Effect of Vesicular-Arbuscular Mycorrhizal Fungi on the Growth, Photosynthesis, Transpiration and the Distribution of Photosynthates of Bearing Satsuma Mandarin Trees

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Summary

The response of satsuma mandarin (Citrus unshiu Marc. cv. Okitsu wase) on trifoliate orange (Poncirus trifoliata Raf.) rootstocks to inoculation of vesicular-arbuscular mycorrhizal (VAM) fungi, such as Glomus ambisporum Smith and Schenck, Glomus fasciculatum (Thaxter) Gerdemann and Trappe emend. Walker and Koske, Glomus mosseae (Nicolson and Gerdemann) Gerdemann and Trappe, and Gigaspora margarita G. Spain was studied at low phosphorus (P) application.

Under high air temperature stress conditions in August, the photosynthetic (Pn) and transpiration (Tr) rates of VAM-inoculated trees were faster than non VAM ones. But in September when air temperature decreased, the Pn and Tr rates did not differ significantly between VAM-inoculated trees and non infected ones. In general, infected trees had larger leaf area and higher leaf P concentration and the tree growth was more vigorous than was that of non infected ones.

When 13CO2 was fed to fruit-bearing trees under the optimal air temperature conditions, fruits were the strongest sink for photosynthates and new leaves were the second strongest. The leaves of VAM and non VAM trees assimilated 13CO2 equally fast per unit leaf area, and the distribution of 13C into various parts of the bearing trees did not differ. VAM trees, however, had 3 times more photoassimilates per tree than uninfected ones because the former had a leaf area 3 times larger than the latter and grew more vigorously.

Introduction

The beneficial effects of VAM symbiotic association on growth of plants are well-known, and there are many reports on its effect on growth of citrus plants (Hattingh and Gerdemann, 1975; Krikun and Levy, 1980; Menge et al., 1978). Although growth responses of the VAM plants vary with soil fertility especially with P content in the soil, mycorrhizal dependency might be affected by soil type (Baylis, 1967; Hayman and Mosse, 1971; Mosse, 1972a), species of mycorrhizal fungus (Graham et al., 1982; Mosse, 1972a, b; Pope et al., 1983), and plant cultivars (Azcon and Ocampo, 1981; Menge et al., 1978). Syvertsen and Graham (1990) reported that VAM colonization does not affect net gas exchange of citrus plants that are comparable in size, growth rate, and nutritional status compared with non VAM plants. In contrast, Johnson (1984) reported that high P levels in leaf tissue as a result of colonization or heavy P application could be expected to increase rate of Pn and subsequent growth response. Brown and Bethlenfalvay (1987) also showed that higher rates of CO2 exchange in VAM plants are associated with P nutrition. Parke et al. (1983) reported that drought-stressed ectomycorrhizal Douglas-fir seedlings fixed CO2 10 times faster than non-inoculated seedlings. There are very few reports on photosynthetic activity and distribution of photosynthates in VAM fungi-inoculated, bearing citrus trees.
The objective of this experiment is to clarify the effect of VAM infection on Pn and Tr and the distribution of photosynthates on bearing satsuma mandarin trees grown in infertile, marginal soil.

Materials and Methods

Two-year-old satsuma mandarin trees on trifoli- ate orange rootstocks were used with a random- ized block design with 5 replicates per treatment. All trees were dipped in iprodione solution (5 g/50 liter of water) for a few minutes and transplanted in 45 liter pots on April 14, 1990. Inoculation took place on May 12, 1990. Different isolates of *Glomus ambisporum*, *Glomus fasciculatum*, *Glomus mosseae*, and *Gigaspora ramiisporophora* spores were obtained from a citrus-growing area of Ehime prefecture, Japan, and had been cultured in pots with Bahia grass (*Paspalum notatum* Flügge) sod for two years. The cultures, containing spores, mycelia, and infected root fragments, were used as an inoculum. Consequently, 100 to 700 spores of a VAM fungus were administered to each pot. The inoculum was mixed thoroughly with the perlite-vermiculite mixture (1:1, v/v) as a plant growth medium. The same process was repeated on May 19, 1992, but this time a unit inoculum ranged from 200 to 800 VAM spores per pot. Soils of control trees were uninoculated, but drenched with a fungicide solution (Iprodione 0.2 g/2 liter of water pot) on April 21, 1990. Fruits were thinned to a leaf/fruit ratio of 20 to 25 on June 7, 1992. Trees were grown under natural conditions and watered every other day.

Both inoculated and non-inoculated trees were supplied with 2 g of N, 0.5 g of P, and 2 g of K per pot in 1990, 6.45 g of N, 5.5 g of P, and 5.15 g of K in 1991, and 2 g of N, 1 g of P, and 1.1 g of K per pot in 1992. Since the growth of non- inoculated trees was very poor in 1990, the amount of fertilizer was increased in 1991. Ammonium phosphate was the main P fertilizer. Other nutrients were applied as a mixture of CaCO₃ and MgSO₄ (10 g/tree-year) and a micro- nutrient solution (10 ml/tree-year) containing 0.001 % Mn and 0.005 % B was applied in the early spring. The concentrations of other micro- nutrients such as Fe, Cl, Zn, Co, Cu, and Mo were below 0.001 %.

Experiment 1. Tree growth, photosynthesis and transpiration.

On a clear day from 8.00 to 10.00 AM, Pn and Tr of leaves on the experimental trees were measured with an Intelligent Portable Porometer (Koito KODIC-50) on August 7, 14, and 21, September 4 and 26, 1992. The light intensity ranged from 1200 to 1400 μE·m⁻¹·S⁻¹. The 3rd or 4th leaf from the apices of nonbearing shoots was chosen, the Pn and Tr of a 5.0 cm² portion of a leaf enclosed in a chamber were measured for one minute. Tree growth was measured in December 1992, and the percentage of leaf P was determined. The average leaf area was determined on 25 leaves with a personal computer equipped with an image processor (Epson GT-20). Leaves were collected, oven-dried (80 °C for 48 hr), and ground to pass a 20 mesh sieve. The ground tissue samples were ashed at 550 °C over night, and the residues were dissolved in 2.4 N HCl. The concentration of leaf P was measured colorimetrically by the method of Deniges (1920, 1921).

Experiment 2. The distribution of 13C photosynthates.

On October 13, 1992, 4 VAM (*Gigaspora rami- sporophora*) infected trees and 4 non infected ones used in Experiment 1 were exposed to 13CO₂ with the system shown in Figs. 1 and 2. The assimila- tion chamber (140 cm height × 105 cm length × 85 cm width, with an acrylic sheet) was designed as a tightly closed system; the air temperature was con- trolled at 26 °C with an air conditioner. 13CO₂

Fig. 1. A photograph of 13CO₂ assimilation system.
was evolved from 6 g Ba\(^{13}\)CO\(_3\) (99 atom %) using 150 ml of 50 % lactic acid, which was injected manually with a 50 ml syringe (50 ml lactic acid/hr) for 4 hours in the assimilation chamber. At the end of \(^{13}\)CO\(_2\) assimilation, the acrylic cover was removed. Three days later the trees were harvested sequentially. The leaf area and the weight of leaves, fruits, shoots, stems, and roots were measured before and after oven drying. All samples were dried at 60 °C and ground into a fine powder with a vibrating mill (Model T1-1, Heiko Co. Ltd, Iwaki, Japan) to pass a 20 mesh sieve. The concentration of \(^{13}\)CO\(_2\) in the tissue samples was determined with a \(^{13}\)CO\(_2\) infrared analyzer (JASCO, Ex-130) as described by Kumazawa and Yanagisawa (1981) and Okano et al. (1983). Total carbon content in the tissues was determined with a C-N coder (Sumitomo Chemical, Sumigraph NC-80).

### Results

#### Experiment 1.

Comparison among trees free of VAM and those inoculated with different VAM fungi in Experiment 1 revealed no significant differences in their height, but the leaf area and the tree canopy area of the inoculated ones were larger than those which were uninoculated. Leaf P concentration of non VAM trees was lower than VAM trees (Table 1). The photosynthetic activity of non VAM trees on August 7 was 2.3 ± 0.4 mg CO\(_2\)•dm\(^{-2}\)•hr\(^{-1}\), whereas that of VAM trees was 2 to 3 times faster (Fig. 3). A significant difference between VAM trees and non VAM trees on P\(_n\) existed on August

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**Table 1.** Effect of VAM fungi-infection on tree growth and leaf P concentration of satsuma mandarin trees.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leaf area (cm(^2))</th>
<th>Tree height (cm)</th>
<th>Tree canopy area (m(^2))</th>
<th>Leaf P concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non VAM</td>
<td>10.3±1.1(^a)</td>
<td>78.7±1.7</td>
<td>0.15±0.01</td>
<td>0.123±0.002</td>
</tr>
<tr>
<td>Gigaspora ramisporophora</td>
<td>19.0±1.5</td>
<td>77.5±5.5</td>
<td>0.27±0.02</td>
<td>0.142±0.004</td>
</tr>
<tr>
<td>Glomus ambigus</td>
<td>13.2±1.0</td>
<td>83.5±1.7</td>
<td>0.30±0.01</td>
<td>0.145±0.001</td>
</tr>
<tr>
<td>Glomus fasciculatum</td>
<td>15.3±1.1</td>
<td>87.3±4.8</td>
<td>0.21±0.02</td>
<td>0.138±0.003</td>
</tr>
<tr>
<td>Glomus mosseae</td>
<td>17.6±0.8</td>
<td>80.5±3.5</td>
<td>0.29±0.01</td>
<td>0.151±0.001</td>
</tr>
</tbody>
</table>

\(^a\) Mean±standard error (SE), n=4.
In September when air temperature became cool, the photosynthetic activities of both VAM and non VAM trees were lower than were the rates in August. No significant difference in Pn between VAM trees and non VAM tree was observed, but leaf P concentration of control trees was lower.

Tr of non VAM trees was about 3 g H$_2$O•dm$^{-2}$•hr$^{-1}$ during the experimental period (Fig. 4). However, Tr rate of VAM tree was faster than that of non VAM trees on August 21 and 28, 1992; Tr of VAM trees on August 21, was 3 to 4 times faster than that of non VAM trees. In late September, no significant differences in Tr of leaves were observed between them.

**Experiment 2.**

Effects of VAM inoculation on tree growth are shown in Table 2. Total fresh weights of shoot, root, and fruit and total leaf area of VAM trees were higher than those of non VAM ones. Particularly, total fresh weight of fruits was 3 times larger than that of the non mycorrhizal ones. A significant different between VAM and non VAM trees on shoot/root ratio was also observed.

Large amounts of assimilated $^{13}$C were accumulated in the fruit (Table 3). Fruit was the strongest sink for photoassimilated carbon at this growing stage, followed by new leaves. The data indicated that VAM and non VAM trees assimilated $^{13}$C equally fast per unit leaf area under normal air temperature condition (Table 4). The accumulation of $^{13}$C by different parts of fruit trees did not differ between VAM trees and non VAM trees (Table 5). However, the incorporation of $^{13}$C on VAM trees was 3 times higher in fruit and 2.5 times higher in leaves than it was on non VAM trees.

**Table 2.** Effect of VAM fungi-infection on leaf area and fresh weight of satsuma mandarin trees.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leaf area per tree (cm$^2$)</th>
<th>Total fresh weight (g)</th>
<th>Shoot/Root ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Shoot</td>
<td>Root</td>
</tr>
<tr>
<td>Non VAM</td>
<td>3097.4±914.3*</td>
<td>237.9±35.9</td>
<td>204.0±43.4</td>
</tr>
<tr>
<td><em>Gigaspora ramosiporophora</em></td>
<td>8892.2±648.4</td>
<td>490.9±36.7</td>
<td>587.7±29.9</td>
</tr>
</tbody>
</table>

* Mean±SE, n=4.
Discussion

It is well-documented that the inoculation of low P soil with VAM fungi stimulates the growth of citrus seedling (Allen, 1982; Allen and Boosalis, 1983; Edriss et al., 1984; Graham et al., 1987a; Levy and Krikun, 1980; Levy et al., 1983; Nemec, 1978). Trifoliate orange seedlings are the main rootstocks for Japanese citrus; VAM fungi-inoculated trifoliate orange seedlings are reported to grow more vigorously than do non VAM seedlings (Ishii and Kadoya, 1989; Tang et al., 1982). In this report, the growth of bearing satsuma mandarin trees on trifoliate orange rootstocks growing in P-deficient soil was stimulated by being inoculated with 4 different types of VAM fungi even though the root infection percentages differed among and within different species of VAM fungi.

It has not been reported whether VAM fungi maintain an adequate Pn under high temperature

<table>
<thead>
<tr>
<th>Table 3. The amount of $^{13}$C (mg) in various parts of VAM and non VAM fungi-inoculated satsuma mandarin trees.</th>
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</thead>
<tbody>
<tr>
<td>Treatment</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Non VAM</td>
</tr>
<tr>
<td>VAM$^y$</td>
</tr>
<tr>
<td>Sd$^x$</td>
</tr>
</tbody>
</table>

$^x$ Big root: > 4mm, Small root: 2-4mm, Rootlet A: < 2mm (except for the apical 20mm). Rootlet B: < 2mm (the apical 20mm only).

$^y$ Gigaspora ramosiporophila

$^x$ Significant difference: ns = no significant, * = 5% level, ** = 1% level.

<table>
<thead>
<tr>
<th>Table 5. The percentage of assimilated $^{13}$C in various parts of VAM and non VAM fungi-inoculated satsuma mandarin trees.</th>
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</tr>
<tr>
<td>VAM$^y$</td>
</tr>
<tr>
<td>Sd$^x$</td>
</tr>
</tbody>
</table>

$^x$ Big root: > 4mm, Small root: 2-4mm, Rootlet A: < 2mm (except for the apical 20mm). Rootlet B: < 2mm (the apical 20mm only).

$^y$ Gigaspora ramosiporophila

$^x$ Significant difference: ns = no significant.

<table>
<thead>
<tr>
<th>Table 4. The percentage of carbon$^4$ (per unit leaf area) assimilated on VAM and non VAM fungi-inoculated satsuma mandarin trees after feeding $^{13}$CO$_2$.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Non VAM</td>
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</tr>
<tr>
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</tr>
</tbody>
</table>

$^x$ Carbon dioxide concentration was calculated from the data of total carbon contents measured by a C-N coder and $^{13}$C atom percent measured by a $^{13}$CO$_2$ infrared analyzer.

$^y$ Gigaspora ramosiporophila.

$^x$ Significant different: ns = no significant.
stress conditions. Several studies have suggested that ectomycorrhizal fungi act as a carbon sink indirectly stimulating the rate of Pn of the host tree (Parke et al., 1983; Reid et al., 1983; Rousseau and Reid, 1990). Parke et al. (1983) observed that ectomycorrhizal Douglas-fir seedlings under a drought-stress condition fixed CO₂ 10 times faster than did non-mycorrhizal seedlings. Johnson (1984) reported that high P levels in leaf tissue as a result to colonization by endomycorrhiza, or heavy P application could be expected to increase the rate of Pn and subsequent tree growth. Brown and Bethlenfalvay (1987) showed that the higher rates of CO₂ exchange in VAM plants are associated with P nutrition. Our experiments also showed that the Pn per unit leaf area of temperature-stressed, VAM fungi-inoculated satsuma mandarin trees was faster than that of equally stressed, non inoculated ones.

Syvertsen and Graham (1990) reported that VAM colonization does not affect net gas exchange of citrus plants that are of comparable size, growth rate, and nutritional status with non VAM plants. In their experiment, seedlings were fertilized weekly with standard Hoagland's solution without P for VAM plants or with fivefold P (KH₂PO₄) added Hoagland's solution for non VAM plants. Such a heavy P fertilization in the early growing stage of citrus seedlings enhanced the growth rates and net gas exchange rates of plants comparable with no P-applied VAM plants. In contrast, our experiment on VAM and non VAM satsuma mandarin trees under low levels of P fertilizer resulted in higher Pn and Tr outdoors. Pn and Tr rates per unit leaf area on temperature-stressed VAM trees were 3 to 4 times higher than they were on non VAM trees. This finding is attributed to higher concentrations of P in leaf tissue as a result of VAM colonization and high hydraulic conductivity of VAM fungi development. The photosynthetic rate of non VAM trees was very low in August, when air temperature ranges from 30 °C to 40 °C. This range is far beyond the optimal temperature for Pn; Kadoya (1974) reported that 15 °C to 30 °C is optimal for photosynthetic activity of C₃ plants such as citrus.

Under drought stress conditions, several studies have indicated that stomatal conductance or Tr of VAM plants was increased as compared to similarly-sized non VAM plants (Allen et al., 1981; Allen and Boosalis, 1983; Hardie and Leyton, 1981; Levy and Krikun, 1980). In our study Tr rate of VAM trees was six times higher than that of non VAM trees. We attribute that to the hydraulic conductivity of VAM fungi-inoculated trees which may affect the total fresh weight of the heat-stressed trees by maintaining high levels of Pn and Tr. The infection by the mycorrhizal fungi resulted in an increased P uptake and water conductivity because of the increased water-absorbing surface area provided by the fungal hyphae (Allen et al., 1981; Hardie and Leyton, 1981). Hyphae may also bridge the gap between soil and root under dry conditions when roots shrink away from the soil granules (Graham et al., 1987a). In our experiment root fresh weight of VAM fungi-inoculated trees was about three times greater than that of non VAM trees so that the greater root volume enhanced Tr. The higher rate of Tr helps to decrease the leaf temperature and provides favorable conditions for Pn and Tr. By relieving drought stress, wilting and defoliation of leaves should be reduced.

Under well-watered conditions, VAM fungi appear to have little influence on the hydraulic conductivity of roots and Tr and on P nutrition of the host plant (Graham and Syvertsen, 1984, 1985; Koide, 1985; Nelsen and Safir, 1982; Safir et al., 1972). However, in our experiment under well-watered conditions with low P fertilizer, Pn and Tr were faster under temperature-stressed conditions and P content in leaf tissue was higher than those of the non VAM trees. The ability of mycorrhizal hyphae to maintain a good P delivery to the root at low soil moisture content was the basis for improved drought tolerance (Graham and Syvertsen, 1987b). Ishii et al. (1993) found that drought tolerance of satsuma mandarin trees was increased by the mycorrhizal (Gigaspora ramiispora) hyphae's ability to maintain P delivery to the roots under high temperature and water stress conditions.

Snellgrove et al. (1982) reported that VAM and non VAM plants have equal ratios of carbon assimilation per unit leaf area. In contrast, data from Experiment 1, which was conducted during the hottest season (August to September) in Ehime prefecture, Pn and Tr per unit leaf area on VAM-inoculated satsuma mandarin trees were 3 to 4 times faster than those for non VAM trees (Figs. 3
Kadoya (1974) reported that 15 °C to 30 °C is optimal photosynthetic activity of C3 plants and that newly developed shoots were the most powerful sinks and that a large amount of 14C was translocated to the shoot during its actively growing period. Data in Experiment 2 which was carried out under the optimally controlled air temperature (26 °C) condition revealed that VAM fungi were also active in October (Ishii et al., 1993). These results suggest that although no significant difference on carbon assimilation per unit leaf area existed between VAM and non-VAM plants grown under optimal air temperatures, carbon assimilation on VAM plants may be significantly influenced by air temperature.

Data in Experiment 2 indicate that fruits are the most powerful sinks on bearing trees; a large amount of 13C is translocated to fruits during their growing period. We found that the absorption and translocation of 13C-photoassimilates may be affected by active growth by VAM and non-VAM trees (Table 3). The total fresh weight of VAM trees was 3 times heavier; whereas the total leaf area and fresh weight of roots were 2.5 times greater than those of non-VAM trees. Hence, 13CO2 assimilation on VAM trees was stimulated because of increased vigor. Non-VAM trees were unable to maintain normal Pn and Tr during the hot summer months in Japan, whereas VAM trees were able to do so.

Literature Cited


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VA菌根菌がウンシュウミカン結実樹の樹体生長、光合成、蒸散、ならびに光合成産物の分配に及ぼす影響

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摘要

数種類のVA菌根菌が、ウンシュウミカン（津早生）4年生結実樹（カラタチ台）の樹体生長、光合成、蒸散、ならびに光合成産物の分配に及ぼす影響を、リン酸少量施用土壤において実験開始2年後に調査した。

菌根菌接種区では、菌根菌無接種区と比べて、葉内のリン濃度は高く、樹体生長も良好となる傾向がみられた。8月の高温時における光合成および蒸散速度は、菌根菌接種区の方が無接種区に比べて高かったが、気温が低下した9月では、両区の光合成速度や蒸散速度の間には明らかな有意差は観察されなかった。

10月に最適な温度条件下で樹体に^{13}CO₂をとりこませ、^{13}Cの分布と移行量を調査したところ、両区ともに、果実への分配率が最大で、葉への分配率がこれに次いだ。^{13}Cの分布についてはいずれの器官においても両区間に明らかな有意差はなく、単位葉面積当たりの^{13}C同化量にも差はなかった。なお、菌根菌接種区の全葉面積は無接種区の3倍で、1樹当たりの^{13}C同化量も3倍に近かった。