

# Increased Geranium Growth Using Pretransplant Inoculation with a Mycorrhizal Fungus

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**Abstract.** Inoculation of the various potting media with the mycorrhizal fungus *Glomus fasciculatum* (Thaxter) Gerd. & Trappe resulted in root colonization of geranium (*Pelargonium X hortorum* L.H. Bailey) by the endophyte that persisted after transplanting. By flowering time, mycorrhizal transplants grown at 11 or 43 ppm P were more uniform in growth than nonmycorrhizal transplants grown at the same nutrient regimes, had greater leaf areas and leaf weights, had increased root and shoot weights, and had lower foliar Mn concentrations. Mycorrhizae were sufficiently well-established on most seedlings at a minimum of 2 weeks after emergence to persist and continue to develop after transplanting. Pretransplant inoculation with *G. fasciculatum* increased subsequent geranium growth over that of nonmycorrhizal controls when both were transplanted into soil heavily infested with mycorrhizal fungus inoculum, even though size of mycorrhizal and nonmycorrhizal plants did not differ at the time of transplanting. Posttransplant inoculation with *G. fasciculatum* did not affect the growth of plants which were already mycorrhizal at transplant, but increased the growth of previously nonmycorrhizal transplants. Pretransplant inoculation in soil, peat, or vermiculite resulted in larger plants than posttransplant inoculation in these media.

Many horticultural crops are grown in containers, including nursery stock, bedding plants, and vegetable transplants. The high value of container-grown plants (3) may make inoculation with mycorrhizal fungi cost-beneficial. Vesicular-arbuscular mycorrhizae (VAM) occur on roots of many species of horticultural plants, and may improve host plant growth through increased uptake of P, Zn, and other minerals, reduced incidence of soil-borne plant diseases, and increased tolerance of drought stress (13). A potential use for VAM on container-grown plants is the improvement of transplant survival and regrowth, as has been reported by Barrows and Roncadori (4) for poinsettia, and by Cooper (7) for tamarillo.

Use of soilless growth media and high levels of soluble P fertilizer inhibits establishment of, and host growth response to, VAM (6), but is common in container plant production. It is possible that even though no host growth benefits are obtained when container-grown plants are inoculated under conditions unfavorable for mycorrhizal development, mycorrhizal colonization may increase survival and induce more rapid regrowth when hosts are transplanted into soil and given less fertilizer (6).

The objectives of this study were to determine the following, using geranium as a test plant: 1) whether VAM spread rapidly to new roots which grow after transplanting; 2) how long a period is required following inoculation before mycorrhizae are well enough established on host roots to persist after transplanting; 3) how growth of mycorrhizal transplants is affected by the use of soilless media and high levels of soluble P fertilizer in con-

tainer growing systems; and 4) whether pretransplant inoculation with VAM increased host growth more than posttransplant inoculation.

## Materials and Methods

*Expt. 1: VAM spread to roots formed after transplanting and effect of pretransplant inoculation on host growth.* Seeds of 'Sprinter Scarlett' geranium were surface-sterilized 20 min in 0.5% sodium hypochlorite solution, placed in running tap water 15 hr, then germinated on water agar plates for 48 hr in darkness at 21°C. Seedlings were then planted individually in 6-cm cell packs in medium-grade vermiculite. The inoculum, mixed into the medium before planting at the rate of 1.5 g inoculum/liter medium, consisted of washed and finely chopped subterranean clover (*Trifolium subterraneum* L.) roots colonized by *Glomus fasciculatum* or roots of nonmycorrhizal subterranean clover plants treated previously with *G. fasciculatum* spore washings, which had been passed through a 37- $\mu$ m sieve. Plants were fertilized weekly with 20 ml/6 cm cell or 75 ml/10 cm pot Long Ashton nutrient solution (11) at full strength P (43 ppm P) or one-quarter strength P (11 ppm P) from monobasic sodium phosphate.

Throughout all experiments, plants were watered daily and the greenhouse was maintained at 16°C (night) and 20° to 22° (day) temperatures. Supplemental lighting with an intensity of 160  $\mu$ E s<sup>-1</sup>m<sup>-2</sup> was provided from 6 AM to 10 PM daily by high-pressure sodium vapor lamps.

After 6 weeks of growth, shoot dry weights (60°C for 72 hr) were determined and percentage of root length with mycorrhizal colonization (5) was measured for 10 plants in each treatment. Plants were then transplanted into pasteurized (aerated steam at 60°C for 30 min) silt loam soil in 10-cm round plastic pots and grown for an additional 6 weeks, at which times almost all were in bloom. At that time the number of flowering stems per plant were counted and plant leaf area was measured with a LI-COR model LI 3000 leaf area meter. Then leaf blades were dried 72 hr at 60°C and leaf dry weight was determined. Foliar nutrient concentrations were determined by the Oregon State Univ. Dept.

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of Soil Science Plant Analysis Lab. Samples were digested in perchloric acid. N and P concentrations were determined colorimetrically (2), and other elements were analyzed with a Perkin-Elmer Model 400 atomic absorption flame spectrophotometer.

Root samples were collected separately from both the pretransplant mass in vermiculite and from the new roots which had grown out into the soil. These roots were distinguished by their spatial distribution in the 2 media. Roots were cleared and stained (14) and colonization was measured as percentage of root length with mycorrhizal colonization (5).

Data are based on 6 replications of 5 plants each (30 plants/treatment), except for foliar nutrient analysis, which was on a single sample of 12 pooled plants.

*Expt. 2: Determination of the minimum duration of pretransplant inoculation allowing mycorrhizae to persist after transplanting.* Surface-sterilized geranium seeds were planted directly into pasteurized silt loam soil in 6-cm cell packs (2 seedlings per cell) and inoculated with *Glomus fasciculatum* or a control treatment as described in expt. 1. Plants were fertilized with Long Ashton nutrient solution at the low P level (11 ppm) as in expt. 1. Emergence was 10 days after seeding. At 1, 2, 3, 4, or 5 weeks after emergence, seedlings were washed free of soil and transplanted into 10-cm round plastic pots containing pasteurized soil. At each time of transplanting, data were taken as in expt. 1. Plants were harvested 9 weeks after seedling emergence and final data taken on shoot dry weight and percentage of root length with mycorrhizal colonization (5). Each treatment had 10 single-plant replications.

*Expt. 3: Effect of pretransplant growth medium and P fertilizer on formation of mycorrhizae and host growth response after transplanting, and comparison of effects of pre- and post-transplant inoculation.* Surface-sterilized seeds were germinated and planted in growth media containing *G. fasciculatum* inoculum as described in expt. 1. The media used were pasteurized sphagnum peat with pH adjusted to 6.4 with dolomitic lime, medium-grade vermiculite, or silt loam soil (pH 6.1). Plants were fertilized weekly with Long Ashton nutrient solution with P at either full or one-quarter strength (43 or 11 ppm P), and transplant data taken 6 weeks after planting as in expt. 1. Plants grown in vermiculite were transplanted into vermiculite or soil, those in peat into peat or soil, and those in soil into soil. In some treatments, the soil into which they were transplanted had been in-

oculated with *G. fasciculatum* as described above. From transplanting until harvest 5 weeks later, plants in all treatments were fertilized weekly at 11 ppm P. Data collection and replications were as in expt. 2.

## Results

*Expt. 1.* Geraniums grown in vermiculite at either fertilizer P level and inoculated with *G. fasciculatum* were no larger than the controls at the time of transplanting, even though the roots had been colonized (Table 1). Colonization was less at the high than at the low P level. After transplant and growth in soil for 6 weeks, mycorrhizal plants at both fertilizer P levels had greater root and shoot weights, leaf areas, and leaf dry weights. The number of flower stems was increased by inoculation with *G. fasciculatum* only at the low fertilizer P level to equal that of nonmycorrhizal plants at the high P fertilizer level. Fewer flowering stems occurred on nonmycorrhizal plants at the low P level than in the other 3 treatments. Nonmycorrhizal plants were less uniform (higher coefficient of variability) in root and shoot weight, leaf area, and leaf weight compared to inoculated plants (Table 1).

VAM spread rapidly to new roots formed after transplanting. Mycorrhizae were equally abundant on the inoculated older portion of the root system and on the new roots which had grown out into the noninoculated medium. The percentage of root length colonized at 11 ppm P was 70.2 on older roots and 74.7 on new roots; at 43 ppm P, it was 50.0% on older roots and 52.0% on newer roots. Foliar concentrations of N, K, Ca, Mg, or Zn did not differ greatly between the treatments, but P and Na concentrations were much lower in nonmycorrhizal transplants grown at the low fertilizer P level (Table 2). Foliar concentrations of Mn were 3 and 5 times higher in nonmycorrhizal than mycorrhizal geraniums at the high and low fertilizer P levels, respectively.

*Expt. 2.* Of the *Glomus*-inoculated seedlings, 40% had detectable mycorrhizal infection one week after seedling emergence and 90% after 2 weeks. Inoculated plants which did not become mycorrhizal had shoot dry weights no different from those of nonmycorrhizal controls. At 3, 4, and 5 weeks after emergence, all inoculated plants were mycorrhizal. The proportions of geranium plants mycorrhizal after the various pretransplant inoculation periods was correlated significantly

Table 1. Effect of pretransplant inoculation with *Glomus fasciculatum* and fertilizer phosphorus on growth of geranium.

| Treatment |    | At transplanting  |                             | Six weeks after transplanting                  |                   |                     |                              |                 |
|-----------|----|-------------------|-----------------------------|--|-------------------|---------------------|------------------------------|-----------------|
|           |    | Shoot dry wt (mg) | Root length mycorrhizal (%) | Shoot fresh wt (g)                             | Root fresh wt (g) | No. in-florescences | Leaf area (cm <sup>2</sup> ) | Leaf dry wt (g) |
| -         | 11 | 157 a'            | 0 a                         | 4.7 a  | 3.3 a             | 0.33 a              | 48 a                         | 0.34 a          |
| -         | 43 | 210 b             | 0 a                         | 30.0 b   | 7.9 b             | 1.88 b              | 287 b                        | 1.50 b          |
| +         | 11 | 146 a             | 53.2 c                      | 31.6 b   | 8.0 b             | 1.90 b              | 320 c                        | 1.70 c          |
| +         | 43 | 206 b             | 22.1 b                      | 35.3 c   | 9.9 c             | 1.98 b              | 352 d                        | 1.76 c          |
|           |    |                   |                             | Coefficients of variability<br>(SD × 100/mean) |                   |                     |                              |                 |
| -         |    |                   |                             | 49.6   | 36.4              | ---                 | 43.8                         | 49.3            |
| -         |    |                   |                             | 17.3   | 20.9              | ---                 | 22.9                         | 20.8            |
| +         |    |                   |                             | 8.6  | 17.3              | ---                 | 11.0                         | 9.2             |
| +         |    |                   |                             | 13.6   | 20.9              | ---                 | 17.3                         | 11.4            |

'Mean separation within columns by Duncan's multiple range test, 5% level.

Table 2. Effect of pretransplant inoculation with *Glomus fasciculatum* and fertilizer phosphorus on foliar mineral concentration of geraniums at flowering.

| Treatment | Foliar mineral concn<br>(% dry wt or ppm) |          |          |          |           |           |             |             |             |
|-----------|---|----------|----------|----------|-----------|-----------|-------------|-------------|-------------|
|           | P<br>(ppm)                                | N<br>(%) | P<br>(%) | K<br>(%) | Ca<br>(%) | Mg<br>(%) | Zn<br>(ppm) | Mn<br>(ppm) | Na<br>(ppm) |
| -         | 11  | 2.8      | 0.12     | 3.1      | 1.3       | 0.30      | 32          | 120         | 12          |
| -         | 43  | 2.7      | 0.21     | 2.1      | 1.5       | 0.46      | 24          | 154         | 55          |
| +         | 11  | 2.8      | 0.18     | 2.1      | 1.3       | 0.42      | 24          | 36          | 55          |
| +         | 43  | 2.7      | 0.22     | 1.9      | 1.6       | 0.48      | 26          | 44          | 55          |

(slope = 1.02,  $r = .980$ ,  $P = 1\%$ ) with the proportion that were mycorrhizal at harvest. Mycorrhizal plants weighed more at transplanting only after the longest (5 weeks) inoculation period (Fig. 1). At the end of the experiment, mycorrhizal plants weighed more than nonmycorrhizal plants after all inoculation periods. Fungal colonization of roots at transplanting increased with length of inoculation period (Fig. 2), but final colonization levels were high regardless of inoculation period. Low levels of colonization one or 2 weeks after emergence resulted in colonization of most of the root system at harvest.

*Expt. 3.* At the time of transplanting, geraniums showed no growth response to *G. fasciculatum* in peat or vermiculite at

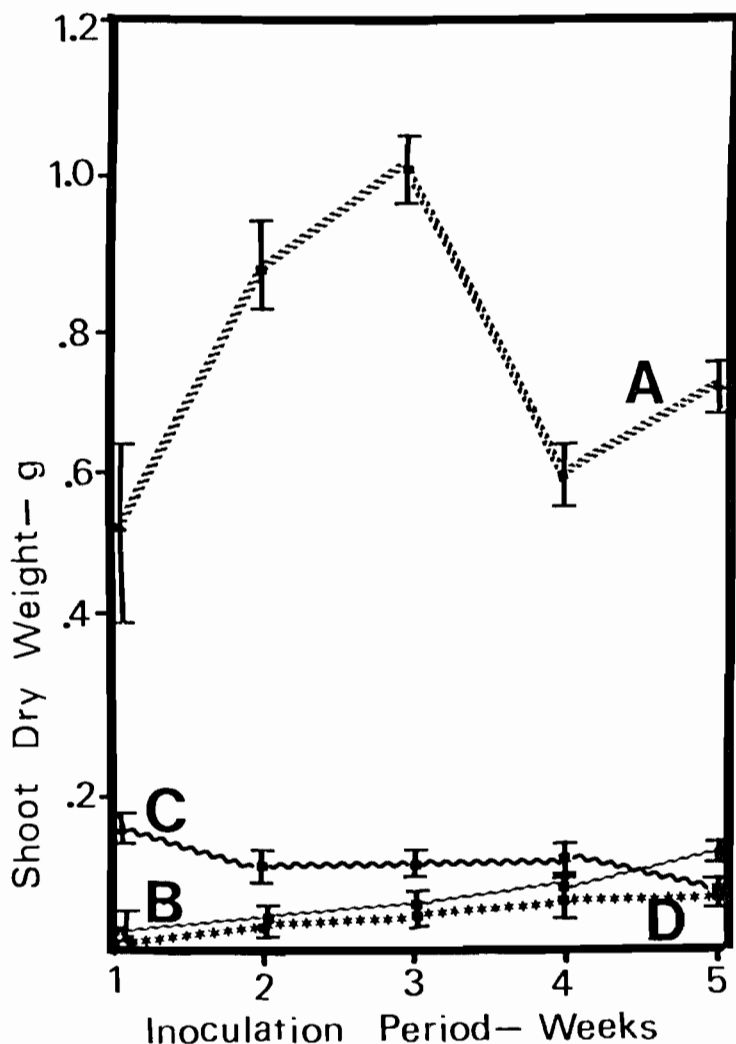


Fig. 1. Effect of inoculation with *Glomus fasciculatum* and duration of pretransplant inoculation period on shoot dry weight of geraniums at transplanting and at harvest. (A = at harvest, mycorrhizal; B = at transplanting, mycorrhizal; C = at harvest, nonmycorrhizal; D = at transplanting, nonmycorrhizal.) Vertical lines show SD.

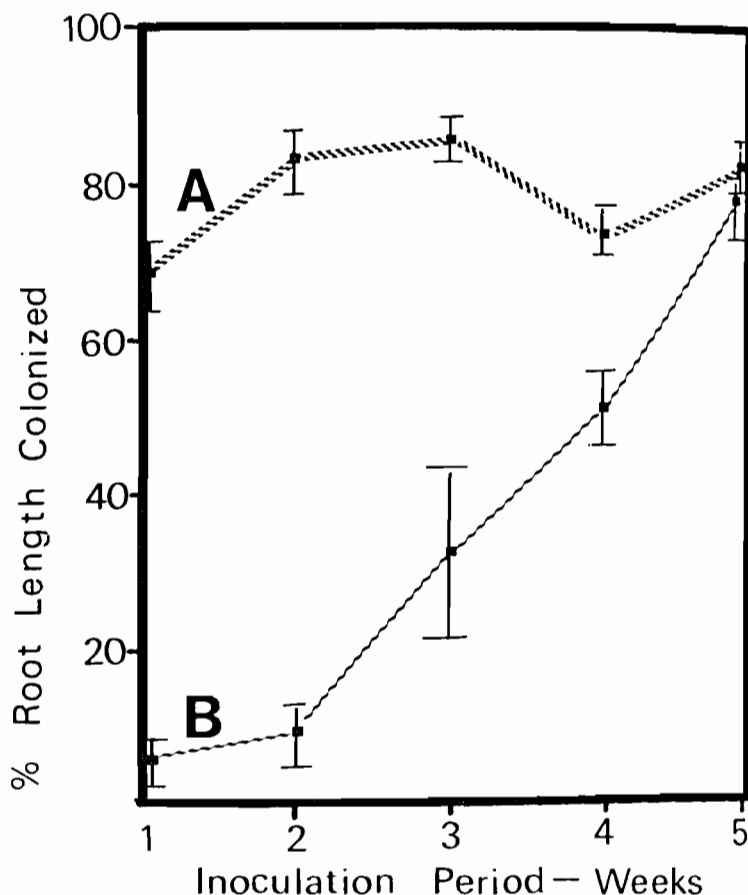


Fig. 2. Effect of duration of pretransplant inoculation with *Glomus fasciculatum* on colonization of geranium roots at transplanting and at harvest. (A = at harvest; B = at transplanting.) Vertical lines show SD.

either high or low P or in soil at the higher fertilizer P level; host growth response only occurred when grown in soil at the low P level (Table 3). Roots of transplants were colonized more heavily when they had been grown in soil (Table 4). Colonization in peat and vermiculite was reduced further by the higher fertilizer P treatment. Shoot dry weight at harvest had been enhanced by *G. fasciculatum* on plants grown in all media and at both fertilizer P levels if they had been transplanted into soil (Table 3). Mycorrhizal growth response of plants grown in vermiculite was not increased after transplanting into vermiculite; however, a small increase occurred when plants grown in peat were transplanted into peat.

Posttransplant inoculation with *G. fasciculatum* did not affect growth of plants which were already mycorrhizal, but increased the growth of nonmycorrhizal plants in all growth media (Table 3). Regardless of this growth increase, pretransplant inoculation in all media and at both P levels resulted in larger plants than posttransplant inoculation.

Colonization of roots in all 3 treatments had reached relatively high levels by the end of the experiment (Table 4). In a few cases, pretransplant inoculation resulted in greater fungal colonization of roots than posttransplant inoculations, but there was no consistent pattern. Control plants in all experiments remained nonmycorrhizal.

#### Discussion

Inoculation with VAM fungi at the time of seeding may be beneficial to horticultural crops which are transplanted into soil, even if the soil already contains abundant propagules of VAM fungi. We observed that pretransplant inoculation with *G. fasciculatum* increased geranium growth over that of noninoculated

Table 3. Effect of pre- or post-transplant growth medium, pretransplant fertilizer phosphorus level, and pre- or post-transplant inoculation with *Glomus fasciculatum* on geranium growth.

| Growth medium | Treatment |               | Shoot dry wt at transplanting (mg) | Final shoot dry wt (g)        |                                     |                                   |
|---------------|-----------|---------------|------------------------------------|-------------------------------|-------------------------------------|-----------------------------------|
|               | P (ppm)   | Inoculation   |                                    | Transplanted into same medium | Transplanted into uninoculated soil | Transplanted into inoculated soil |
| Soil          | 11        | Noninoculated | 96 a <sup>c</sup>                  | ---                           | 0.17 a                              | 0.73 a                            |
|               | 11        | Inoculated    | 145 bc                             | ---                           | 3.48 g                              | 3.47 c                            |
|               | 43        | Noninoculated | 151 bc                             | ---                           | 0.43 b                              | ---                               |
|               | 43        | Inoculated    | 157 bc                             | ---                           | 3.52 g                              | ---                               |
| Vermiculite   | 11        | Noninoculated | 127 b                              | 1.28 a                        | 0.47 b                              | 1.14 b                            |
|               | 11        | Inoculated    | 129 b                              | 1.23 a                        | 2.77 f                              | 2.67 d                            |
|               | 43        | Noninoculated | 168 c                              | ---                           | 1.31 d                              | ---                               |
|               | 43        | Inoculated    | 163 c                              | ---                           | 3.01 fg                             | ---                               |
| Peat          | 11        | Noninoculated | 185 c                              | 1.50 a                        | 0.67 c                              | 1.67 c                            |
|               | 11        | Inoculated    | 191 c                              | 2.10 b                        | 3.19 fg                             | 3.14 dc                           |
|               | 43        | Noninoculated | 349 d                              | ---                           | 2.02 c                              | ---                               |
|               | 43        | Inoculated    | 330 d                              | ---                           | 3.57 g                              | ---                               |

<sup>c</sup>Mean separation within columns by Duncan's multiple range test, 5% level.

controls transplanted into heavily inoculated soil, even when there was no growth difference at the time of transplanting. In some cases the growth differences were quite dramatic, and nonmycorrhizal plants which were apparently healthy at the time of transplanting ceased growing after transplanting (Fig. 4). Barrows and Roncadori (4) and Cooper (7) observed that pre-established VAM increased transplant survival and speed of regrowth of poinsettia and tamarillo, respectively.

Another reported benefit of pretransplant inoculation is that preinoculation with mycorrhizal fungi has reduced disease incidence from many soil-borne pathogens, whereas plants exposed simultaneously to soil-borne pathogens and VAM generally are not protected (17, 18). Pretransplant inoculation may also permit selection and establishment of more efficient strains of the endophyte. Plants grown in soilless media benefited from pretransplant inoculation with mycorrhizae if grown later in soil. Posttransplant benefits may be a more important reason to inoculate container-grown plants in soilless media than benefits during production (6).

Effectiveness of VAM in increasing host growth and P uptake has been related to the speed and extent of root colonization by these fungi (1, 8, 10, 16). Data presented here show that new roots can be colonized extensively by *G. fasciculatum* transfer-

ring from older roots. New roots may be colonized more quickly in this manner than from soil-borne propagules. This is supported by our observation that the growth response from pretransplant inoculation was greater than that from posttransplant soil inoculation. Final levels of root colonization were similar, probably because they were near the maximum for the system. Development of the external hyphal network, which has also been related to host growth response (9), may also have been more rapid when plants were inoculated already. Further research comparing rates of spread from roots of mycorrhizal transplants and from naturally occurring soil inoculum in field situations would better indicate their relative ability to colonize transplants. Confinements of roots within a pot may facilitate spread of the endophyte within the root system.

The presence of barely detectable signs of mycorrhizal colonization after the various pretransplant incubation and growth period resulted in plants that were mycorrhizal at harvest. The smallest amount of mycorrhiza—about 1 mm of colonized root—was sufficient to spread and colonize the entire root system after transplanting (Fig. 2). The minimum pretransplant period following inoculation, which resulted in persistence of mycorrhizae on most seedlings, was 2 weeks after emergence. This corresponds with the recommended time for transplanting seedling

Table 4. Effect of pre- or post-transplant growth medium and pretransplant fertilizer phosphorus level on colonization of geranium roots inoculated with *Glomus fasciculatum*.

| Growth medium | Treatment |               | Root length mycorrhizal at transplanting (%) | Final root length mycorrhizal (%) |                                     |                                   |
|---------------|-----------|---------------|--|-----------------------------------|-------------------------------------|-----------------------------------|
|               | P (ppm)   | Inoculation   |  | Transplanted into same medium     | Transplanted into uninoculated soil | Transplanted into inoculated soil |
| Soil          | 11        | Noninoculated | ---  | ---                               | ---                                 | 70.2 ab                           |
|               | 11        | Inoculated    | 81.8 e <sup>z</sup>                          | ---                               | 86.6 b                              | 83.7 c                            |
|               | 43        | Inoculated    | 80.2 e                                       | ---                               | 85.9 b                              | ---                               |
| Vermiculite   | 11        | Noninoculated | ---  | ---                               | ---                                 | 64.6 a                            |
|               | 11        | Inoculated    | 31.5 c                                       | 64.5 a                            | 75.1 a                              | 77.5 bc                           |
|               | 43        | Inoculated    | 0.3 b  | ---                               | 79.8 ab                             | ---                               |
| Peat          | 11        | Noninoculated | ---  | ---                               | ---                                 | 73.3 ab                           |
|               | 11        | Inoculated    | 54.2 d                                       | 66.4 a                            | 73.8 a                              | 71.1 ab                           |
|               | 43        | Inoculated    | 11.0 a                                       | ---                               | 77.3 ab                             | ---                               |

<sup>z</sup>Mean separation within columns by Duncan's multiple range test, 5% level.

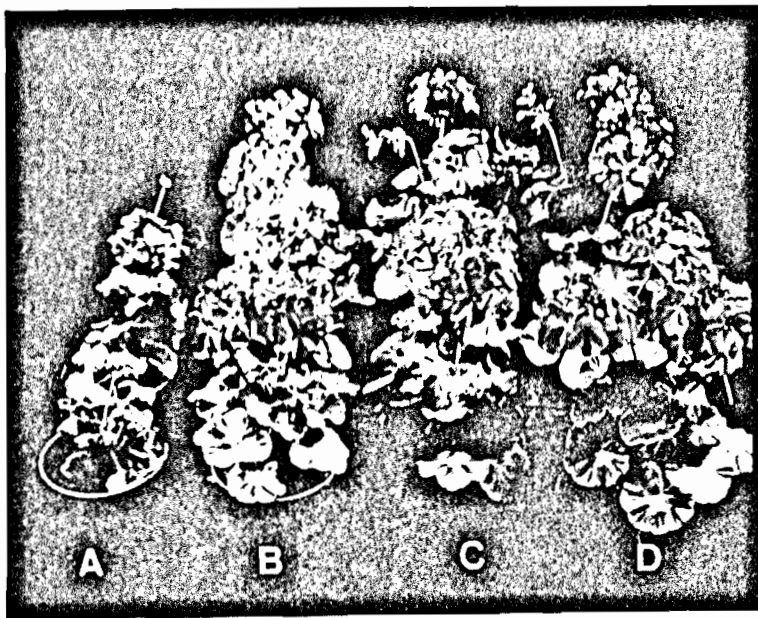


Fig. 3. Effect of pretransplant inoculation with *Glomus fasciculatum* and fertilizer phosphorus level on growth of geranium. (A = noninoculated, 11 ppm P; B = inoculated, 11 ppm P; C = noninoculated, 43 ppm P; D = inoculated, 43 ppm P.) Photograph taken 6 weeks after seedling transplant.

geranium crops, when 2 or 3 true leaves have developed (15). Seed-flat inoculation may be the most practical way to establish VAM on container-grown plants, since less inoculum is necessary than at later stages.

The increase in crop uniformity which we observed when plants were mycorrhizal is a benefit not reported previously. However, Janos (12) did find that various species of tropical tree seedlings grew at a more even rate (compared with other species) in soil inoculated with VAM fungi. It should be determined if this also applies to vegetatively propagated plants of the same clone, which would indicate whether the effect was due to host genetic variation or to environmental factors.

A slight reduction in Mn uptake caused by inoculation with VAM fungi was also observed by J.A. Chatfield, L.H. Rhodes, and C.C. Powell, Jr. (personal communication). Large differ-

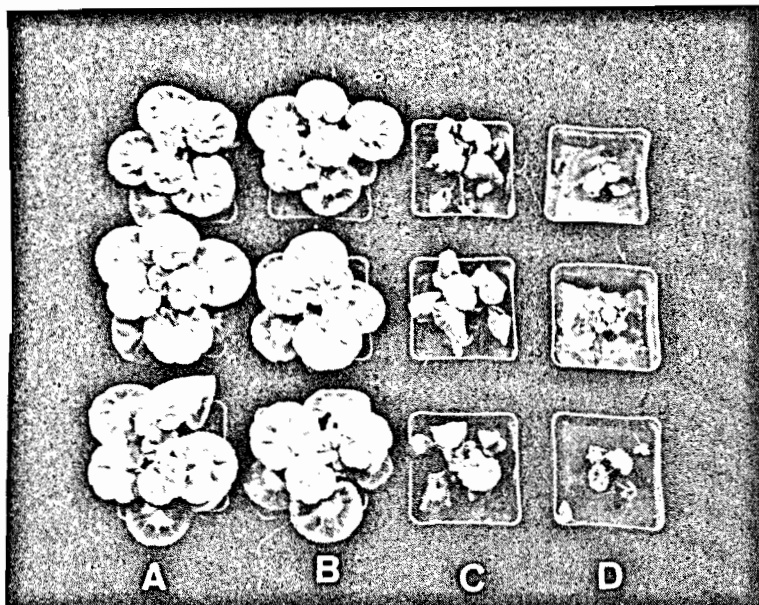


Fig. 4. Effect of pretransplant inoculation with *Glomus fasciculatum* and fertilizer phosphorus level on growth of geranium. (A = inoculated, 43 ppm P; B = inoculated, 11 ppm P; C = noninoculated, 43 ppm P; D = noninoculated, 11 ppm P.) Photograph taken 4 weeks after seedling transplant.

ences in Mn concentration were observed here, even at a fertilizer P level where mycorrhizal and nonmycorrhizal plants were of equivalent size. If this reduction in Mn uptake by mycorrhizal plants occurred at higher Mn levels, it could be useful in reducing toxicity from excess Mn, which may occur in heat-treated soils but is rare in field situations.

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