土壌溶液中のリン濃度,窒素施用,およびアーバスキュラー菌根菌の感染がクロタラリア(Crotalaria Juncea L.)の生育に及ぼす影響
Reactions of sun hemp (Crotalaria juncea L.) to phosphorus concentration in soil solution, nitrogen fertilization, and arbuscular mycorrhizal colonization

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Abstract  Greenhouse experiments were undertaken to determine the degree to which sun hemp (Crotalaria juncea L.) was dependent on arbuscular mycorrhizal fungi (AMF) for nutrient uptake and growth and to assess the extent to which this dependency might be modified by nitrogen fertilization. Seeds of sun hemp were grown in soil inoculated or not inoculated with AMF at 0.02, 0.04, 0.08, and 0.2 mg P L⁻¹ in soil solution. Mycorrhizal inoculation stimulated dry matter yield at all but the highest P concentrations in soil solution. Mycorrhizal plants were higher in leaf P content than nonmycorrhizal plants at all P concentrations in soil solution except the initial and the highest ones. Calculation of mycorrhizal dependency at the different P concentrations in soil solution revealed that sun hemp is highly dependent on the mycorrhizal condition for phosphorus uptake and growth. In a follow up study in which we tried to assess the effect of N fertilization rates on mycorrhizal dependency of sun hemp, we were unable to evaluate it fully because of the high level AMF colonization in soil which was not inoculated with AMF. However, the tendency of N fertilization to increase P demand resulting from N fertilization would likely to need more mycorrhizal activity. We, therefore, conclude that N fertilization is likely to alter the mycorrhizal dependency of sun hemp.

Key words: mycorrhizal dependency, nitrogen fertilization, nodulation, soil solution P, sun hemp

Introduction

Most plant species whether in natural ecosystems or in non-intensively managed agroecosystems function under the influence of a group of soil fungi known as arbuscular mycorrhizal (AM) fungi. AM fungi play numerous roles that impact plant health and productivity; e.g., enhancement of the uptake of diffusion-limited nutrients such as P is most prominent (Smith and Read, 1997). Plant species and even varieties within a species are known to differ in the degree to which they react to mycorrhizal colonization. By and large, these differences among plant species reflect the differences in the efficiency with which the species take up nutrients from the soil solution (Miyasaka and Habte, 2001). The term 'mycorrhizal dependency' is often used to...
describe this relationship even when plants are grown under conditions in which P concentrations in soil solution are not determined. Manjunath and Habte (1991) highlighted the importance of basing mycorrhizal research on P concentration in soil solution and demonstrated that they could delineate the mycorrhizal dependency of plant species differing in their root morphological properties by growing at different P concentrations in soil solution in the presence or absence of an AM fungus known to effectively colonize a wide array of plant species. Since then the AM dependency of a few plant species has been determined using the approach (Miyasaka and Habte, 2001). However, the AM fungal dependency of a vast number of commercially and ecologically important plant species remains yet to be determined. It is also known that the responses to nitrogen application are different among plant species (Blanke et al., 2011).

Sun hemp (Crotalaria juncea L.) is generally considered to be indigenous to India where it is extensively used for soil improvement, fiber, and forage. It is widely used as a nematode resistant green manure crop in the tropics (Hoshiai, 1986; Yuasa and Maekawa, 1987; Germani and Plenchette, 2004). Although interest is developing to understand the impacts that sun hemp might have on indigenous AM fungi when it is used as a green manure crop, studies on the effects of the fungi on the growth and nutrient uptake of sun hemp itself are hard to come by. In a recent study, Germani and Plenchette (2004) evaluated the mycorrhizal responsiveness of several sun hemp species and noted that most of them had very high mycorrhizal dependency. However this information is of a limited value since the researchers carried out their test using a single level of extractable P instead of P concentration in soil solution. Even when one uses P concentration in soil solution, at least two levels of P concentrations in soil solution are required before one can separate different host species into distinct mycorrhizal dependency categories (Habte and Manjunath, 1991; Miyasaka and Habte, 2001). The aims of this study were 1) to clearly define the AM fungal dependency of sun hemp and 2) to assess the degree to which N fertilization might influence the AM dependency of the legume.

**Materials and Methods**

**Experiment 1: Determination of dependency**

The soil used for evaluating the mycorrhizal dependency was a subsurface sample (15-25 cm) from the Leilehua series (Typic Kandihumult, clayey, oxidic, isothermic). The soil was sieved (< 4 mm), limed to pH 6.0. Portions (2.25 kg) of the limed soil were transferred into 3L plastic pots. The soil was amended with P to obtain concentrations in the soil solution of 0.02, 0.2, 0.4 and 0.8 mg L⁻¹ based on the P adsorption isotherm of the soil. It was fumigated to eradicate the indigenous AM fungi, as described by Habte and Manjunath (1987). After standing it for two more weeks in open air to dissipate toxic residues from the fumigant, a crude inoculum of *Glomus aggregatum* was mixed with the soil at the rate of 30 g per kg of soil. The mycorrhizal inoculum was produced using Habte's modification (Habte and Osorio, 2001) of the pot culture technique described previously (Habte and Manjunath, 1987). In the modified approach, a mixture of 90% crushed basal (Mansand ™) and 10% calcined montmorillonite (Turface ™; Applied Industries Corporation, Deerfield, IL) were used instead of Mansand alone. Phosphorus-free Hoagland's solution (Habte and Osorio, 2001) was used instead of nutrient solution used by Habte and Manjunath (1987) and *Leucaena leucocephala* var. K636 was used as the nurse plant instead of corn. The control medium received 30 g kg⁻¹ of sterilized Mansand + Turface and a filtrate of the crude inoculum obtained by suspending 100 g of the crude inoculum in a liter of de-ionized water and passing this suspension through Whatman No.1 filter paper. The soil was then moistened to 50% of maximum water holding capacity.

Two days later, 2.5 by 2.5 cm depressions were made on the surface of the potted soil in order to accommodate the germinated seeds. Seeds of sun hemp were placed in the depressions and covered with soil. Treatments were arranged on greenhouse benches in a randomized complete block design with three replicates per treatment. Watering was achieved by sprinkler irrigation. A phosphorus-free blanket nutrient (Aziz and Habte, 1989) was added at the rate of 200 ml per pot 10 days after emergence. Plants were grown for 49 days under natural light (21° 51' N and 156° 22' W) during early March to the end of April, 2002. The development of AM effectiveness was determined at 28 days after planting by determining the P content of leaf disks as described by Aziz and Habte (1987).

At harvest, the extent to which sun hemp roots were colonized by the test AM fungus and dry weight of shoot were determined. The proportion of sun hemp roots colonized by the AM fungus was estimated by the grid-line intersect method (Giovannetti and Mosse, 1980) after clearing roots with 10% KOH and staining them with acid fuchsin in a lactic acid-glycerol solution as described by Kormanik et al. (1982) except using a 0.15% acid fuchsin solution. We also carried out the staining process at 22°C and the clearing, staining and de-staining steps for 24 h duration. Dry weight of shoot was determined after drying samples at 70°C for 96
Experiment 2: Effect of nitrogen on AM dependency

In this experiment, a non-fumigated subsoil (45-70 cm) of the Waialua series (Vertic Haplustolls, very fine, kaolinitic, isohyperthermic) with a pH of 6.6 was used. The soil was crushed to pass through a 2-mm aperture sieve, air-dried, and portions (2.23kg) of the soil were put into 3-L plastic pots, and the P concentration of the soil solution was adjusted to 0.02 and 0.2 mg L⁻¹ as in Experiment 1. Nitrogen was added as NH₄NO₃ at the rate of 0, 50, 100, 200, 400 mg kg⁻¹ soil and a nitrogen and phosphorus-free Hoagland's solution was also applied as in Experiment 1.

Before planting, soil in half of the pots was inoculated with 50g portions of the same inoculum of AM fungus by mixing the contents of each pot, as in Experiment 1. The remaining soil was also mixed with same amount of a sterilized Mansand. *Bradyrhizobium* sp. TAL309 was inoculated to the plants grown in soil without N fertilization. For this purpose, the *Bradyrhizobium* was grown on yeast mannitol agar slants at 30°C for one week. The growth was scraped and suspended in sterile water. The volume of the suspension was increased by diluting it with 100 ml, then 8 ml portions of the diluted suspension was added to depressions made to accommodate the sun hemp seeds. Surface-sterilized seeds of sun hemp were sown in the pots. The pots were arranged on greenhouse benches in a randomized complete block design with three replicates per treatment. Plants were grown under natural light from early October to the end of November, 2001. They were watered as needed to maintain the moisture contents of the soil up to approximately 90% of maximum water holding capacity.

Fifty one days after planting, shoots were harvested at the soil line. Roots were excised and washed, and nodules were removed from them. After removing 0.5 g portion of root samples for AM fungal colonization, the rest of the roots, shoots, and nodules were dried at 70°C for 3 days for dry weight determination. Shoots were then ground for analysis of N and P contents in tissues. N contents in tissues were measured by means of Nitrogen Auto Analyzer (Leco CN-2000, LECO Corporation, St. Joseph, MI, USA). Tissue P concentration was determined after 10 mg of the ground tissue was ashed in a Pyrex test tube at 500°C for 3 h. The proportion of root length colonized by AM fungi was determined as in Experiment 1.

**Results**

Experiment 1: Determination of dependency

Plants grown in soil which was not inoculated with *Glomus aggregatum* showed no evidence of AM fungal colonization on their roots after 49 days of growth. On the other hand, those grown in the inoculated soil showed AM fungal colonization levels, ranging from 14 to 43% at various P concentrations in soil solution (Table 1). The lowest AM fungal colonization was noted at the highest P concentration in soil solution while the highest one was noted at 0.04 mg P L⁻¹ in soil solution. Dry weight of shoot in mycorrhizal plants was significantly higher than that of non-mycorrhizal ones at all P concentration in soil solution except the highest one. Leaf P status of sun hemp showed that mycorrhizal inoculation had enhanced P content at all P concentrations in soil solution except the initial and the highest ones. Mycorrhizal dependency values were 36.1, 81.8, 76.4, and 37.3% for plants grown at the first four P concentrations in soil solution.

Experiment 2: Effect of nitrogen on AM dependency

Nitrogen application significantly decreased nodulation at added N concentration of 400 mg kg⁻¹ (Fig.1), and increased total nitrogen content (Fig.2), total phosphorus content (Fig.3), and total dry weight (Fig.4) of sun hemp, irrespective of AM fungal inoculation and P concentration in soil solution. Phosphorus amendment increased total tissue nitrogen, phosphorus content, and dry weight of sun hemp (Figs.2-4). AM colonization also tended to increase with increase in the rate of nitrogen application, especially at 0.02mg P L⁻¹ in soil solution, particularly if soil was inocul-

### Table 1

<table>
<thead>
<tr>
<th>Soil solution P (mg L⁻¹)</th>
<th>Colonization (%)</th>
<th>Shoot dry weight (g pot⁻¹)</th>
<th>Leaf P content (µg disk⁻¹)</th>
<th>Mycorrhizal dependency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>none</td>
<td>29.7 bc</td>
<td>0.13 a</td>
<td>0.663 a</td>
<td>36.1</td>
</tr>
<tr>
<td>0.02</td>
<td>37.3 cd</td>
<td>0.46 a</td>
<td>0.747 a</td>
<td>81.8</td>
</tr>
<tr>
<td>0.04</td>
<td>43.0 a</td>
<td>0.47 a</td>
<td>0.747 a</td>
<td>81.8</td>
</tr>
<tr>
<td>0.08</td>
<td>21.7 ab</td>
<td>1.54 b</td>
<td>2.173 b</td>
<td>37.3</td>
</tr>
<tr>
<td>0.20</td>
<td>14.0 a</td>
<td>2.75 c</td>
<td>2.560 c</td>
<td>36.1</td>
</tr>
</tbody>
</table>

*Means followed by the same small letters within a column are not significantly different using least significant difference test (P<0.05). -AMF: without arbuscular mycorrhizal fungi. +AMF: with arbuscular mycorrhizal fungi.
ed with AM fungus (Fig.5). Although the experimental soil was collected after removing the top 45 cm layer, roots of sun hemp grown in the soil were nodulated and reasonably well colonized by AM fungi, even if the soil was not inoculated by Bradyrhizobium or AM fungus (Figs.1,5). The highest level of AM fungal colonization values was obtained in the tests using AM fungus and its P concentration in soil solution was adjusted to 0.02 mg L\(^{-1}\) (Fig. 5).

**Discussion**

Sun hemp did not depend on the AM fungus for P uptake and growth at the highest P concentration in soil solution. Sun hemp was most dependent on the mycorrhizal condition for P uptake and growth at 0.02 mg P L\(^{-1}\) in soil solution.

The mycorrhizal dependency values of sun hemp calculated according to the formula of Plenchette et al. (1983) at 0.02 mg P L\(^{-1}\) in soil solution was 81.8% in this study. The value is somewhat higher than the value required for placing a plant species into the category of highly mycorrhizal dependent species (Habte and Manjuanth, 1991). However, since AM fungus did not significantly stimulate growth of sun hemp at 0.2 mg P L\(^{-1}\) in soil solution, it cannot be classified as very highly mycorrhizal dependent species. Therefore, we

![Fig. 1. Effect of nitrogen application on nodulation of sun hemp. Bar at the top right of graph indicates the least significant difference value for comparing treatment means. P concentrations in soil solution were 0.02 or 0.2 ppm. AMF: arbuscular mycorrhizal fungi.](image1)

![Fig. 2. Effect of nitrogen application on total nitrogen content of sun hemp. Bar at the bottom left of graph indicates the least significant difference value for comparing treatment means. P concentrations in soil solution were 0.02 or 0.2 ppm. AMF: arbuscular mycorrhizal fungi.](image2)

![Fig. 3. Effect of nitrogen application on total phosphorus content of sun hemp. Bar at the bottom left of graph indicates the least significant difference for comparison between treatments. P concentrations in soil solution were 0.02 or 0.2 ppm. AMF: arbuscular mycorrhizal fungi.](image3)

![Fig. 4. Effect of nitrogen application on dry weight of sun hemp. Bar at the bottom left of graph indicates the least significant difference value for comparing treatment means. P concentrations in soil solution were 0.02 or 0.2 ppm. AMF: arbuscular mycorrhizal fungi.](image4)

![Fig. 5. Effect of nitrogen application on AMF colonization of sun hemp. Bar at the bottom left of graph indicates the least significant difference value for comparing treatment means. P concentrations in soil solution were 0.02 or 0.2 ppm. AMF: arbuscular mycorrhizal fungi.](image5)
can confidently place the legume among plant species that are highly dependent on the mycorrhizal condition.

According to Colozzi and Sequeira (1986), the minimum colonization level required for positive host response to AM fungal colonization is 30%. Our findings negate this earlier finding since growth of sun hemp was significantly stimulated by AM fungal colonization level of 21.7% in Experiment 1.

High level of phosphorus generally suppresses the formation of mycorrhiza (Habte and Manjunath, 1987), and our findings are in agreement with this general observation since we noted that AM fungal colonization was significantly reduced by P concentration in soil solution above 0.04 mg L⁻¹. Since soil P exerts its influence on AM colonization through its effect on tissue P content (Tawaraya et al. 1994), the concentration of tissue P associated with these soil P concentrations were sufficient to inhibit AM colonization in roots of sun hemp. The decline in AM dependency with increase in P concentrations in soil solution suggest that more and more of the P was accessible to the unaided plant as P concentrations in soil solution was increased and hence mycorrhizal inoculation effect diminished with increase P concentration in soil solution. The soil P concentration at which sun hemp was most dependent on mycorrhizal colonization for growth was 0.02 mg L⁻¹. This is a characteristic of species that are highly or very highly dependent on AM fungi for growth and nutrient uptake (Habte and Manjunath, 1991).

Our data suggests that AM fungi can play significant roles in the productivity of sun hemp, and provides useful information on its AM dependency category, necessary for managing AM fungi in order to enhance the productivity of the legume with predictable efficacy.

It is possible that a variety of soil and management factors could influence the dependency of a host plant to AM fungal colonization. Our effort to determine the extent to which nitrogen fertilization modifies the AM dependency of sun hemp was only partially successful because the subsurface soil we thought to be free of AM fungi had significant numbers of indigenous AM fungi. This is contrary to the earlier studies of Habte (1989) which illustrated that mycorrhizal activity in an Oxisol subjected to surface soil removal of above 38 cm was nil. Nevertheless, the tendency of N fertilization to stimulate AM fungal colonization and tissue P content suggests that any increase in P demand resulting from N fertilization would likely need additional mycorrhizal activity. We, therefore, conclude that N fertilization is likely to alter the mycorrhizal dependency of sun hemp.

**References**


