Mychorrhizal Density Differences in a Costa Rican Reforestation Project

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Abstract

Root samples were taken from *Sapium pachystachys* and *Inga sierrae* trees from both the plantation and forested sections of the Cloudbridge Nature Reserve in San Gerardo de Rivas, Costa Rica and these were stained in order to determine the degree of mycorrhizal colonization in the roots. The degree of colonization between plantation and forest trees was compared to determine if there is a difference in mycorrhizal density between the two areas. It was found that the *Sapium pachystachys* trees from the plantation were much more heavily colonized than those *Sapium pachystachys* from the forest, but no difference was seen between the *Inga sierrae* of the plantation and those of the forest.

Introduction

Mycorrhizae are fungi which colonize the roots of trees, growing structures both inside and outside of the roots. The fungus has a symbiotic relationship with the trees, receiving nutrients such as amino acids and sugars and benefiting the tree by increasing the effective surface area of it's roots, enabling it to absorb water and minerals more easily. Thus, the presence of mycorrhizae is beneficial to trees, plants that are colonized have overall better nutrition, faster growth, and increased resistance to drought, cold, and root pathogens (Dalpe).

The distribution of mycorrhizae within the trees of the Cloudbridge Reserve is of interest since it is an indication of the health of the trees. Tree roots from both the forested sections (naturally occurring trees) and the plantation areas (trees planted for

reforestation purposes) were sampled and the degree of colonization in these samples was determined. This data can be used to determine if there is a difference in the health of the plantation trees compared to that of the forest trees.

Methods

Two species of trees were sampled, *Sapium pachystachys* and *Inga sierrae*. These two types of trees were chosen because they are easy to identify, their roots stain well, and they are abundant in both the forest and plantations. Five trees from each species were sampled from the forest and five plantation trees of each species were sampled. Small, young, healthy-looking trees were selected from all areas of the reserve, the heights of the trees sampled ranged from less than a meter to five meters.

The roots were collected by digging into the ground until a root was encountered, following the root back to the tree trunk to ensure that it was from the desired tree, then following the root to the end where a sample of a few centimeters was cut. Only the finest, youngest roots were collected since this is where the mycorrhizae are most likely to be growing. Samples were taken from a few roots of each tree. Care was taken to do as little damage to the tree as possible, so only roots close to the surface of the ground were examined and all of the uncovered roots were reburied after the samples were collected. GPS points were also taken at the site of the tree and the approximate height and health of the tree was noted.

These root samples were then stained using the ink and vinegar technique (Veirheilig) due to the fact that it requires less toxic substances and is easier to execute in a rural Costa Rica setting than other staining techniques. The type of ink used was Sheaffer black Skrip® ink. First the roots were rinsed with water to remove dirt and debris and then they were placed in 10% potassium hydroxide in a 90 degree Celsius water bath for 40 minutes. The KOH clears the roots and makes it possible to see inside of the cells and examine them for mycorrhizae. The roots were then removed from the KOH and rinsed several times with water. Next they were placed into a 5% ink and 95% distilled white vinegar solution and placed in the hot water bath for 10 minutes. This process stains the roots and the fungi inside. The roots were then rinsed with water that

contained a few drops of vinegar and were then ready to be examined. The samples were examined using a microscope and the density of mycorrhizae was estimated using a 5 point scale. The scale corresponds to approximately the following densities:

0- no mycorrhizae present
1- less than 5% of cells colonized
2- 5-10% of cells colonized
3- 10-15% of cells colonized
4- 15-20% of cells colonized
5- More than 20% of cells colonized

The roots were stained brown or greenish brown and the mycorrhizae stained a dark navy blue color. Cells that were clear or brownish did not have mycorrhizae growing in them and cells that were blue were colonized by mycorrhizae. A number from the five point scale was assigned to each sample after it had been examined.

Results

All but one of the trees sampled had mycorrhizae present in the roots stained. The roots all had the same pattern of colonization, very heavy in some areas and no colonization in other areas, but the overall amount of colonization varied largely between samples.

The most heavily colonized group were the *Sapium pachystachys* from the plantation, with all trees rating at either 4 or 5 on the density scale. The mean value for these trees was 4.4. Conversely, the *Sapium pachystachys* from the forest were the least colonized group and the one tree with was not colonized was in this group. The range of values for these trees was 0-3 and the mean density was 1.8. The *Inga sierrae* from the forest had a mean value of 2.4 with a range of 2-3 and the *Inga sierrae* from the plantation had a mean value of 2.8 with a range of 2-5.

There was a large difference between the *Sapium pachystachys* of the forest and those of the plantation, with the plantation trees being much more colonized. However, the *Inga sierrae* showed no considerable difference in mycorrhizae density between the forest and plantation trees.

Data collected from root samples:

Sample #	Species	Area	Density	GPS point	Height	Location
SF1	*S. pachystachys*	Forest	3	0218054, 1048838	3m	Cloudbridge North
SF2	*S. pachystachys*	Forest	0	0217414, 1048139	3m	main trail before bench
SF3	*S. pachystachys*	Forest	2	0217804, 1048037	4m	Robert's Trail
SF4	*S. pachystachys*	Forest	1	0217587, 1048189	5m	River loop trail near bench
SF5	*S. pachystachys*	Forest	3	0217884, 1049087	4m	Cloudbridge North
IF1	*I. sierrae*	Forest	2	0217721, 1048129	4m	Robert's Trail
IF2	*I. sierrae*	Forest	2	0217645, 1048165	1m	Near main trail after first river crossing
IF3	*I. sierrae*	Forest	3	0217653, 1048167	0.5m	Near main trail after first river crossing
IF4	*I. sierrae*	Forest	3	0217846, 1049007	1m	Cloudbridge North
IF5	*I. sierrae*	Forest	2	0217872, 1049077	0.5m	Cloudbridge North
SP1	*S. pachystachys*	Plantation #187	5	0217762, 1048280	2m	River loop trail near maintenance trail
SP2	*S. pachystachys*	Plantation #I-31	4	0217760, 1048243	1.5m	Maintenance Trail
SP3	*S. pachystachys*	Plantation #A104	4	0217195, 1048186	4m	Amanzimtoti Trail
SP4	*S. pachystachys*	Plantation #126	5	0217981, 1048159	5m	Ridge Trail
SP5	*S. pachystachys*	Plantation #S7	4	0217691, 1048266	4m	near Cloudbridge Falls
IP1	*I. sierrae*	Plantation no #	2	0217925, 1048242	2m	Ridge Trail
IP2	*I. sierrae*	Plantation #103	2	0217917, 1048239	2m	Ridge Trail
IP3	*I. sierrae*	Plantation #A46	3	0217148, 1048159	1.5m	Meditation Garden
IP4	*I. sierrae*	Plantation #212	5	0218020, 1048303	2m	Lower plantations
IP5	*I. sierrae*	Plantation #221	2	0217984, 1048292	2m	Lower plantations

Discussion

The abundance of mycorrhizae in the *Sapium pachystachys* plantation trees compared to those of the forest suggests that in this respect the trees of the plantation are healthier than the trees of the forest. One explanation for this could be that the plantation trees have less competition for root space than the forest trees. The plantation trees are weeded and other vegetation is cut back to give them more light and space, and this also decreases the amount of roots from other plants that the tree's roots must compete with. This lack of competition could lead to more mycorrhizae colonization. However, this explanation does not account for the fact that a difference in colonization was not seen in the *Inga sierrae* trees.

The difference in results between the two tree species studied is surprising and would be an interesting problem to study further. It would be useful to study more species of trees in order to get a better idea of the differences in mycorrhizal abundance between the forest and plantation trees. Another study which would be interesting is to determine if those plantation trees that have high mycorrhizae density grow faster than those with low mycorrhizae density. This would have to be a long term study with root samples taken and height measured several times in order to determine if there is a correlation.

References

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2. Vierheilig, Horst. "Ink and Vinegar, a Simple Staining Technique for Arbuscular-Mycorrhizal Fungi," Applied and Environmental Microbiology, December 1998, p. 5004-5007.