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ASSESSMENT OF MYCORRHIZAL INFECTIVITY IN TERRESTRIAL BAHAMIAN PLANT ROOTS

Gregory D. Miller, Mel Wrigley, and Garriet W. Smith
Department of Biology
University of South Carolina at Aiken
Aiken, SC USA 29801.

ABSTRACT

Mycorrhizal associations generally aid in nutrient (especially phosphorus) uptake, and are necessary for plants growing in nutrient-poor environments. Vesicular-arbuscular mycorrhizal hyphae enhance the uptake of phosphate from the soil and distribute it throughout the root cortical tissue, vesicles are used as stroage organs for organs for phosphorus, and arbuscules are responsible for phosphorus exchange with the host plant cell. In this study, a total of 39 plant roots representing 20 different species were collected from two sites on the Bahamian Island of San Salvador. Due to the nutrient poor soil present on the island, it was hypothesized that the island plants would have a significant amount of mycorrhizal infection. After clearing and staining, the roots were assessed for mycorrhizal infection using a subjective visual assay. Results were tabulated and plants were ranked according to their degree of mycorrhizal infection. Highest degrees of infection were found with *Croton linearis*, *Grundlachia corymbosa*, *Cenchrus incertus*, *Mimosa bahamensis*, and *Urechites lutea*.

INTRODUCTION

Vesicular-arbuscular mycorrhizae (VAM) produce a number of structural components ranging from spores and sporocarps to arbuscules where nutrient exchange between the plant and fungus occurs. Plant species vary in their susceptibility to VAM infection. In addition, different plant parts also exhibit variable susceptibility patterns and these can change with the age of the tissue. Hepper (1984) showed that 21-day old clover roots did not immediately become infected with VAM, but infection was immediate on newly formed roots. Smith and Walker (1981) showed that infection of apical regions of 35-day old clover roots was 10-times greater than the rest of the root system.

Phosphorus uptake in VAM infected plants has been shown to be enhanced in sudangrass, strawberries, onions, tomato, corn, apple, soybean, and many other plant species (Allen, 1991). The mechanism presumably involves the stimulation of a phosphorus-solubizing system that actively reduces unavailable inorganic phosphorus. This mechanism, however, was questioned by Abbot and Robson (1984). They suggest that an increase in nutrient uptake due to VAM is primarily the result of the shorter distance nutrients must travel to the root. Mosse (1975) observed increased Cu, Fe, Mg, Mn, and Zn uptake in VAM infected apple tissue over uninfected plants.

VAM infection appears to enhance plant growth mostly during soil stressed conditions. In some cases, VAM infection depressed plant growth in soils in which phosphorus was not limiting (Allen, 1991). This was probably due to a carbon drain by the endophyte. This effect was most apparent in seedlings. Non-nutritional effects of VAM associations have been shown to occur, although the mechanisms are not clear (Graham, 1987). In VAM infected sweetgum plants, the dry-weight biomass was 40 times higher than non-VAM plants. Addition of various fertilizers did not reduce the biomass differences (Schultz *et al.*, 1979).

Although VAM are relatively host nonspecific, certain VAM-host combinations are more effective than others (Mosse, 1975). VAM infected plants have been observed from

a wide variety of environments including alpine ecosystems north of the Arctic Circle and in Antaractica (Allen *et al.*, 1987). Nicolson (1960) reported that plants on coastal dunes or near the sea tended to be nonmycotrophic and that VAM infection tended to increase proceeding inland. The degree of VAM infection varies depending on a number of soil environmental conditions including temperature, fertility, composition of microorganisms and seasonal variations (Allen, 1991). VAM probably enhance the fitness of most plant species, but not in all environmental situations. Given the wide range of areas in which they occur and the potential advantage of these fungi, VAM could have a substantial impact on agriculture and environmental restoration.

MATERIALS AND METHODS

Plant roots were collected from two sites at the north end of San Salvador Island, Bahamas, in July of 1992. Site 1 was a coastal site which received sea spray during storms and, therefore, contained salt tolerant plants. Site 2 was approximately 0.5 km south of the coast. Both sites had carbonate soils and were extremely low in phosphorus. The plants were removed from the soil and root samples were preserved in a formalin-acetic acid-ethanol (FAA) fixing solution (Schenck, 1982). Samples were collected of 39 different plants from 20 species and transported to the laboratory.

Ten roots were randomly selected from each sample, rinsed in tap water to remove excess FAA and placed in beakers for clearing and staining. Roots were immersed in a 10% KOH solution covered with aluminun foil, and autoclaved for two minutes at 15 psi. Samples were then repeatedly rinsed in tapwater until the wash water was clear. The entire clearing procedure was repeated for dark roots until they began to clear. Roots were then bleached in alkaline hydrogen peroxide for 20-30 minutes to allow stain penetration. After removal of the peroxide, roots were rinsed three times in tapwater and acidified in 0.8% HCI for three to five minutes. Roots were then drained and submerged in a solution of 0.1% acid fuchsin-lactic acid and autoclaved for one to two minutes to force stain into the tissue (Schenck, 1982).

Roots were then sectioned into 1.0 cm segments and ten of these were placed on a microscope slide. Thirty root segments from each plant sample were analyzed for VAM structures and a number assigned to each segment corresponding to the degree of infection. An assessment scale ranging from 0 to 3 was used in increments of 0.5 corresponding to the density of VAM structures (0=none, 3=very dense).

RESULTS AND DISCUSSION

Plant species tested for mycorrhizal infection are listed by site in Tables 1 and 2. In most cases, these were also the most dominant plants at these sites. The average degree of infection is listed in Table 3 by species. Species showing VAM infection levels greater than 1.0 were classified as heavily infected. The most heavily infected was *Croton linearis* with an average VAM infection level of 1.58. This species is shown in Figure 1. Figure 1a shows extensive hyphal development in the root tissue. Extensive arbuscular development can be seen in the cortical cells (deeply stained cells). The most heavily infected individual sample was a *Grundlachia corymbosa* (Table 4, E-1), closely followed by a *Croton linearis* (B-1). The third most heavily infected plant was also a *C. linearis*, which may indicate that this species could be less variable than others. Figure 2 shows *G. corymbosa* while Figure 2a shows the high density of VAM vesicules found in the roots of this species. Figures 3-5 show the three remaining plants that were ranked among the most heavily infected. Figure 5a shows a VAM spore connected to a hyphal strand.

The plant species used in this study fell into rather broad general groups, or natural divisions, based on the degree of VAM infection. These divisions were: heavy (>1.15), medium (0.84-0.88), and light (0.21-0.67). Although all species tested showed some VAM infection, only five showed heavy infection levels. These were *Croton linearis*, *Grundlachia corymbosa*, *Cenchrus incertus*,

TABLE 1. PLANTS TESTED FOR MYCORRHIZAL ASSOCIATIONS. SITE 1.

Plant	Common Name	Scientific Name
A-1	Granny Bush	*Croton linearis*
B-1	Granny bush	*Croton linearis*
C-1	Coastal Sand Spur	*Cenchrus Incertus*
D-1	Coastal sand Spur	*Cenchrus Incertus*
E-1	Horsebush (older)	*Grundlachia corymbosa*
F-1	Horsebush (younger)	*Grundlachia corymbosa*
G-1	Wooly Corchorus (older)	*Corchorus hirsutus*
H-1	Wooly Corchorus (younger)	*Corchorus hirsutus*
I-1	Bay Geranium	*Ambrosia hispida*
J-1	Bay Geranium	*Ambrosia hispida*
K-1	Wild Unction	*Urechites lutea*
L-1	Wild Unction	*Urechites lutea*
M-1	Ink Berry	*Scaevola plumieri*
N-1	Ink Berry	*Scaevola plumieri*
O-1	Passion Flower	*Passiflora*
P-1	Sea Oats	*Uniola paniculata*
Q-1	Sea Oats	*Uniola paniculata*
R-1	Railroad Vine	*Ipomea pes-caprae*
W-1	Railroad Vine	*Ipomea pes-caprae*
S-1	Sea Grape	*Coccoloba unifera*
T-1	Sea Grape	*Coccoloba unifera*
U	Bay Cedar	*Suriana maritima*
V	Bay Cedar	*Suriana maritima*

TABLE 2. PLANTS TESTED FOR MYCORRHIZAL ASSOCIATIONS. SITE 2.

Plant	Common Names	Scientific Name
A-2	Jim Bay	*Leucaena leucocephala*
B-2	Jim Bay	*Leucaena leucocephala*
C-2	Pencil Flower	*Stylosountnes hamata*
D-2	Pencil Flower	*Stylosountnes hamata*
E-2	Haulback	*Mimosa bahamensis*
F-2	Haulback	*Mimosa bahamensis*
G-2	Hardhead	*Phylloanthus epiphyllanthus*
H-2	Hardhead	*Phylloanthus epiphyllanthus*
I-2	Wild Sage	*Lantana involucrata*
O-2	Wild Sage	*Lantana involucrata*
J-2	Strong Back	*Bourreria ovata*
K-2	Strong Back	*Bourreria ovata*
L-2	Bahamian Buttercup	*Tunera ulmifolia*
P-2	Bahamian Buttercup	*Tunera ulmifolia*
M-2	Wild Tomato	*Rivina humulis*
N-2	Wild Tomato	*Rivina humulis*

TABLE 3. SPECIES LISTED BY AVERAGE DEGREE OF MYCORRHIZAL INFECTION.

Species		Degree of Infection
Croton linearis		1.58
Grundlachia corymbosa		1.51
Cenchrus incertus	(Heavy)	1.38
Mimosa bahamensis		1.22
Urehites lutea		1.16
Passiflora		.88
Phylloanthus epiphyllanthus		.87
Stylosountnes hamata	(Medium)	.87
Lantana involucrata		.84
Corchorus hirsutus		.67
Tunera ulmifolia		.64
Leucaena leucocephala		.64
Bourreria ovata		.60
Coccoloba unifera	(Light)	.48
Scaevola plumieri		.45
Rivina humulis		.28
Uniola paniculata		.26
Suriana maritima		.21

Figure 1. *Croton linearis* (Granny Bush).

Figure 1a. Vesicles and hyphal regions in Granny Bush (40X).

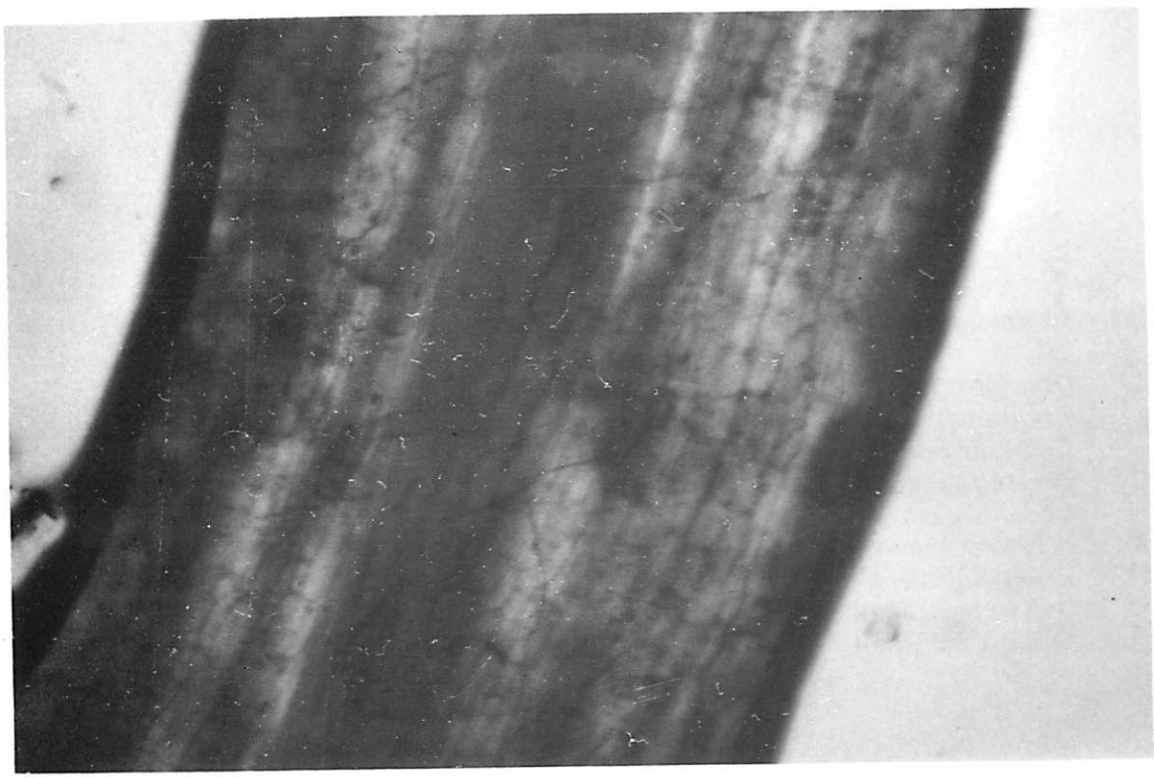


TABLE 4. PLANT ASSAYED BY DEGREEE OF MYCORRHIZAL INFECTION

Plant	# Segments		Degree Infectivity		Scientific Name
			AVG.	S.D.	
E-1	30		1.63	0.84	*Grundlachia Corymbosa*
B-1	29		1.62	0.80	*Croton linearis*
A-1	37		1.53	0.64	*Croton linearis*
C-1	20	(Heavy)	1.47	0.50	*Cenchrus incertus*
E-2	30		1.38	0.49	*Mimosa bahamensis*
K-1	30		1.38	0.66	*Urechites lutea*
F-1	30		1.36	0.79	*Grundlachia Corymbosa*
D-1	30		1.29	0.74	*Cenchrus incertus*
F-2	30		1.05	0.63	*Mimosa bahamensis*
H-2	30		1.03	0.82	*P. epiphyllanthrus*
K-2	29		1.02	0.68	*Bourreria ovata*
C-2	30		0.95	0.52	*Stylosountnes hamata*
L-1	30		0.93	0.52	*Urechites lutea*
S-1	30		0.90	0.71	*Coccoloba unifea*
O-1	30	(Medium)	0.88	0.76	*Passiflora*
I-2	30		0.85	0.62	*Lantana involucrata*
O-2	30		0.83	0.49	*Lantana involucrata*
D-2	30		0.78	0.57	*Stylosountnes hamata*
H-1	30		0.77	0.50	*Corchorus hirsutus*
I-1	29		0.76	0.56	*Ambrosia hispida*
R-1	30		0.75	0.56	*Ipomea pes-caprea*
L-2	29		0.71	0.56	*Tunera ulmfolia*
G-2	30		0.70	0.81	*P. Epiphyllanthus*
A-2	30		0.67	0.60	*Leucaena leucocephala*
B-2	30		0.60	0.40	*Leucaena leucocephala*
P-2	28		0.57	0.38	*Tunera ulmifolia*
G-1	30		0.56	0.43	*Corchorus hirsutus*
N-1	30		0.46	0.47	*Scaevola plumieri*
W-1	30		0.43	0.45	*Ipomea pes-caprea*
M-1	30		0.43	0.42	*Scaevola plumieri*
J-1	35	(Light)	0.39	0.32	*Ambrosia hispida*
V-1	30		0.35	0.44	*Suriana maritima*
N-2	30		0.35	0.39	*Rivina humulis*
P-1	30		0.35	0.35	*Uniola paniculata*
M-2	30		0.20	0.24	*Rivina humulis*
J-2	30		0.18	0.17	*Bourreria ovata*
Q-1	15		0.17	0.24	*Uniola paniculata*
U-1	30		0.07	0.17	*Suriana maritima*
T-1	16		0.06	0.17	*Coccoloba unifera*

Figure 2. *Grandlachia corymbosa* (Horsebush).

Figure 2a. Vesicular regions in Horsebush (40X).

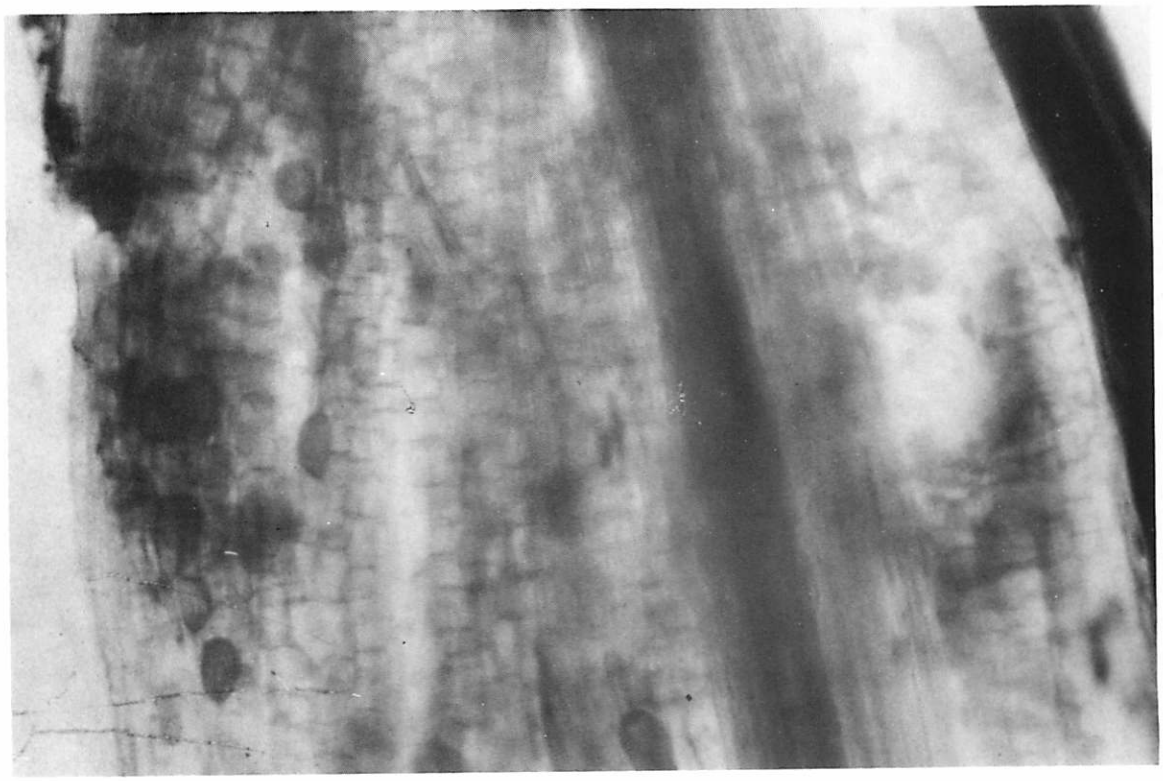


Figure 3. *Cenchrus incertus* (Coastal Sandspur).

Figure 3a. Hyphal regions of Coastal Sandspur (40X).

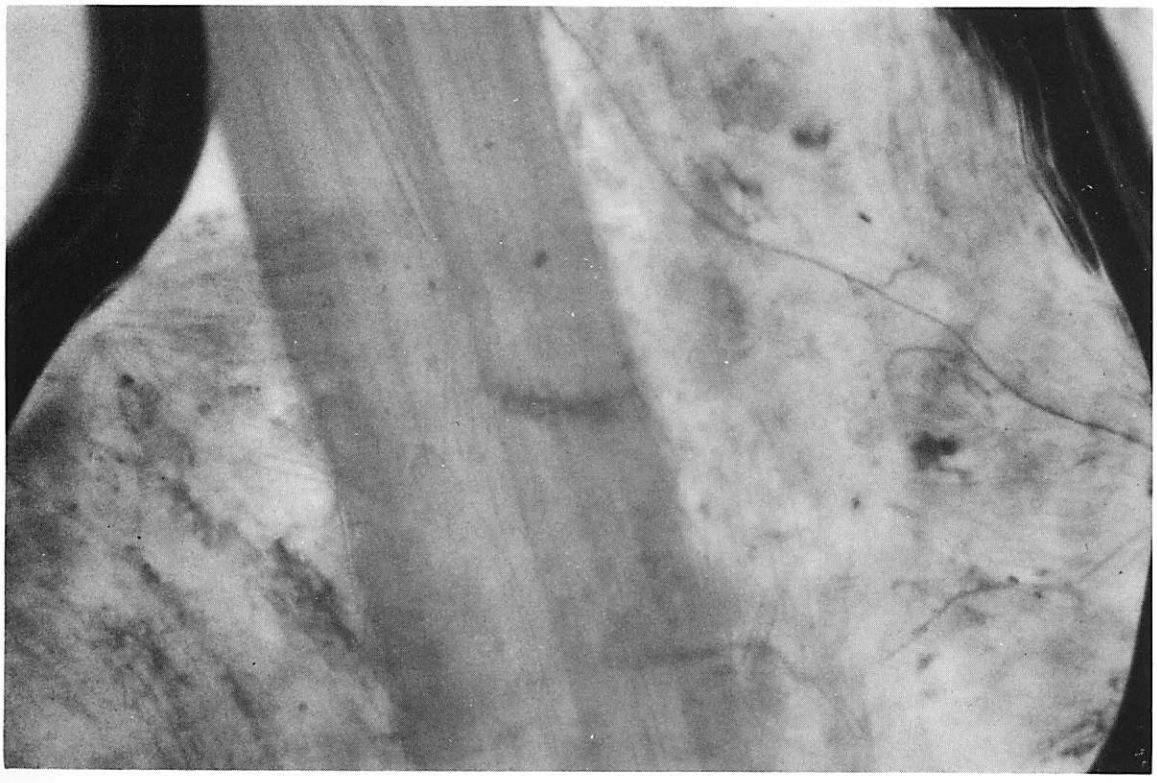


Figure 4. *Mimosa bahamenis* (Haulback).

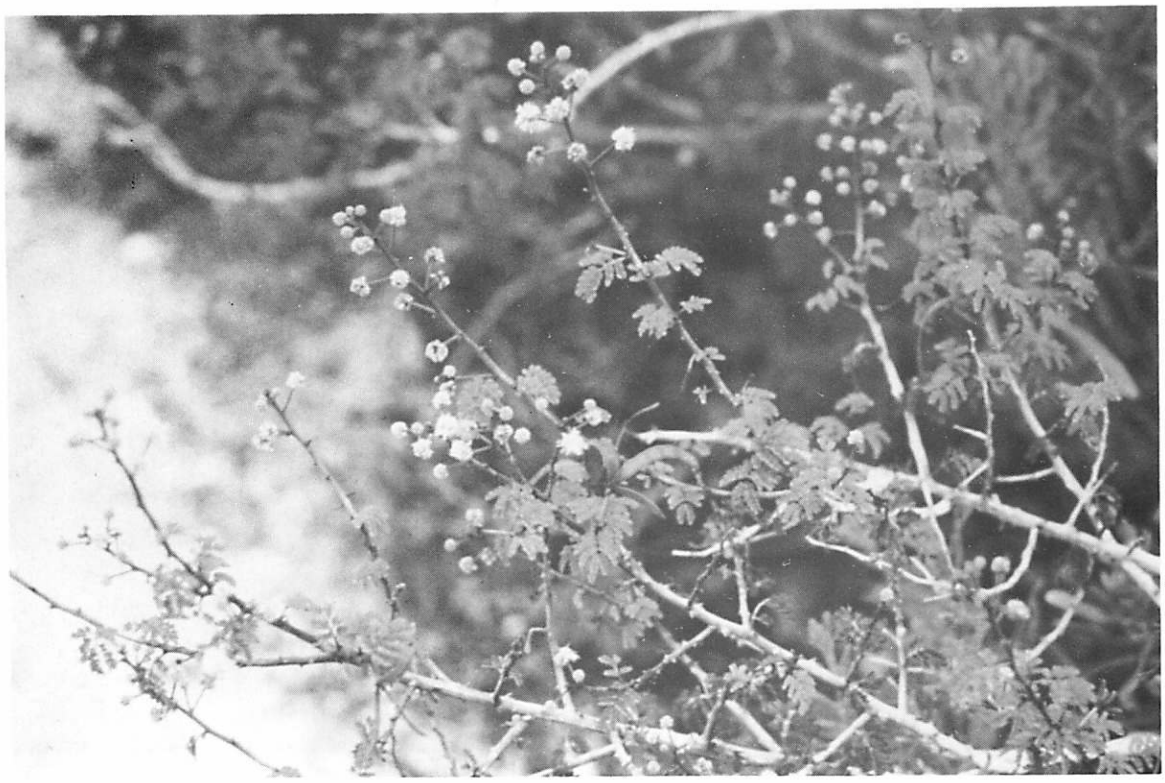


Figure 4a. Vesicular and hyphal regions in Haulback (40X).

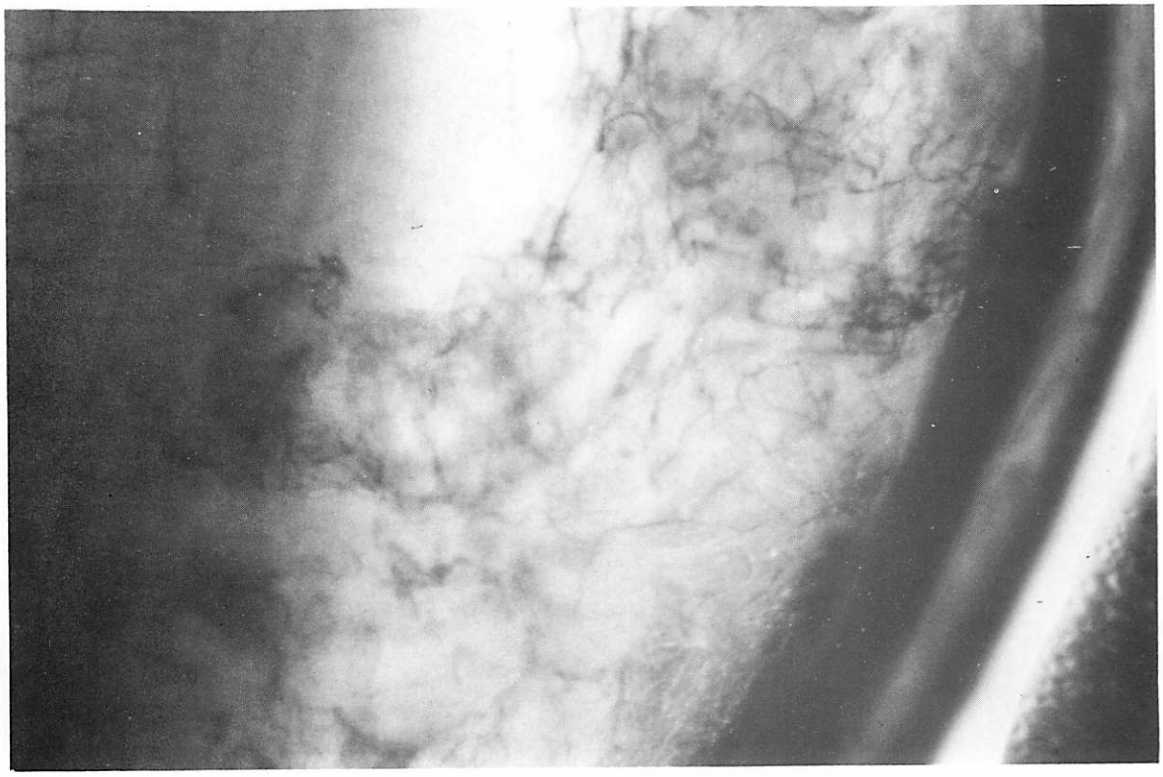
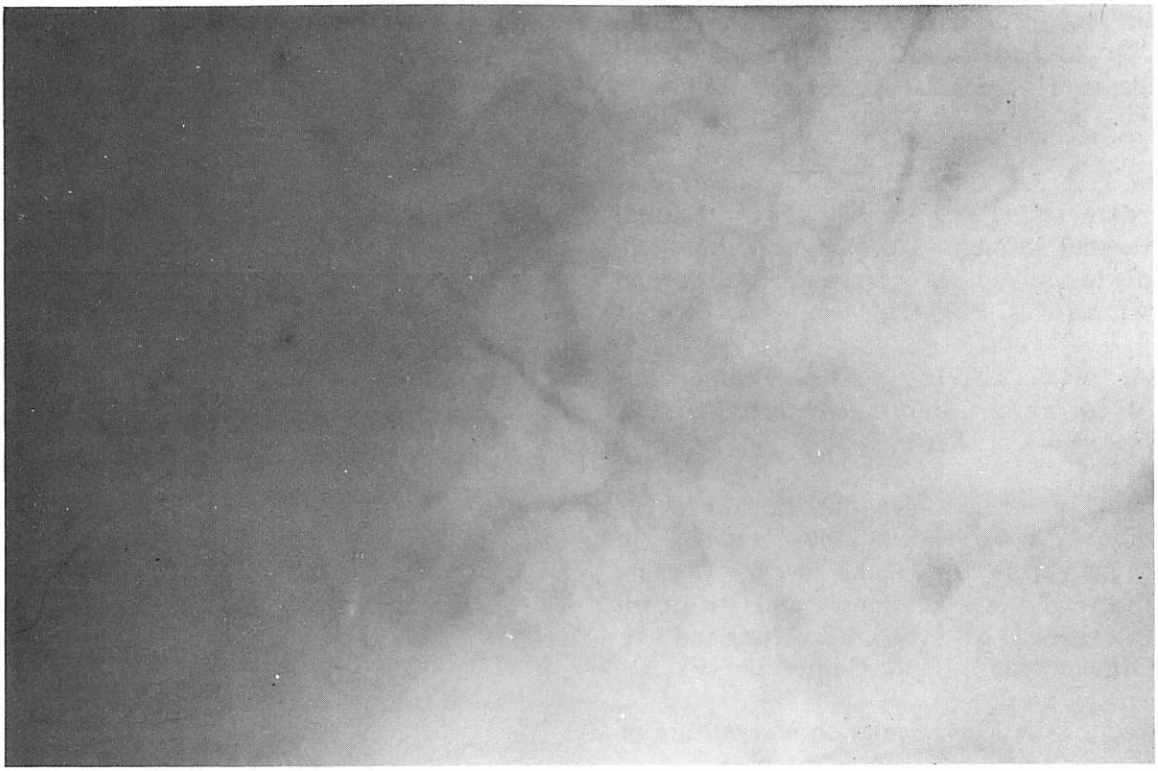


Figure 5. *Urechites lutea* (Wild Unction).

Figure 5a. Vesicular and hyphal regions in Wild Unction (400X).



Mimosa bahamensis, and *Urechites lutea.* All of these plants except *M. bahamensis* were collected from site 1. Our observation that coastal plants (site 1) were more heavily infected than interior (site 2) plants conflicts with Nicolson's (1960) observations on coastal plants in Australia. Bahamian plants, growing in carbonate soils, may have a much greater need for VAM due to the low soil phosphorus content. Although the degree of VAM infection does not always translate to increased growth for the plant, our study found that the most heaily VAM infected plants were also the most dominant, particularly along the coast.

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