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Discovery and Percent Colonization of Vesicular-Arbuscular Mycorrhizae in Pueraria lobata

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ABSTRACT

Pueraria lobata, more commonly known as Kudzu, was brought from Japan to the United States in 1876. Although originally planted for erosion control, *P. lobata's* vigorous growth facilitated its escape and caused it to spread over vast areas of land. Today *P. lobata* is found predominately in the Southeastern United States where it thrives during the summer months, growing up to a foot a day. This growth leads to a vine-like canopy several layers thick with deep set roots which makes removal of the plant extremely difficult.

Research on *P. lobata* is limited and has focused mainly on its role in erosion control. No literature has been found to provide insights into *P. lobata's* prolific growth. Mycorrhizae may, however, hold the answer. This fungus, present in the roots of many plants, enhances the uptake of essential nutrients and could provide *P. lobata* with the vigor it needs for such an incredible growth rate.

To determine if mycorrhizal association was present, *P. lobata* roots were collected, stained, and viewed for fungal structures. The presence of vesicular-arbuscular mycorrhizal fungi (VAM) was established. To further understand *P. lobata*'s dependence on this symbiotic relationship, two factors are important. First, the extent of association between the plant and fungus can be determined by percent colonization of VAM using the magnified intersections method (McGonigle *et al.*, 1990). Second, experimental growth rate differences between *P. lobata* planted in sterile soil versus mycorrhizae rich soil would provide direct evidence of its dependence on VAM.

INTRODUCTION

Pueraria lobata is a deciduous perennial plant that was first introduced to the United States through the Japanese pavilion at the 1876 Philadelphia Centennial Exposition. Commonly called Kudzu, this legume became popular as a porch covering due to its rapidly growing vines with broad leaves and beautiful reddish purple, grape-scented flowers (Hipps, 1994). During the 1930s, the U.S. Department of Agriculture imported *P. lobata* for erosion control in the South (Wilson and Ferris, 1989). Soon its rapid growth and extensive vine-like canopy became difficult to control. Today *P. lobata* covers much of the Southeastern United States and continues to engulf vast areas of land.

Reportedly growing up to a foot a day, *P. lobata* thrives during the summer months. During this time of rapid growth, young vines may travel over 100 feet and produce a canopy between two and eight feet thick (Hipps, 1994). Now covering over 2 million acres of forest land in the South, *P. lobata* continues to envelope trees and cause their death by blocking out sunlight with its 16 inch broad leaves. These leaves also hide the wisteria-like blossoms that flower from June into the fall. However, during the first killing frost, *P. lobata's* leaves drop and the vigorous vines are subdued. Although *P. lobata* is sensitive to cold temperatures, deep set roots can easily survive mild Southern winters and thus return a larger crop of vines each summer (Wilson and Ferris, 1989).

Root growth of *P. lobata* is also rapid and extensive during the summer. New roots branch out from stem nodes that touch the ground and form crowns. These roots may dive 10 feet or more into the ground and weigh several hundred pounds (Hipps, 1994). *Pueraria lobata* roots thrive in well-drained acidic and neutral soils, but the plant is weakened in wet soils and high

pH. The roots also contain an essential starch material that supports beginning growth during the spring and vine repair after damage by mowers or cattle grazing during the summer. This stored food is vital for *P. lobata's* vigorous growth and maintenance (*Farmers' Bulletin No. 1840*).

Removing *P. lobata's* immense canopy and root system is extremely difficult and time consuming. Overgrazing cattle can reduce top foliage in two years but the roots will remain viable underground. If cattle are not available, complete eradication can take up to five years with consistent work. Fire may also be used, but burning off young plants encourages dormant seeds to sprout (Hipps, 1994). So far, defoliation is the most successful method. If top foliage removal is frequent and consistent, the roots will be killed eventually as well.

Pueraria lobata gains strength from nutrients stored in its fleshy roots. These nutrients are essential to *P. lobata's* rapid growth rate. Therefore, any method that would increase the uptake of nutrients would be facilitating this plant's success. Mycorrhizae, which participates in a mutual symbiotic association with plants, is found in a broad range of habitats and abundant within the root systems of an array of plant species (Allen, 1991). Vesicular-arbuscular mycorrhizal (VAM) fungi is the most widely distributed type of mycorrhizae. Three distinct fungal structures are present in VAM: hyphae, vesicles, and arbuscules. The hyphae provides a structure and way of connection for the mycorrhizae within the root. The vesicles are balloon-shaped structures that serve as storage organs and arbuscules provide a point of interaction where the hyphae branches for exchange of nutrients with the plant (Hussey and Roncadori, 1982).

If *P. lobata* contains this symbiotic relationship, the presence of VAM in the roots would enhance the uptake of essential nutrients and thus provide the vigor needed for such an extensive growth habit. The following questions were entertained. Does *P. lobata* contain vesicular-

arbuscular mycorrhizal fungi? If so, what is the percent colonization of the various fungal structures and overall interaction?

METHODS AND MATERIALS

To test the hypothesis that *P. lobata* contains VAM, an experiment to collect, stain, and view the roots began in late December of 1995. Samples were taken from a road-side *P. lobata* patch in Collegedale, Tennessee. Six sites were randomly chosen and the roots were shoveled from the ground and placed in plastic bags. At the Biology research laboratory, the roots were washed free of dirt and placed in deionized water. All six samples were then rinsed again thoroughly, cut into pieces approximately ½ inch long, and placed back into deionized water. Next, the samples were transferred to six small bottles containing formyl acetic alcohol (FAA) to be fixed until further preparation for viewing. A 1000 ml recipe of FAA contains the following: 350 ml deionized water, 500 ml 95% ethanol, 50 ml glacial acetic acid, 100 ml 37-40% formalin.

In order to view the roots for fungal structures, the mycorrhizae needed to be colored. A mycorrhizae positive stain was prepared from 1.2 grams of Chlorazol Black E and 400 ml of 85% lactic acid. In order to dissolve the acid, the mixture was stirred magnetically for approximately 2 hours. Next, 400 ml of glycerine was added and the mixture was stirred for an additional 10 minutes. Four hundred ml of deionized water was then added, the beaker was covered with punctured parafilm, and the mixture was stirred overnight. In the morning, the solution was allowed to settle and then decanted to leave residue behind. The stain was now ready for use.

All six root samples were removed from the FAA solution and rinsed with deionized water. The samples were then soaked in a 10% KOH (potassium hydroxide) solution and

autoclaved at 121 degrees Celsius for 10 minutes to clear the roots. After cooling, the KOH solution was removed and the roots were washed with deionized water. Finally, just enough stain was added to cover the roots and the samples were placed in an incubator at 90 degrees Celsius for 2 hours.

To remove excess stain, deionized water was added to the bottles and mixed gently. This solution was decanted and the roots were washed again before replacing them into their rinsed bottles. Next, glycerine was added to the samples and swirled well to replace water remaining in the roots. This solution was then drained and fresh glycerine was added. The roots were now ready for viewing.

A slide from site 1 was prepared and viewed for the presence of mycorrhizae. Three slides were also prepared from each of the six samples in preparation for determining percent VAM colonization. Each slide contained several horizontal rows of sample which were mounted with glycerine and covered with a 22 x 44 mm cover glass. Percent colonization of various VAM structures and the overall association was determined using the magnified intersections method (McGonigle *et al.*, 1990).

The following two statistical tests were used to compare colonization values: A one-way analysis of variance (ANOVA) (Triola and Flynn, 1995) and Mainland's minimal table of contrasts (*Laboratory Manual, Biology 203F*).

RESULTS

After staining was complete, a slide was prepared from site 1 so the roots could be viewed for mycorrhizae. A large network consisting of hyphae and vesicles could easily be seen. Thus,

VAM presence was established.

Next, percent colonization of VAM was determined using the magnified intersections method (McGonigle *et al.*, 1990). The following five categories were determined under 200x magnification: arbuscular colonization, vesicular colonization, arbuscular and vesicular colonization, hyphae colonization, and the lack of mycorrhizal structures. Total percent colonization was then calculated as the sum of the above mentioned fungal structures, excluding the percentage of roots lacking VAM. Refer to Appendix I for a graphical comparison of fungal structures within and between sites.

Table 1 shows the percent colonization of all five categories for each of the six study sites. Data from site 1 revealed 6.25% more vesicles than arbuscules. Total hyphae and VAM absence were equivalent at 50.00%. Site 2 also revealed an increase in vesicular colonization over arbuscular colonization by 20.75%. Total hyphae colonization was approximately 80%. Vesicular colonization remained high for site 3 and total hyphae returned to just above 50%. Site 4 revealed the greatest percentage of vesicles at 39.58%. Total hyphae count for site 4 was also outstanding at 83.33%.

Site 5 revealed a 10% increase in vesicles to arbuscules and total colonization returned to about 50%. Total hyphae count for site 6 was 82.00%, vesicular colonization was 28.00%, and the presence of arbuscules was a low 2.00%. All six sites remained under 6.50% for arbuscular plus vesicular colonization and sites 1, 3, 4, and 6 were less than 2.50%.

Overall percentages for the various VAM structures and total root count are listed in Table 2. Total hyphae colonization was greater than half at 67.11%. For an in depth review of percentages found in the three samples of each site, see Appendix II.

Table 1. Percent VAM colonization in six sample sites of *Pueraria lobata.*

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Arbuscules	8.33	0.00	1.59	4.17	2.38	2.00
Vesicles	14.58	20.75	14.29	39.58	11.90	28.00
Arb + Ves	2.08	3.77	1.59	6.25	2.38	0.00
No Structures	50.00	20.75	44.44	16.67	47.62	18.00
Hyphae Only	25.00	54.72	38.10	33.33	35.71	52.00
Hyphae Total	50.00	79.25	55.56	83.33	52.38	82.00

Table 2. Total root count and VAM colonization for all six sites.

	Totals
Arbuscules	2.96%
Vesicles	21.38%
Arb + Ves	2.63%
No Structures	32.89%
Hyphae Only	40.13%
Hyphae Total	67.11%
Root Total	304

Figure 1 presents a graphical comparison of the percent colonization of VAM. Mainland's minimum table of contrasts was utilized at a 5% confidence level to provide an analysis of the similarity within categories (ie. vesicles, hyphae, etc.). The letters above each bar in Figure 1 represent similarities between bars having the same letters. A change in letter represents a statistical difference.

According to Mainland's statistical test, arbuscular colonization for site 2 (0.00%) was significantly different from all other sites (Figure 1, Graph A). Significant differences were also apparent in vesicular colonization on (Graph B) on several levels. Overall, sites 1 and 3 were not significantly different to each other as were sites 4 and 6. Sites 2 and 5, however, were significantly different from all other sites.

Hyphae colonization also revealed overlapping under statistical analysis. Sites 1, 4, and 5 produced no statistical differences, but sites 2, 3, and 6 were each significantly different from all other sites. Additional overlapping grouped sites 1, 3, 4, 5, and 6 as similar and excluded site 2 with 54.72% hyphae colonization. However, site 2 was statistically similar to site 6 (52.00%) as noted by the letter b in Figure 1, Graph D.

Values for arbuscular and vesicular colonization (Figure 1, Graph C) were low in all sites and never rose over 6.50%. Therefore all six sites were not significantly different. Total hyphae (Graph E) and the lack of VAM structures (Graph F) are tied closely together through the following equation: 100% - percentage of roots lacking VAM = percent total hyphae. Therefore sites 1, 3, and 5 in both colonizations were significantly different from sites 2, 4, and 6.

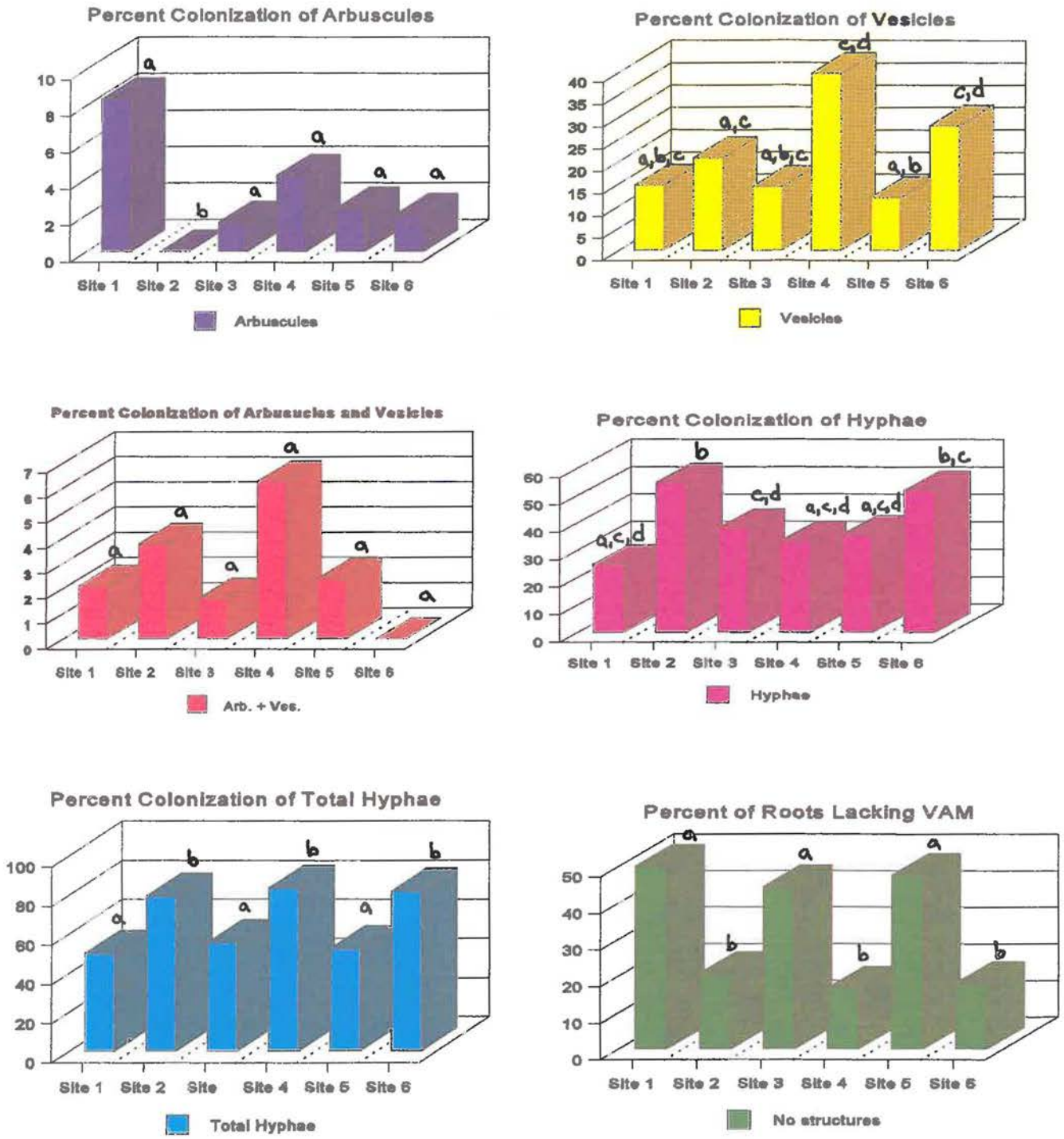


Figure 1. These graphs compare the percent colonization of arbuscules (A), vesicles (B), arbuscules and vesicles (C), hyphae (D), total hyphae (E), and the lack of VAM (F) between sites. Bars containing the same letter within a graph are not significantly different. A change in letter represents a significant difference according to Mainland's minimal table of contrasts.

DISCUSSION

Vesicular-arbuscular mycorrhizae was ubiquitous in *P. lobata*. Vesicular colonization was more prevalent than arbuscular colonization in all six study sites. Two reasons could be suggested to support this finding. First, the difference in percentages could be an accurate representation of colonization regardless of the time of year. Secondly, this difference between vesicular and arbuscular colonization could be due to the fact the roots were collected in December, a time when *P. lobata* is metabolically inactive and arbuscules are nonfunctional (Perumal, pers.comm.). This assumption may explain the lack of arbuscular colonization in site 2 even though total VAM colonization was 79.25% (Table 1).

Site 4 had an even greater increase in vesicular colonization as compared to arbuscular colonization which confirms our earlier suggestion. Furthermore, vesicular colonization in site 4 was the largest of all six sites, yet it was only significantly different to site 5 (Figure 1, Graph B). This broad range of similarity may be due to a small sample size. The greater number of samples tested, the narrower a statistical analysis becomes which allows less room for error (Zar, 1984).

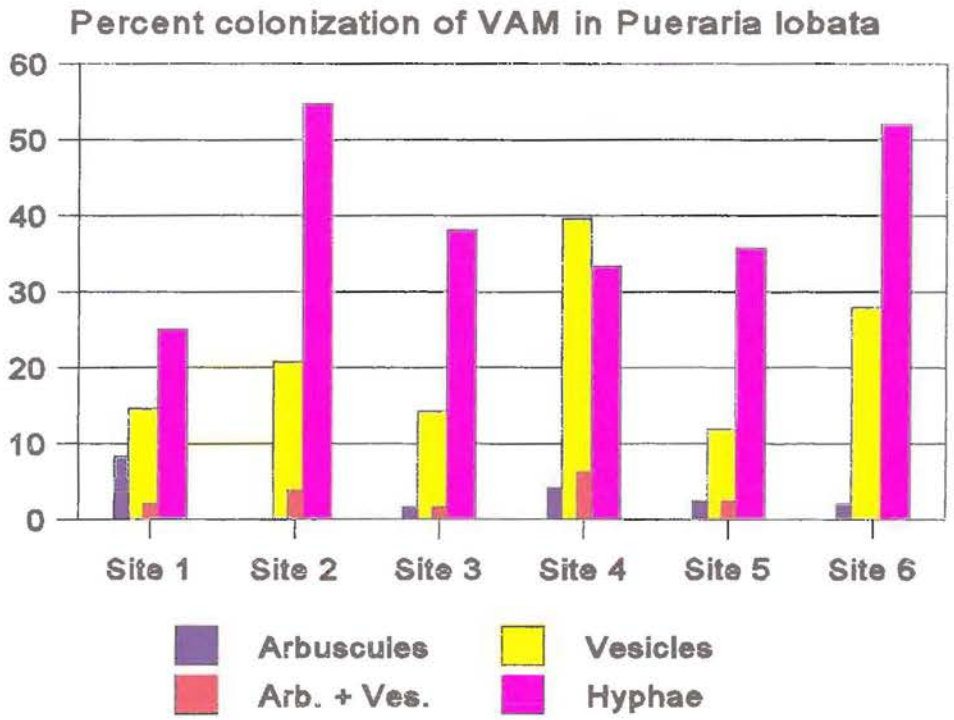
To test for similarity between VAM structural colonization, ANOVA (Triola and Flynn, 1995) was performed. This test accepted the null hypothesis that colonization values were not significantly different. With confirmed similarity, the overall totals were then analyzed. Total hyphae colonization infiltrated the roots at 67.11%. Therefore less than 33% of the roots were uncolonized by VAM. Such a high percentage of colonization suggests that vesicular-arbuscular mycorrhizal fungi plays an important role in *P. lobata* through the uptake of nutrients. This increase in nutrients facilitates growth and development (Allen, 1991) and appears to be the secret beneath *P. lobata's* aggressiveness.

Unfortunately, little research has been done on *P. lobata.* However, this experiment has paved the way for further research in determining the role played by VAM. Additional investigation into *P. lobata's* reliance on mycorrhizae needs to be pursued. Growing *P. lobata* in controlled soils (sterile, mycorrhizae rich, and mixtures) would provide direct evidence to *P. lobata's* degree of VAM dependence. Another question that needs to be addressed is whether planting *P. lobata* in wet, dry, acidic, and/or alkaline soils will effect the percentage of VAM colonization.

Research opportunities in the field of *P. lobata* extend beyond the scope of VAM. For example, daidzin, which was found to be an active ingredient in *P. lobata*, may soon be used medically to suppress alcohol cravings (Sharris, 1993). These research interests are only a few of the many possibilities that await exploration. However, we cannot hope to harness the full potential in this field of research without first understanding the ecophysiology of *P. lobata.*

APPENDIX I

A graphical comparison of VAM structure colonization within and between sites.



APPENDIX II

The following tables list percentages of vesicular-arbuscular mycorrhizae found in individual samples taken from the six study sites. Total colonization per site is also shown.

Table 1. Percent VAM colonization in site 1.

	K1S1	K1S2	K1S3	TOTAL
Arbuscules	7.69	0.00	15.79	8.33
Vesicles	15.38	6.25	21.05	14.58
Arb + Ves	0.00	0.00	5.26	2.08
No Structures	46.15	62.50	42.11	50.00
Hyphae Only	30.77	31.25	15.79	25.00
Hyphae Total	53.85	37.50	57.89	50.00

Table 2. Percent VAM colonization in site 2.

	K2S1	K2S2	K2S3	TOTAL
Arbuscules	0.00	0.00	0.00	0.00
Vesicles	22.22	11.11	29.41	20.75
Arb + Ves	5.56	0.00	5.88	3.77
No Structures	27.28	16.67	17.65	20.75
Hyphae Only	44.44	72.22	47.06	54.72
Hyphae Total	72.22	83.33	82.35	79.25

Table 3. Percent VAM colonization in site 3.

	K3S1	K3S2	K3S3	TOTAL
Arbuscules	6.25	0.00	0.00	1.59
Vesicles	25.00	4.35	16.67	14.29
Arb + Ves	6.25	0.00	0.00	1.59
No Structures	31.25	56.52	41.67	44.44
Hyphae Only	31.25	39.13	41.67	38.10
Hyphae Total	68.75	43.48	58.33	55.56

Table 4. Percent VAM colonization in site 4.

	K4S1	K4S2	K4S3	TOTAL
Arbuscules	6.67	0.00	5.56	4.17
Vesicles	26.67	33.33	55.56	39.58
Arb + Ves	0.00	6.67	11.11	6.25
No Structures	20.00	26.67	5.56	16.67
Hyphae Only	46.67	33.33	22.22	33.33
Hyphae Total	80.00	73.33	94.44	83.33

Table 5. Percent VAM colonization in site 5.

	K5S1	K5S2	K5S3	TOTAL
Arbuscules	0.00	6.67	0.00	2.38
Vesicles	0.00	20.00	13.33	11.90
Arb + Ves	0.00	6.67	0.00	2.38
No Structures	66.67	20.00	60.00	47.62
Hyphae Only	33.33	46.67	26.67	35.71
Hyphae Total	33.33	80.00	40.00	52.38

Table 6. Percent VAM colonization in site 6.

	K6S1	K6S2	K6S3	TOTAL
Arbuscules	0.00	0.00	5.00	2.00
Vesicles	27.78	25.00	30.00	28.00
Arb + Ves	0.00	0.00	0.00	0.00
No Structures	11.11	25.00	20.00	18.00
Hyphae Only	61.11	50.00	45.00	52.00
Hyphae Total	88.89	75.00	80.00	82.00

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