected relative field growth for those species. No growth differences due to inoculation are seen for four of the leguminous species. The differences which do appear can be explained as one or two unusual individuals having undue influence on a value. These instances can be identified by the low survival percentage associated with them. Inoculation significantly ($p<0.05$) increased C. glauca survival by quadrupling it, and almost significantly ($p<0.10$) increased C. equisetifolia survival.

The only species to survive well no matter the inoculation treatment was A. auriculiformis (Table 6). Outplanted A. auriculiformis also differed by developing growth differences. For height increase, inoculation treatment giving the least growth to treatment giving the most growth follows the sequence

+Niftal < 0 < +local < +Niftal+TSP,

and all the differences in the sequence are significant. Root collar diameter increase follows the same sequence, but only the difference between non-inoculated and +local is significant. Analysis of variance for those tests, including contrasts of the adjacent treatments in the sequence, are presented as Table 7.

Conclusions

Several conclusions can be drawn from these observations. First, enough variability occurs among seedlings within a treatment combination that apparent differences may not be statistically significant. The second general conclusion is that nursery seedlings are larger when inoculated. This size increase appears to be related to colonization by the nitrogen-fixing microorganisms. Even when _C. calothyrsus_ seedlings were smaller with inoculation (Tables 1 and 3), individuals with more nodules were larger (Table 1), and the one inoculated seedling which survived three months after outplanting was much bigger than the non-inoculated calliandra (Table 5). Since root colonization by microsymbionts is a carbohydrate drain on the plant, observing smaller seedlings with inoculation is common, but they were not seen here.

The results from the _A. auriculiformis_ study should be evaluated cautiously. This smaller study suggests local inoculum can substitute for Niftal inoculum on _A. auriculiformis_, but benefits of any inoculation are not evident until seedlings have been outplanted. The larger study suggests inoculation with Niftal _Rhizobium_ is beneficial immediately. Two other surprising results were less field growth resulting from Niftal inoculum than from local inoculum, and much greater growth resulting from inoculation plus a one-time addition of triple-super phosphate. The TSP addition was expected to increase growth by increasing nodulation. Since an increase in nodulation was not seen, the phosphorus response may reflect a phosphorus deficiency in seedlings in the other treatments. These unexpected results suggest this experiment should be repeated, and more and different species should be included.

The significant differences seen within the two Casuarina species indicate these species must be inoculated with Frankia inoculum in the nursery to get good growth and outplanting survival. Not seeing significant differences with most of the legume species does not mean they do not need inoculation, however. Increased growth was seen when these species were inoculated, and they should be inoculated as long as Niftal inoculum is affordable. Although differences were not significant in the nursery, they may be after a year in the field. Nursery inoculation of legumes should be continued at least until data from that portion of the study are collected and analyzed.

Table 1. Mean shoot height, root collar diameter, and nodule number for inoculated (+Niftal or +Frankia) and non-inoculated (0) seedlings of six nitrogen-fixing species grown in a Port-au-Prince nursery. Values are means of four replications containing ten individuals each.

Species	Inoculation	Shoot height	Root collar diameter	Nodule number
		- cm -	- mm -	- # -
Acacia magium -				
	0	13.7	2.2	4.0
	+Niftal	19.9	2.7	11.9
Calliandra calothyrsus -				
	0	9.6	2.6	30.6
	+Niftal	7.9	2.1	13.6
Gliricidia sepium -				
	0	24.0	6.5	21.2
	+Niftal	25.7	7.1	27.6
Lysiloma latisiliqua -				
	0	9.2	2.9	1.8
	+Niftal	10.3	2.6	2.0
Casuarina equisetifolia -				
	0	23.3	2.0	0.8
	+Frankia	39.6	3.8	8.6
Casuarina glauca -				
	0	33.8	1.7	1.9
	+Frankia	50.9	2.3	15.3

Table 2. Analysis of variance of nursery seedling measurements for selected species grown with and without inoculation.

Species	Measurement	ANOVA source	df	mean square	F	prob>F
Acacia magium	height	block	3	3.0106	0.1759	>0.10
		inoc.	1	75.8472	4.4309	>0.10
		error	3	17.1178	-	-
	root collar diameter	block	3	0.0447	0.3159	>0.10
		inoc.	1	0.5967	4.2170	>0.10
		error	3	0.1415	-	-
	number of nodules	block	3	2.9433	0.1869	>0.10
		inoc.	1	124.4122	7.8986	>0.05
		error	3	15.7511	-	-
Gliricidia sepium	height	block	3	14.5593	3.2900	>0.10
		inoc.	1	4.4253	0.2654	>0.10
		error	3	16.6721	-	-
	root collar diameter	block	3	0.1633	0.3253	>0.10
		inoc.	1	0.8128	1.6191	>0.10
		error	3	0.5020	-	-
	number of nodules	block	3	22.2025	0.1472	>0.10
		inoc.	1	82.5613	0.5474	>0.10
		error	3	150.8346	-	-
Casuarina equisetifolia	height	block	3	37.5526	0.6010	>0.10
		inoc.	1	528.4501	8.4577	>0.05
		error	3	62.4818	-	-
	root collar diameter	block	3	0.0750	0.2222	>0.10
		inoc.	1	6.8450	20.2815	<0.025
		error	3	0.3375	-	-
	number of nodules	block	3	0.6292	0.2046	>0.10
		inoc.	1	122.4613	39.8300	<0.01
		error	3	3.0746	-	-
Casuarina glauca	height	block	3	44.0216	6.5108	>0.05
		inoc.	1	585.6753	86.6217	<0.005
		error	3	6.7613	-	-
	root collar diameter	block	3	0.0283	3.9859	>0.10
		inoc.	1	0.7813	110.0423	<0.005
		error	3	0.0071	-	-
	number of nodules	block	3	4.2333	0.0579	>0.10
		inoc.	1	359.1200	4.9121	>0.10
		error	3	73.1100		

Table 3. Initial shoot height and root collar diameter of inoculated (+Niftal and +Frankia) and non-inoculated (0) seedlings of six nitrogen-fixing species outplanted near Port-au-Prince. Values are means of four replications containing ten individuals each.

Species	Inoculation	Shoot height	Root collar diameter
		- cm -	- mm -
Acacia magium -			
	0	12.6	1.9
	+Niftal	18.2	2.3
Calliandra calothyrsus -			
	0	9.5	2.2
	+Niftal	8.0	1.9
Gliricidia sepium -			
	0	22.6	4.7
	+Niftal	21.5	4.6
Lysiloma latisiliqua -			
	0	7.4	2.6
	+Niftal	8.7	2.3
Casuarina equisetifolia -			
	0	21.0	1.6
	+Frankia	38.7	3.1
Casuarina glauca -			
	0	32.5	1.6
	+Frankia	49.6	1.9

Table 4. Mean shoot height, root collar diameter, and nodule number for non-inoculated, inoculated, and inoculated+TSP *Acacia auriculiformis* seedlings at harvest and immediately after outplanting. Values are means of four replications containing ten individuals each.

Inoculum	Shoot height	Root collar diameter	Nodule number
	- cm -	- mm -	- # -
Harvested -			
0	27.0	2.8	34.0
+Niftal	28.8	3.1	29.3
+Niftal+TSP	26.8	3.1	27.7
+Local	26.7	3.0	23.4
Outplanted -			
0	23.9	2.3	-
+Niftal	26.3	2.3	-
+Niftal+TSP	21.9	2.2	-
+Local	24.3	2.3	-

Table 5. Three-month survival and mean growth of shoot height and root collar diameter for inoculated (+Niftal and +Frankia) and non-inoculated (0) seedlings of six nitrogen-fixing species outplanted near Port-au-Prince. Survival is percentage of planted seedlings still alive. Other values are average increments for surviving seedlings.

Species	Inoculum	Survival	Shoot height	Root collar diameter
		- % -	- cm -	- mm -
Acacia magium -				
	0	5.0	2.5	0.3
	+Niftal	20.0	0.4	0.9
Calliandra calothyrsus -				
	0	22.5	20.9	5.8
	+Niftal	2.5	64.9	7.1
Gliricidia sepium -				
	0	47.5	-8.2	3.5
	+Niftal	62.5	-7.0	2.7
Lysiloma latisiliqua -				
	0	20.0	22.7	3.3
	+Niftal	20.0	7.1	2.6
Casuarina equisetifolia -				
	0	25.0	10.3	3.3
	+Frankia	77.5	4.5	3.3
Casuarina glauca -				
	0	10.0	13.4	2.9
	+Frankia	47.5	2.3	2.5

Table 6. Three-month survival and mean growth of shoot height and root collar diameter for non-inoculated, inoculated, and inoculated +TSP *Acacia auriculiformis* seedlings outplanted near Port-au-Prince. Survival is percentage of planted seedlings still alive. Other values are average increments for surviving seedlings.

Inoculum	Survival	Shoot height	Root collar diameter
	- % -	- cm -	- mm -
0	75.0	25.3	5.0
+Niftal	62.5	21.0	4.1
+Niftal+TSP	70.0	42.1	6.2
+Local	80.0	31.3	5.3

Table 7. Analysis of variance of three-month growth of *Acacia auriculiformis* seedlings receiving various inoculation treatments in the nursery.

Measurement	ANOVA source	df	mean square	F	prob>F
survival	block	3	2.396	0.878	0.488
	inoculation	3	2.229	0.817	0.516
	error	9	2.729	-	-
height	block	3	205.554	6.512	0.012
	inoculation	3	334.105	10.585	0.003
	+Niftal & 0	1	211.562	6.703	0.029
	0 & +local	1	888.522	28.150	0.000
	+local & +Niftal+TSP	1	225.792	7.154	0.025
	error	9	31.563	-	-
root collar diameter	block	3	3.013	3.672	0.056
	inoculation	3	3.031	3.694	0.056
	+Niftal & 0	1	2.761	3.365	0.100
	0 & +local	1	8.904	10.851	0.009
	+local & +Niftal+TSP	1	0.667	0.813	0.391
	error	9	0.821	-	-