GROWTH, ROOT ALTERATION, AND NUTRIENT UPTAKE OF NEEM TREE (*Azadirachta indica* A. Juss) SEEDLINGS IN RESPONSE TO VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGI AND PHOSPHORUS NUTRITION

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SUMMARY

Growth and nutrient uptake of neem tree (*Azadirachta indica* A. Juss) seedlings were studied in response to vesicular-arbuscular mycorrhizal (VAM) fungi *Glomus intraradices* Schenck and Smith, and 2 levels of phosphorus (P). Extensive VAM colonization in neem roots occurred at both P levels: 0.65 and 1.30 mM P. Growth of VAM plants at both P levels was similar, while growth of noncolonized (Non-VAM) plants increased with increasing P supply. At the low P level, VAM plants had greater leaf area, shoot, root and leaf dry weight, and a greater root:shoot ratio than Non-VAM plants. Low P, VAM plants had a greater leaf area and root dry weight and a comparable leaf and shoot dry weight and root:shoot ratio, when compared with high P, Non-VAM plants. VAM inoculation also altered dry mass partitioning to root systems resulting in greater root length and dry weight of suberized roots in VAM plants. The length of nonsuberized roots increased with increasing P supply regardless of VAM inoculation. VAM inoculation increased leaf tissue concentration of N, P, S and B. There are benefits of utilizing VAM inoculation on growth enhancement and nutrient uptake of neem tree seedlings under low phosphorus conditions.

INTRODUCTION

The neem tree (*Azadirachta indica* A. Juss) is of reforestation, ornamental, medicinal and biomass value in India, Myanmar, Southeast Asia, and Africa. The compound azadirachtin is extracted from neem seeds and is commercially used as an insecticide for controlling insect pests (National Research Council, 1992). Oil extracted from neem seeds can control plant fungal diseases such as powdery mildew and rust (Becker, 1994). Neem is drought resistant with a deep root system that tolerates poor soils of semi-arid to arid regions (Benge, 1989). Neem has a fairly coarse root system with very few root hairs. The survival of neem trees under low fertility conditions, particularly low phosphorus (P) availability, which is a common characteristic of many soils where it is planted, may depend on vesicular-mycorrhizal fungi (VAM). VAM are commonly associated with tropical trees (Redhead, 1980). Little information is known about naturally occurring VAM association in neems. Plants in the same family as neem (*Meliaceae*) such as *Melia azadarach*, *Cedrela odorata*, and *Swietenia macro-
phylla have been reported to form VAM colonization under natural conditions (Herrera and Ferrer, 1980). The benefits of VAM on growth enhancement, improvement of nutrient uptake, and diminishing abiotic stresses have been reported in a wide range of host plants from annual crops to woody perennials (Smith and Gianinazzi, 1988). The VAM symbiosis can increase P-uptake in low P available soils and subsequently benefit host plants (Abbott and Robson, 1984; Smith and Gianinazzi, 1988). Neem is commonly propagated by seed and grown in nurseries for 3-5 months before it is transplanted to production sites (Benge, 1989). Preinoculation of neem seedlings with efficient VAM fungi would potentially enhance growth, reduce transplant shock and increase rate of survival and growth after transplanting, as reported in the production of other woody plant species (Strong and Davies, 1982; Graham, 1986; Davies and Call, 1990).

The objective of this study was to determine the effect of VAM fungi Glomus intraradices Schenck and Smith and phosphorus nutrition on the growth, nutrient uptake, root alteration and VAM colonization of neem tree seedlings.

MATERIALS AND METHODS

A 2x2 factorial experiment with 2 levels of VAM inoculation (inoculated or noninoculated) and 2 levels of phosphorus fertility (0.65 or 1.30 mM P) was arranged in a completely randomized design in a glasshouse with 10 replicate plants per treatment. The four treatment combinations consisted of:

1) VAM inoculation + 0.65 mM P,
2) VAM inoculation + 1.30 mM P,
3) Noninoculated + 0.65 mM P, and
4) Noninoculated + 1.30 mM P.

Three month old neem seedlings were transplanted into 3.8 liter containers containing sterilized sandy loam soil and coarse sand (1:1 v/v). The container medium was very low in phosphorus and soluble salts (Table 1). Plants were either inoculated at transplanting with the vesicular-arbuscular mycorrhizal fungi (VAM) Glomus intraradices Schenck and Smith or were not inoculated as nonmycorrhizal (Non-VAM) controls. Each inoculated plant received approximately 10,000 spores of the fungus and the inoculum was banded halfway down the container. Non-VAM plants received sieved (11 µm) inoculum solution to equalize background microflora associated with the mycorrhizal inoculum. VAM and Non-VAM plants were fertilized twice a week at the rate of 300 ml per plant with Long Ashton Nutrient Solution (LANS) (Hewitt, 1966) modified to supply phosphorus (P) at either 0.65 mM (half strength P of LANS) or 1.30 mM (full strength P of LANS). Plants were grown for 20 weeks in a glasshouse (30/25 C average day/night temperature with supplemental light for a 16-hr photoperiod, and maximum photosynthetic photon flux at the plant canopy of 1,100 µmol m⁻¹ sec⁻¹) and irrigated as needed.

<table>
<thead>
<tr>
<th>Properties of a</th>
<th>Sand (%)</th>
<th>Silt (%)</th>
<th>Clay (%)</th>
<th>pH</th>
<th>Electrical conductivity (µS cm⁻¹)</th>
<th>Total N (µg g⁻¹)</th>
<th>Bicarbonate extractable P (µg g⁻¹)</th>
<th>Exchangeable K (µg g⁻¹)</th>
</tr>
</thead>
</table>

Twenty weeks after transplanting, the number, leaf area, and shoot dry weight, suberized roots (light brown to white color) and root length was determined (Melbourne, Australia). Dry weights were determined after root length measurements from 10 plants per treatment (= 5 plants per treatment cleared with acidic glycerol (Koske and Goss) and root length with VAM colonized. Biermann and Linderman (1981) used an inductively coupled plasma atomic emission spectrometer (Warick, New York), using 3 composite sample of leaves and stems of each sample of Variance Procedure (SAS Inst.

RESULTS

VAM inoculation significantly increased root growth, and root shoot ratio of plants at high P (1.30 mM) level (Table 2). Leaf area was greater than that of Non-VAM plants at high P (1.30 mM) level greater than than that of Non-VAM plants. The difference between P levels on shoot growth was significant. Leaf number, SLA, and LAR were significantly higher in VAM plants at high P level.
TABLE 1

Properties of a growing media used in this experiment

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Sand (%)</td>
<td>81</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>5</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>14</td>
</tr>
<tr>
<td>pH</td>
<td>7.7</td>
</tr>
<tr>
<td>Electrical conductivity (S m⁻¹)</td>
<td>0.021</td>
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<tr>
<td>Total N (µg g⁻¹)</td>
<td>3.18</td>
</tr>
<tr>
<td>Bicarbonate extractable P (µg g⁻¹)</td>
<td>1.65</td>
</tr>
<tr>
<td>Exchangable K (µg g⁻¹)</td>
<td>11.45</td>
</tr>
</tbody>
</table>

Twenty weeks after transplanting, plants were harvested and evaluated for leaf number, leaf area, and shoot dry weight. Root systems were visually separated into suberized roots (light brown to brown in color) and nonsuberized roots (white in color) and root length was determined using a Comair Rootlength Scanner (Melbourne, Australia). Dry weight of suberized and nonsuberized roots was also determined after root length measurement. All growth parameters were determined from 10 plants per treatment (n=10). From these measured growth parameters, leaf area ratio [LAR: leaf area (cm²)/total plant dry weight (g)], specific leaf area [SLA: leaf area (cm²)/leaf dry weight (g)], and root to shoot ratio [root dry weight (g)/shoot dry weight (g)] were calculated. Subsamples of nonsuberized roots were taken from 5 plants per treatment, cleared with 2.5% KOH, and stained with 0.05% trypan blue in acidic glycerol (Koske and Gemma, 1989) to access VAM colonization. Percent root length with VAM colonization was determined using the techniques of Biermann and Linderman (1981). Leaf tissue mineral analysis was conducted on an inductively coupled plasma atomic emission spectrometer (Fison Horticulture Inc., Warick, New York), using 3 composite samples per treatment (n=3). Each composite sample was a pool of leaves from 3 plants. All data were analyzed by Analysis of Variance Procedure (SAS Institute Inc., 1988).

RESULTS

VAM inoculation significantly increased leaf area, leaf, shoot, and root dry weight, and root to shoot ratio of neem seedlings, particularly at the low P (0.65 mM P) level (Table 2). Leaf area of VAM plants at both P levels was comparable and was much greater than leaf area of Non-VAM plants. Dry weight partitioning to leaves, stems, and roots of VAM plants at both P levels and of Non-VAM plants at high P (1.30 mM P) level was comparable and was significantly greater than that of Non-VAM plants at the low P level. There was no significant difference between P levels on overall growth and development of VAM plants. Leaf number, SLA, and LAR were not significantly different between VAM and Non-VAM plants at both P levels.
TABLE 2

Effects of VAM and different phosphorus levels on growth and development of neem seedlings

<table>
<thead>
<tr>
<th>Mycorrhiza</th>
<th>P (mM)</th>
<th>Leaf no.</th>
<th>Leaf area (cm²)</th>
<th>SLA (cm² g⁻¹)</th>
<th>LAR (g)</th>
<th>Leaf dry wt (g)</th>
<th>Shoot dry wt (g)</th>
<th>Root dry wt (g)</th>
<th>Root:shoot ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>0.65</td>
<td>22.1±0.9</td>
<td>684±34</td>
<td>139.2</td>
<td>65.4</td>
<td>4.7±0.3</td>
<td>7.0±0.5</td>
<td>3.1±0.3</td>
<td>0.45±0.04</td>
</tr>
<tr>
<td></td>
<td>1.30</td>
<td>24.3±0.6</td>
<td>759±49</td>
<td>134.6</td>
<td>61.2</td>
<td>5.7±0.4</td>
<td>8.3±0.5</td>
<td>4.2±0.4</td>
<td>0.51±0.04</td>
</tr>
<tr>
<td>Yes</td>
<td>0.65</td>
<td>25.0±0.9</td>
<td>877±34</td>
<td>144.6</td>
<td>61.0</td>
<td>6.1±0.4</td>
<td>9.1±0.4</td>
<td>5.4±0.5</td>
<td>0.59±0.05</td>
</tr>
<tr>
<td></td>
<td>1.30</td>
<td>23.6±0.9</td>
<td>885±52</td>
<td>144.8</td>
<td>63.0</td>
<td>6.2±0.5</td>
<td>9.1±0.6</td>
<td>5.1±0.4</td>
<td>0.56±0.03</td>
</tr>
</tbody>
</table>

Significance:
- VAM: NS, NS, **
- P: NS, NS, NS, NS
- Interaction: NS, NS, NS

NS = Nonsignificant, ** and * = Significant at P ≤ 0.01 and 0.05, respectively. Data represents a mean of 10 plants per treatment ± SE.

TABLE 3

Effects of VAM and different phosphorus levels on partitioning of nonsuberized and suberized roots of neem seedlings and percentage of root length colonized by VAM.

<table>
<thead>
<tr>
<th>Mycorrhiza</th>
<th>P (mM)</th>
<th>Root length (cm)</th>
<th>Root dry wt (g)</th>
<th>Colonization %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonsuberized</td>
<td>Suberized</td>
<td>Total</td>
<td>Nonsuberized</td>
</tr>
<tr>
<td>No</td>
<td>0.65</td>
<td>3160±162</td>
<td>624±97</td>
<td>3784±139</td>
</tr>
<tr>
<td></td>
<td>1.30</td>
<td>3498±513</td>
<td>641±66</td>
<td>4139±567</td>
</tr>
<tr>
<td>Yes</td>
<td>0.65</td>
<td>2840±300</td>
<td>862±76</td>
<td>3702±348</td>
</tr>
<tr>
<td></td>
<td>1.30</td>
<td>4132±186</td>
<td>840±80</td>
<td>4972±257</td>
</tr>
</tbody>
</table>

Significance:
- VAM: NS, **
- P: NS, NS, NS
- Interaction: NS

NS = Nonsignificant, ** and * = Significant at P ≤ 0.01 and 0.05, respectively. Data represents a mean of 10 plants per treatment ± SE.
* Percent root length colonized by VAM determines from 5 plant per treatment.

The length of nonsuberized roots and total roots increased with increasing P supply regardless of VAM inoculation (Table 3). The greatest length of nonsuberized roots and total roots occurred with VAM plants at the high P level. In contrast, VAM inoculation significantly increased the length of suberized roots. VAM plants at low and high P levels had more roots, respectively, than Non VAM plants. Phosphorus inoculation enhanced the development of VAM plants at low and high P levels, respectively. The interaction of VAM and P level was significant, indicating that increasing P fertility increased plant root dry weight of VAM plants but had no effect on nonsuberized root dry weight of VAM plants. The percent root length colonized by VAM was greater than that of Non-VAM inoculated plants (Table 3).

Regardless of P-fertility levels, Ca and Mg concentration (data not shown) were higher in plants at the high P level. VAM inoculation increased K concentration (data not shown) and Al concentration (data not shown) in VAM plants. No decrease in K concentration was observed in VAM plants inoculation, which suggested that VAM colonization suggested that VAM plants with increased P-levels had higher K concentrations.
at low and high P levels had a 38% and 31% greater suberized root length, respectively, than Non VAM plants at the same P level. Dry weight partitioning to nonsuberized and suberized roots was significantly altered by VAM association. VAM inoculation enhanced the dry weight of suberized roots regardless of P fertility. VAM plants at low and high P levels had 63% and 24% greater dry weight of suberized roots, respectively, than Non-VAM plants at the same P levels. At the low P level, VAM inoculation also enhanced the dry weight of nonsuberized roots.

The interaction of VAM and P factors on nonsuberized root dry weight indicated that increasing P fertility increased nonsuberized root dry weight of Non-VAM plants but had no effect on nonsuberized root dry weight of VAM plants. The total root dry weight of VAM plants at both P levels was comparable and was much greater than that of Non-VAM plants at either P level.

The percent root length colonized by *Glomus intraradices* was very high in inoculated plants (Table 3). The different P levels did not affect VAM colonization. There was no evidence of VAM colonization in noninoculated plants.

Regardless of P-fertility, VAM inoculation increased leaf tissue N, P, S, and B concentration (Table 4). At high P-levels, VAM plants also had greater tissue Ca and Mg than Non-VAM plants. Tissue macronutrient concentrations of VAM plants at both P levels were comparable except for P and Ca which were higher at the high P level. VAM inoculation had no effect on tissue Fe, Mn, Zn, Cu, Mo, and Al concentration (data not shown). Increasing P fertility had no effect on leaf tissue macro- and micronutrient concentration of Non-VAM plants, except for a decrease in K concentration. The interaction of VAM and P factors on tissue P concentration suggested that tissue P concentration of Non-VAM plants remained low at both P levels, while enhanced tissue P concentration occurred in VAM plants with increasing P fertility.

| TABLE 4 |

| Effects of VAM and different phosphorus levels on leaf tissue elemental concentration of neem seedlings. |
|-----------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| Mycorrhiza | P (nmM) | N (%) | P (%) | K (%) | Ca (%) | Mg (%) | S (%) | B (mg g⁻¹) |
| No | 0.65 | 2.72±0.12 | 0.08±0.00 | 1.63±0.15 | 3.09±0.23 | 0.23±0.02 | 0.35±0.03 | 33.7±1.33 |
| 1.30 | 3.17±0.03 | 0.07±0.01 | 1.30±0.06 | 2.56±0.13 | 0.22±0.01 | 0.32±0.01 | 32.73±0.62 |
| Yes | 0.65 | 2.77±0.03 | 0.12±0.00 | 1.58±0.05 | 3.15±0.11 | 0.26±0.02 | 0.45±0.03 | 37.7±1.71 |
| 1.30 | 3.53±0.14 | 0.14±0.00 | 1.51±0.05 | 4.21±0.70 | 0.28±0.03 | 0.46±0.04 | 36.93±2.14 |

Significance

| VAM | ** | ** | NS | * | * | ** | * |
|** | NS | NS | * | NS | NS | NS | NS |

NS = Nonsignificant, ** and * = Significant at P ≤ 0.01 and 0.05, respectively. Data represents a mean of 3 composite samples per treatment. Each composite sample is a pool of leaves from 3 plants.
DISCUSSION

This is one of the first reports of mycorrhizal enhancement of neem tree seedling growth and development. Extensive VAM colonization of neem roots occurred at both P levels in this research, which contrasts to previous reports of decreasing VAM colonization with increasing soil fertility or increasing tissue P concentration (Menge et al., 1978; Abbott et al., 1984; Amijee et al., 1989). The same trend with neem has been reported in Volkamer lemon which is highly dependent on VAM colonization when grown in low P soil, and maintains relatively high rates of VAM colonization even at high P supply (Graham et al., 1991).

The results in this experiment demonstrate the growth enhancement benefits of the neem- *Glomus intraradices* symbiosis, particularly at low phosphorus fertility. Greater leaf area and shoot and root dry weight of VAM inoculated plants correlated well with an increase in nutrient uptake, particularly P. This evidence suggests that enhanced growth by VAM inoculation under low P fertility conditions was primarily due to improved P nutrition. Zech (1984) reported leaf P concentration of 0.13% for a healthy neem trees grown under natural conditions; the presence of VAM was not reported. The VAM neem seedlings at both P levels in our experiment were most likely P-sufficient (0.12-0.14 P), while non-VAM neems were P-deficient (0.07-0.08% P). At the high P level, VAM plants had a greater leaf area and root dry weight, and higher P uptake than Non-VAM plants. The symbiosis facilitated the most dramatic increase in tissue elemental levels of P which increased 50% and 100% in VAM plants, respectively, at the low and high P fertility regimes. Low P, VAM plants had a greater leaf area and root dry weight and higher tissue P than high P, Non-VAM plants. P accumulation could provide a storage pool for the later stages of plant growth (Smith and Gianinazzi, 1988). Although LAR and SLA of neems were not affected by either VAM inoculation or P nutrition in this experiment, VAM inoculation significantly increased leaf area, a component of these two measurements. Since leaf number was not different among treatments, increased leaf area of VAM inoculated plants was due to greater leaf expansion capacity which is commonly related to an enhanced shoot P status (Koide and Li, 1989).

VAM alteration of root morphology of host plants has been reported in both monocots and dicots (Berta et al., 1993). Root alteration by VAM association may contribute to enhanced P nutrition, photosynthetic partitioning to the fungal partner, or hormonal factors (Berta et al., 1993). Root alteration by VAM and phosphorus were also observed in neem seedlings. In general, root dry weight was influenced by VAM, while P fertility influenced the increased length of non-scrubbed roots, which contributed to more than 75% of the total root length (Table 3). The effect of P on increased root length and root branching has been reported (Drew and Saker, 1978). Greater dry mass was allocated to scrubbed roots of VAM plants resulting in larger and longer scrubbed roots than Non-VAM plants, regardless of P level. Scrubbed roots contributed to more than 75% of total root dry weight of neems (Table 3). Greater numbers of second and third order lateral roots could be visually observed in VAM plants. This characteristic may be beneficial for survival after transplanting or after the aerial parts of the trees are destroyed and new aerial parts must form (Nambari, 1980; Kormanik, 1985).

At the low P level, larger Non-VAM plants, yet VAM results indicate that VAM plants may have increased nutrient uptake. VAM is frequently attributed to the abilities of the mycorrhizal fungus to solubilize and transport nutrients in the soil. Those studies are conducted under ideal growing conditions, such as well watered, high P soil and relatively high temperature. However, VAM inoculation may not be beneficial for most of the above ground material in other conditions. Increased intake of N, S and B in neem seedlings, the dry matter was in a greater proportion to shoot ratio in VAM plants than in Non-VAM plants at high P concentration.

Besides an increase in the nutrient uptake of N, S and B in neem seedlings, Ca and Mg than Non-VAM plants' uptake by VAM inoculation (Abbott et al., 1984; Davie et al., 1990). However, VAM inoculation may be beneficial for survival after transplanting or after the aerial parts of the trees are destroyed and new aerial parts must form (Nambari, 1980; Kormanik, 1985).

The large scale production of neem for global problems as deforestation, insecticidal, medicinal, and more is increasing. Neem plantations and more are being used on growth enhancement and for the production of trees which are frequently threatened to drought conditions. With VAM inoculated neems, the benefits of VAM inoculation may be even more pronounced.
The enhancement of neem tree seedlingization of neem roots occurred at previous reports of decreasing or increasing tissue P concentration [1989]. The same trend in which is highly dependent on d but maintains relatively high rates [IHM et al., 1991].

The growth enhancement benefits particularly at low phosphorus fertility of VAM inoculated plants under low P fertility conditions. Zech (1984) reported leaf P grown under natural conditions: VAM neem seedlings at high P level, VAM plants higher P uptake than Non-VAM plants. P accumulation in tissue elemental VAM plants, respectively, at the plants had a greater leaf area and a greater leaf area of VAM inoculated shoots which is commonly raised leaf area of VAM inoculation. Plants have been reported in both alteration by VAM association with partitioning to the fungal root. Root alteration by VAM and shift. In general, root dry weight decreased the increased length of non-subsidiary roots than Non-VAM plants. This characteristic may be beneficial for survival and establishment (i.e. better root regeneration after transplanting or after stress recovery), while finer-nonsubersized roots tend to be destroyed and new roots formed from the existing suberized roots [Nambar, 1980; Kormanik, 1986].

At the low P level, length of nonsuberized roots was equal between VAM and Non-VAM plants, yet VAM plants had greater growth and nutrient uptake. These results indicate that VAM inoculation can increase nutrient uptake efficiency. Increased nutrient uptake capacity and subsequent growth of host plants by VAM is frequently attributed to extraradical hyphae of the fungi. There is evidence that extraradical hyphae can enhance nutrient and water uptake of VAM plants that have smaller or equal sized root systems than Non-VAM plants [Abbott et al., 1984; Davies et al., 1992]. Root to shoot ratio of host plants often decreased following VAM inoculation [Abbott and Robson, 1984; Bell et al., 1989]. However, VAM inoculation tended to increase the root to shoot ratio of neems. Although VAM inoculation increased both shoot and root growth of neem seedlings, the dry mass partitioning to roots, particularly suberized roots, was in a greater proportion than shoot dry mass, resulting in an increase in root to shoot ratio in VAM plants. An increased root to shoot ratio was also observed in Non-VAM plants at high P fertility levels.

Besides an increase in tissue P levels, VAM inoculation also increased uptake of N, S and B in neem seedlings. At high P, VAM plants also had a greater tissue Ca and Mg than Non-VAM seedlings. Enhanced P, K, Ca, S, Zn, Cu, Mn and Fe uptake by VAM inoculation was reported with other plant species (i.e. Davies 1987; Kucey and Janzen, 1987; Bell et al., 1989). Various mechanisms of increasing nutrient uptake, particularly P, by VAM include exploration of larger soil volume by extraradical hyphae, faster movement of soil P into VAM hyphae, and solubilization of soil P by the release of organic acids and phosphatase enzymes (Bolan, 1991).

The large scale production of neem trees may partially help alleviate such global problems as deforestation, desertification, soil erosion, and even global warming (National Research Council, 1992). The utilization of neem products for insecticidal, medicinal, and other purposes is increasing and may require larger neem plantations and more efficient cultural techniques. The mycorrhizal benefits on growth enhancement and nutrient uptake have important implications for neem trees which are frequently transplanted into agriculturally poor soils and subject to drought conditions. We plan to study the establishment and growth of transplanted, VAM inoculated neem tree seedlings under field conditions.
REFERENCES


Hewitt E.J., 1966. Sand and...


