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# Relationship Between Cowpea Root Systems and Mycorrhizal Dependency

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***Abstract.*** **A hydroponic experiment was conducted to determine the relationship between mycorrhizal dependency (MD) of cowpea [*Vigna unguiculata* (L.) Walp.] cultivars and their root morphology. Seeds of 19 cowpea cultivars with known MD levels were inoculated with *Glomus fasciculatum* and *Bradyrhizobium* in seedling trays. Twelve-day-old seedlings were transferred to a hydroponic culture system, where they were grown for 5 weeks. Leaf area, length of taproot, total root length, root weight, root abundance, average length of fine roots, number of nodules formed on lateral roots, and total nodule weight differed among cultivars. Less than 5% of the root length was colonized by mycorrhizal fungus in all cultivars. Average length of fine roots was negatively correlated with MD of cowpea cultivars; however, only 27% of the variability in MD was explained by this variable. Therefore, root morphology did not appear to determine MD in cowpea.**

The effectiveness of vesicular-arbuscular mycorrhizal (VAM) fungi in enhancing plant growth is dependent on interactions among fungal, soil, and plant factors. Plant species, as well as cultivars within species, differ in their response to inoculation with VAM fungi (1, 9, 12). Mycorrhizal dependency (MD) is used by mycorrhizasts as an index to compare host response to VAM fungi (4). Different MD levels have been identified among cultivars of citrus (8), wheat (*Triticum L.*) (1), and cowpea [*Vigna unguiculata* (L.) Walp.] (13).

Plant species are thought to differ in MD based on their inherent capacity to absorb P from low P soils (2). Baylis (3) reported that length of root hairs and the thickness of roots can determine MD level of a plant species. The short roots and poorly distributed root hairs of citrus contribute to its increased dependency on mycorrhizae (6, 8). However, the reasons for differences in MD within a species are not clearly understood. The theory proposed by Baylis (3) on length and thickness of root hairs at the species level may explain cultivar variability as well. According to Menge et al. (8), the number of feeder roots in citrus cultivars could be correlated with MD; however, their experiment did not show the basis for true cultivar variability within species. A separate study conducted with five citrus rootstocks showed that leaf P concentration, fineness of roots, growth rate, hydraulic conductivity, transpiration, and $CO_2$ assimilation rate were linked to MD (6). The latter study also illustrated interspecific, not intraspecific, variability. The basis for cultivar differences in response to inoculation with VA mycorrhizae has not been determined. The purpose of our investigation was to determine if intraspecific variability for MD in cowpea could be explained by differences in root morphology.

Nineteen cowpea cultivars (Table 1) with known MD levels (13), when inoculated with both *Glomus fasciculatum* and *Bradyrhizobium,* were used in this experiment. seeds were surface sterilized 20 min in 5% sodium hypochlorite solution, washed with distilled water several times, and grown in seedling trays divided into 4 cm × 4 cm compartments containing steamed (90 min, 80°C) sand. Before seeding, the sand medium was mixed with a peat-based rhizobial inoculum (strain 32-H-1) and inoculum of *G. fasciculatum* (100 g per 500 g sand). Inoculum of *G. fasciculatum* consisted of spores (30–50 g), infected roots, and soil. All seedlings were inoculated with both *Bradyrhizobium* and *G. fasciculatum,* as rhizobial inoculation alters MD (13). When the seedlings were 12 days old, they were removed from the compartmentalized seedlings trays, and the roots were washed with double distilled water. The seedlings were placed immediately in a hydroponic culture system. Hydroponics were used to facilitate recovery of the entire undisturbed root system, so that root morphological characteristics could be accurately evaluated.

Each hydroponic unit consisted of a 0.9 liter (1 quart) glass jar, coated on the outside with black paint to reduce light entry, thus impeding algal growth. Jars also were coated with silver paint to reduce heat buildup and were disinfected with a 5% hypochlorite solution. Nutrient solutions were aerated with plastic tubing connected to an air pump via a manifold. Sponge lids with a slit in the center were cut to fit the mouth of each jar and were used to support plants.

A half-strength, N-free modified Hoagland and Arnon (7) solution was used as the growing medium. Phosphorus concentration in this solution was reduced to 5 ppm, and the pH was adjusted to 6.5 using potassium hydroxide. The nutrient solution in all jars was aerated constantly and changed weekly. Plants were illuminated with fluorescent lights for 12 hr each day. Light intensity varied from 70–90 $\mu mol \cdot s^{-1} \cdot m^{-2}$. Relative humidity averaged 64%. Temperatures ranged from 25° to 27°C. Plants were arranged in a randomized block design with two blocks and grown for 5 weeks. One plant from each cultivar was grown in each block.

At harvest, root morphological characteristics, VAM root colonization, nodule characteristics and total leaf area (Decagon area meter) were measured. Root morphological characteristics measured included taproot length, taproot thickness at the crown, total root length measured by the gridline-intersect method (5), root abundance (number of roots on a plane 5 cm below the crown),

Table 1. Description of cowpea cultivars.

| Cultivar | Origin | Growth habit | Maturity |
|---|---|---|---|
| Mississippi Silver | United States | Indeterminate | Medium |
| Pinkeye Purple Hull | United States | Indeterminate | Medium |
| Calico Crowder | United States | Indeterminate | Medium |
| Cream 12 | United States | Indeterminate | Medium |
| Mississippi Purple | United States | Indeterminate | Medium |
| Chinese Red | United States | Indeterminate | Medium |
| Vining Purple Hull | United States | Indeterminate | Medium |
| Knuckle Purple Hull | United States | Indeterminate | Medium |
| California Blackeye No. 5 | United States | Indeterminate | Medium |
| Lady | United States | Indeterminate | Late |
| Monarch | United States | Indeterminate | Late |
| Blue Goose | United States | Indeterminate | Late |
| Brown Crowder | United States | Indeterminate | Late |
| Bush Purple Hull | United States | Determinate | Early |
| Six Week Browneye | United States | Determinate | Early |
| SEL 74 | Sri Lanka | Determinate | Early |
| SEL 266-1 | Sri Lanka | Determinate | Early |
| MI 35 | Sri Lanka | Determinate | Early |
| TVu 1 (TX 2057) | United States | Determinate | Early |

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Table 2. Cultivar effect on plant variables measured.

| Variable | Probability > F[z] |
|---|---|
| Leaf area | 0.0039 |
| Length of tap root | 0.0365 |
| Thickness of tap root at the crown | 0.1883 |
| Total length of roots | 0.0450 |
| Root weight | 0.0118 |
| Root abundance[y] | 0.0468 |
| Average length of fine roots[x] | 0.0430 |
| Number of fine roots per 5 cm length of root | 0.8914 |
| Number of nodules on tap root and crown | 0.5809 |
| Number of nodules on lateral roots | 0.0500 |
| Nodule weight | 0.0333 |

[z]Results obtained from analysis of variance.
[y]Number of roots across a plane, 5 cm below the crown.
[x]Roots <0.2 mm diameter.

Table 3. Correlation between mycorrhizal dependency and those variables significantly affected by cultivar.

| Variable | Correlation coefficient | Probability > F |
|---|---|---|
| Leaf area | −0.08 | 0.63 |
| Length of tap root | 0.02 | 0.18 |
| Total length of roots | 0.07 | 0.65 |
| Root weight | 0.07 | 0.68 |
| Average length of fine roots[z] | −0.27 | 0.05 |
| Root abundance[y] | 0.02 | 0.89 |
| Number of nodules on lateral roots | −0.20 | 0.25 |
| Nodule weight | −0.01 | 0.95 |

[z]Roots <0.2 mm diameter.
[y]Number of roots across a plane, 5 cm below the crown.

Table 4. Mycorrhizal dependency and average length of fine roots of 19 cowpea cultivars.

| Cultivar | Mycorrhizal dependency[z] | Average length of fine roots (mm) |
|---|---|---|
| Six Week Browneye | 431 a[y] | 0.7 b |
| MI 35 | 282 b | 1.8 ab |
| Knuckle Purple Hull | 240 cb | 1.0 b |
| TVu-1 (Tx 2057) | 220 cd | 1.6 ab |
| Blue Goose | 196 cde | 1.2 b |
| Brown Crowder | 177 efd | 2.2 ab |
| Calico Crowder | 168 defg | 2.6 ab |
| SEL 266-1 | 152 efgh | --- |
| Vining Purple Hull | 140 fgh | 1.7 ab |
| Bush Purple Hull | 132 fgh | 3.6 a |
| Mississippi Purple | 121 ghi | 2.2 ab |
| SEL 74 | 109 hi | 1.6 ab |
| Lady | 105 hi | 1.8 ab |
| California Blackeye No. 5 | 104 hi | 2.3 ab |
| Pinkeye Purple Hull | 102 hi | 1.4 b |
| Mississippi Silver | 100 hi | 0.9 b |
| Monarch | 99 hi | 1.2 b |
| Chinese Red | 93 hi | 1.1 b |
| Cream 12 | 55 J | 2.5 ab |

[z]High values indicate high mycorrhizal dependency and vice versa.
[y]Means separated by least significant difference, $P = 5\%$.

average length of fine roots (roots <0.2 mm in diameter), and number of fine roots per 5 cm length of root. Nodule characteristics measured were nodule distribution on the tap root and lateral roots, and dry weight of nodules (70°C, 24 hr). Root colonization by *Glomus fasciculatum* was observed by cutting roots into 1-cm segments. Random samples were stained (11) and examined under a light microscope.

The significance of the cultivar effect on different variables is presented in Table 2. Leaf area, length of the tap root, total root length, root weight, root abundance, average length of fine roots, number of nodules formed on lateral roots, and total nodule weight varied among cultivars. Cowpea cultivars did not show differences in thickness of tap roots at the crown, number of fine roots per 5 cm of thick root and number of nodules on the tap root and crown.

Correlation coefficients between MD and those variables which were affected significantly by cultivar indicated that only average length of fine roots was significantly and negatively correlated with MD (Table 3). 'Six Week Browneye', which showed the greatest MD among the cowpea cultivars tested, had the shortest fine roots, whereas the cultivar with lowest MD ('Cream 12') had relatively long fine roots (Table 4). Long fine roots (or root hairs) facilitate nutrient absorption and may result in reduced MD as in 'Cream 12'. Short fine roots could limit nutrient absorption and increase dependency on mycorrhizae. Therefore, the theory proposed by Baylis (3) for interspecific variability was also found applicable to intraspecific variability for MD in cowpeas.

Only 27% of the variability in MD was explained by length of fine roots (Table 3). Other factors which were not measured in this experiment, such as sugar content of the roots, alkaline phosphatase activity of the roots, composition of root exudates, etc. also may regulate the MD of a cultivar. In a study conducted by Ollivier et al. (10), using two cowpea cultivars and two VAM fungi, growth stimulation due to VAM fungi was accompanied by the appearance of soluble alkaline phosphatases in extracts of mycorrhizal roots. The number and electrophoretic mobility of these enzymes varied depending on the VAM fungus involved. It was not stated whether or not these enzyme characteristics differed among host cultivars.

Average length of fine roots was negatively correlated with MD of cowpea cultivars. This variable is thought to be linked to MD through absorption of P and could be used as an indicator to select cultivars that benefit most from inoculation with mycorrhizal fungi. The hydroponic method was chosen to facilitate recovery of the entire undamaged root system. However, problems such as lack of root colonization by the VAM fungus and reduced growth were encountered. After 5 weeks of growth in hydroponics, <5% of the root length was colonized by VAM fungi in all cultivars. Nevertheless, all cultivars were profusely nodulated. Therefore, this method appears to be unsuitable for studies involved with VAM root colonization and production of VAM inoculum. Also, the root morphology of hydroponically-grown plants should be viewed with caution, since it might differ from the root morphology of soil-grown plants. In conclusion, since average length of fine roots was the only root morphological variable correlated with MD and this variable only accounted for 27% of the intraspecific variability for MD, root morphology alone does not appear to determine MD in cowpea.

### Literature Cited


1. Azcon, R. and J.A. Ocampo. 1980. Factors affecting the VA infection and mycorrhizal dependency of thirteen wheat cultivars. New Phytol. 87:667–685.
2. Baylis, G.T.S. 1970. Root hairs and phycomycetous mycorrhizas in phosphorus-deficient soil. Plant Soil 3:713–716.
3. Baylis, G.T.S. 1975. The magnoloid mycorrhiza and mycotrophy in root systems derived from it, p. 373–389. In: F.E. Sanders, B. Mosse, and P.B. Tinker (eds.). Endomycorrhizas. Academic, New York.
4. Gerdemann, J.W. 1975. Vesicular-arbuscular mycorrhizae, p. 575–591. In: J.G. Torrey and D.T. Clarkson (eds.). The development and function of roots. Academic, London.

5. Giovannetti, M. and B. Mosse. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84:489–500.
6. Graham, J.H. and J.P. Syvertsen. 1985. Host determinants of mycorrhizal dependency of citrus root stock seedlings. New Phytol. 101:667–676.
7. Hoagland, O.R. and D.I. Arnon. 1938. The water culture method for growing plants without soil. Calif. Agr. Expt. Sta. Circ. 347.
8. Menge, J.A., E.L.V. Johnson, and R.G. Platt. 1978. Mycorrhizal dependency of several citrus cultivars under three nutrient regimes. New Phytol. 81:553–559.
9. Mosse, B. 1981. Vesicular-arbuscular mycorrhiza research for tropical agriculture. Hawaii Inst. Trop. Agr. and Human Res., Univ. of Hawaii, Honolulu, Res. Bul. 194.
10. Ollivier, B., Y. Bertheau, H.G. Diem, and V. Gianinazzi-Pearson. 1983. Influence de la variete de *Vigna unguiculata* dans l'expression de trois associations endomycorrhiziennes a vesicules et arbuscules. Can. J. Bot. 61:354–358.
11. Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. British Myc. Soc. 55:158–161.
12. Powell, C.L. and J. Sithamparanathan. 1977. Mycorrhizae in hill country soils. VI. Infection rate in grass and legume species by indigenous mycorrhizal fungi under field conditions. New Zealand J. Agr. Res. 20:489–502.
13. Rajapakse, S. 1986. Response of cowpea [*Vigna unguiculata* (L.) Walp.] cultivars to vesicular-arbuscular mycorrhizal symbiosis. PhD Diss. Texas A&M Univ., College Station.

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# Effect of Shoot Vigor on Pistillate Flower Production and Abortion in 'Stuart' Pecan

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***Abstract.*** **In pecan [*Carya illinoensis* (Wangenh.) C. Koch)], pistillate flower production is positively and abortion is inversely correlated with growth of the supporting shoot. Shoot growth from 1-year-old branches of 4- and 8-cm lengths was increased by pruning all other branches from the supporting limb. Pistillate flower production increased and subsequent abortion decreased with increasing shoot vigor. The results demonstrate a cause-and-effect relationship between shoot vigor and pistillate development and abortion.**

The pistillate inflorescence in pecan is a spike borne terminally on the shoot. Spike or cluster (i.e., the number of pistillate flowers in the cluster) development is positively correlated with shoot vigor (3, 4, 8, 9). Pistillate abortion, which occurs shortly after full bloom, is inversely correlated with shoot vigor, especially in the 'Stuart' cultivar (8, 9). These correlations imply that pistillate production and retention are closely associated with shoot vigor. However, this relationship has not been verified experimentally. The aborted flowers generally are considered to have been weak and underdeveloped (8, 9, 12), a condition prevalent during the "off" year of the alternate fruit-bearing cycle (7). Their loss constitutes the first of four pistillate or fruit drops that occur during the course of pecan fruit development (9). In the "off" year, as much as 54% of the pistillate flowers may abort during the first drop (8). The entire cluster may abort, which is especially common during the "off" year, or it may be limited to individual flowers within the cluster. When the latter occurs, abortion is primarily from the cluster tip, which is the region with the weakest or most underdeveloped females (8, 9, 12). Although pistillate abortion is greatest on weak shoots, some abortion occurs regardless of shoot vigor (8, 9). Underdeveloped female flowers occur on the distal portion of the inflorescence, regardless of shoot vigor, due to incomplete development of the spike; Hence, the abortion from vigorous shoots.

Studies with $^{14}C$ indicate that the initial spring growth of the pecan shoot, leaves, and pistillate inflorescence is dependent on substrates accumulated the previous season (5). Labeling suggests a decreasing allocation of substrate from the base to the apex of the shoot. The hypothesis was proposed that in instances of low assimilate reserves, pistillate flower development may be prevented or impeded simply because of the terminal position of the inflorescence. If so, then diverting above-normal amounts of substrate into a shoot normally low in vigor should increase subsequent shoot growth and number of females produced in the cluster while decreasing the percentage of females that abort. To test this shoot vigor–fruiting hypothesis, shoot growth was increased by selective pruning and the effects on pistillate production and abortion were recorded.

Just prior to budbreak, 1-year-old branches of 4- and 8-cm lengths were selected on limbs of mature 'Stuart' trees. One-half of these branches in each length category were used as controls. The other half were treated as follows. Lateral branches on the limb supporting the selected 4- or 8-cm 1-year-old branch were removed, leaving only the terminal selected branch. The diameter of the base of the pruned supporting limb averaged 1.6 cm. At the time of pruning, all primary buds, except the apical most lateral bud, on the 1-year-old branch were rubbed off. Pruning and rubbing was done to limit new growth to one lateral bud. Shortly after budbreak, and as needed during the course of the study, any new growth, except that coming from the apical lateral bud, was removed. The four treatments were applied on each of 14 trees.

Counts of pistillate flowers were recorded weekly to determine the time of full bloom, the number of pistillate flowers produced per cluster, and the number of females aborted during the first drop. A pistillate flower was counted only if its stigmatic surface was discernible; i.e., small, underdeveloped, and undiscernible females that are characteristic

Table 1. Effect of pruning and vigor of the 1-year-old branch on shoot and leaf growth and pistillate production and abortion in 'Stuart' pecan.

| 1-year-old branch length (cm) | Shoot length (cm) | Leaf length (cm) | Shoot diam (mm) | Leaves/ shoot (no.) | No. pistillate flowers/cluster (11 May) | Percentage pistillate abortion (11–25 May) |
|---|---|---|---|---|---|---|
| | | | *Control* | | | |
| 4 | 8.9[z] | 15.0 | 4.6 | 8.6 | 3.5 | 58.9 |
| 8 | 14.5 | 18.5 | 5.3 | 10.7 | 3.8 | 26.1 |
| | | | *Pruned to one branch* | | | |
| 4 | 13.2 | 21.7 | 7.9 | 8.0 | 4.7 | 26.7 |
| 8 | 15.2 | 23.7 | 8.4 | 10.4 | 5.6 | 16.4 |

[z]Statistical significance: Control vs. pruned is significant for all comparisons, except leaves/shoot; branch length effect is significant for all comparisons; and the control vs. pruned × branch length interaction is not significant for any comparison, $P \leq 0.05$ level.

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