Effects of Charcoal as a Soil Conditioner on Citrus Growth and Vesicular-Arbuscular Mycorrhizal Development

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Summary

Effects of several kinds of charcoal applied to soil on citrus growth and vesicular-arbuscular mycorrhizal (VAM) development were investigated. Satsuma mandarin (Citrus unshiu Marc.) trees on trifoliate orange (Poncirus trifoliata Raf.) rootstocks were transplanted to root boxes using the soil mixed with charcoal derived from rice husk, citrus juice sediment or western spruce bark. The trees were inoculated with the spores of Glomus fasciculatum (Thaxter) Gerdemann and Trappe emend. Walker and Koske. Elongation of the roots in the charcoal treatments was more vigorous than that in the charcoal-free control. The fresh weights of the root, shoot and the whole tree increased in response to charcoal application. The intensity of VAM infection in any charcoal treatment was higher than that in the control. In particular, the percentage of the infection in the rice husk charcoal plot was 41.5 and P concentration in the leaf exceeded that of the control.

In a Citrus iyo orchard, the percentage of VAM infection was 52% in the rice husk charcoal plot, the highest among plots. The intensity in the Bahia grass (Paspalum notatum Flugge.) plot was next, followed by the third highest rate found in the abandoned plot which had not been cultivated in recent years. The lowest percentage of VAM infection was in a clean-culture plot. A microscopic observation also revealed that in a charcoal-treated plot there were many sites where VAM fungi infected the root.

Introduction

A classic book written in Japan (Miyazaki, 1697) explained that soybean plants vigorously flourished with a minimum of care when their seeds were sowed with charcoal. Recently, Ogawa (1987) reported that charcoal applied to the soil could stimulate the activity of soil microorganisms and promote the formation of root nodules and vesicular-arbuscular mycorrhizae in soybean roots.

VAM symbioses are exceptionally common among terrestrial flowering plants (Harley and Harley, 1987). Among these plants, there is a wide range of dependency on VAM fungi for plant growth. Citrus is also infected by several kinds of VAM fungi and is considered highly dependent on them (Dixon et al., 1988; Edriss et al., 1984; Ishii et al., 1992b; Menge et al., 1978; Nemec, 1979). These fungi improve mineral nutrition of the host by increasing P uptake from a P deficient soil (Antunes and Cardoso, 1991; Ferguson and Menge, 1986; Graham and Timmer, 1985; Krikun and Levy, 1980; Nemec, 1979; Tang et al., 1984). Higher concentration of minor elements, especially Zn (Krikun and Levy, 1980) and Cu (Timmer and Leyden, 1980), were also observed after an inoculation with VAM fungi. Because the fungi provided essential elements for citrus growth, the infected trees could grow more rapidly and appeared healthier than non-infected trees. This phenomenon was especially noticeable in soils of low fertility (Nemec, 1979). Furthermore, VAM fungi inoculation may increase tolerance to water stress by regulating stomatal opening through hormone synthesis (Graham et al., 1987). In our reports, the photosynthesis and transpiration rates of VAM fungi-infected satsuma mandarin trees grew-
ing in P-deficient soil surpassed those of non-VAM trees stressed by high temperatures in August (Shrestha et al., 1992). Interestingly, an inoculation of VAM fungi improved the fruit quality of satsuma mandarin trees. In particular, it enhanced the Hunter’s a/b value of peel color and the sugar content in juice (Ishii et al., 1992b). In the citrus orchards where high quality fruit was produced, the percentage of VAM infection in the root was very high (Shrestha et al., 1993).

On the other hand, phytotoxic substances exist in the bark and sawdust extracts from several woody forest species, especially in the bark extracts of hinoki cypress (Chamaecyparis obtusa Sieb. et Zucc. ex Endl.) which are condensed tannins (Ishii and Kadoya, 1993). In soils to which unfermented organic matter were added, ethylene has often been detected at concentrations high enough to inhibit the growth of citrus trees (Ishii and Kadoya, 1984). The problem of growth inhibition is solved, however, if the organic matter with phytotoxic substances is first transformed into charcoal and then used as a soil amendment.

The purpose of this study is to investigate effects of several kinds of charcoal applied to soil on citrus growth and VAM development.

**Materials and Methods**

**Experiment 1. Effects of charcoal application on citrus growth and VAM development**

In this experiment, we examined the effects of charcoal application on the growth and VAM development of ‘Aoshima’ satsuma mandarin trees on trifoliate orange rootstocks for two years. In early April of 1988, three two-year-old satsuma mandarin trees per plot were transplanted individually to root boxes (40 cm × 40 cm × 40 cm) containing the mixtures of river sand and a specific charcoal. Before planting, the roots were carefully washed to remove the soil which had nourished the trees. The control soil lacked charcoal. The charcoal used was made by using a chimney (15 cm in diameter and 1.8 m in length) with some holes for aeration or an oil drum (200 liter) equipped with a chimney (15 cm in diameter and 1.8 m in length). The charcoal sources were rice husk, citrus juice sediment and western spruce bark. The charcoal derived from western spruce bark was broken into 5~10 mm pieces. The sand and charcoal were mixed in a proportion of 50 to 1 by weight. The pH and electric conductivity (EC) of the mixtures were measured with a pH meter and an EC meter, respectively.

Two months after planting, the roots were treated with 50 g fresh weight of soil inoculum containing 300~340 spores of Glomus fasciculatum. The inoculum was obtained from greenhouse pot cultures of Bahia grass inoculated with Glomus fasciculatum originally isolated from citrus orchards in Matsuyama city, Ehime prefecture, Japan. In 1988, each tree was fertilized with 6.4 g of N, 3.2 g of P, and 3.8 g of K per annum from a mixture of ammonium sulfate, calcium phosphate, and potassium sulfate, respectively; the P and K contents in the charcoal were first deducted. The control trees were supplied with 5 g of calcium carbonate-magnesium sulfate mixture (8 : 2 by weight) to improve the soil pH. A Hoagland minor element solution (1 liter/tree) was administered to each trees. In 1989, P was excluded.

Roots appearing on the glass plates of root boxes were traced onto transparent plastic sheets from which the root lengths were measured, using a personal computer equipped with an image processor (NEC mediagraph MG-10 with a stylus pen, Tokyo, Japan) and a special software program.

In early December of 1989, the trees were removed from the root boxes, and then the total, root, and shoot fresh weights were measured. For the determination of leaf P, leaf samples were ashed at 550 °C overnight, and the residues were dissolved in 2.4N HCl. The P content was measured colorimetrically by the method of Deniges (1920, 1921). Undamaged feeder rootlets were sampled and rinsed with distilled water for a few seconds. After the rootlets were cut into 2-cm segments behind the growing tip, the segments were immediately fixed in FAA (formalin: acetic acid: 50% ethanol, 13 : 5 : 200, v/v/v). Ten segments per treatment, stained by the technique of Phillips and Hayman (1970), were analyzed for the intensity of VAM infection by light microscopy. The percentage of VAM infection was calculated with the following equation:

\[
\text{% of VAM infection} = \frac{\text{Root length infected}}{\text{Root length observed}} \times 100
\]
Experiment 2. Soil management and the intensity of VAM infection in citrus roots

In late April of 1987, 4 experimental plots of 5 trees each were prepared in a Citrus iyo (15-year-old trees on trifoliate orange rootstock) orchard in Matsuyama city, Ehime prefecture. The experimental plots were as follows: 1) charcoal as a soil amendment, 2) abandoned culture, 3) sod culture with Bahia grass, and 4) clean culture by using herbicides such as paraquat dichloride and N-(phosphonomethyl) glycine (3 times in a year). The charcoal plot had two pits (60 × 60 cm in width and 40 cm in depth) circling a tree and filled with 6 kg of rice husk charcoal. Paraquat dichloride was applied once annually. In the Bahia grass plot, the grass was mowed once each summer. Except for the abandoned plot, the rest received 32 kg N, 23 kg P, and 25 kg K per 10 a annually. The application of agrochemicals, such as fungicides and pesticides, followed the guidelines of disease and pest control for Ehime prefecture.

In early September of 1988, root samples were obtained from 3 to 5 places of each plot at a depth of 5–10 cm, and then the intensity of VAM infection was determined by the methods described above. The root structure was observed with a scanning electron microscope (SEM, JEOL type JSM-T200, Tokyo, Japan). The apical 20 mm of 20 elongating roots from each plot were rinsed with distilled water for a few seconds and the apices were immediately fixed in Karnovsky solution (Karnovsky, 1965) at room temperature for 24 hr. After being dehydrated through graded solutions of ethyl alcohol-acetone, they were divided into 4 segments in 100% acetone. These segments were then immersed in acetic acid for 2 hr, critical-point-dried, mounted on aluminum stubs with silver conducting paint, and coated with a thin layer of gold using an ion-coater (Eiko Engineering type IB-2, Tokyo, Japan). The roots were observed in a SEM and photographed.

Results

Experiment 1.

No differences in soil pH among treatments were observed. The EC value of the charcoal treatments was higher than that of the control. The EC in the western spruce bark charcoal treatment was about 13 times higher than that of the control plot (Table 1). This is because NaCl permeated into the bark during sea storage after being imported into Japan from North America.

Soils treated with 3 kinds of charcoal had significant effects on growth, leaf P concentration, and VAM development in roots of satsuma mandarin trees. About 2 months after the onset of this experiment, except in the western spruce bark charcoal treatment, roots appeared on the glass plates, and their elongation rates indicated that roots in the charcoal-treated plots were more vigorous than ones in the control. As of November 8, 1989, the root length in any charcoal treatment was about 1.5 times longer than that in the control. The total fresh weights and the fresh weights of roots and shoots increased with charcoal treatments. The growth increments varied little among the kinds of charcoal (Table 2). The intensity of VAM infection in any charcoal treatment was higher than that in the control; that of the rice husk charcoal treatment attaining 41.5% (Table 3). Hardly any significant differences in leaf P concentration among treatments with western spruce bark charcoal, citrus juice sediments charcoal and the control were observed; but leaf P concentration in the rice husk charcoal treatment, which significantly stimulated VAM infection, was higher than that in the control (Table 3).

Experiment 2.

The intensity of VAM infection in the rice husk charcoal plot was 52%, the highest among plots. The intensity in the Bahia grass sod plot was second highest, whereas that of the abandoned plot was third. The lowest percentage of VAM infection was in the clean culture plot where herbicides were used 3 times a year (Table 4). The hyphae, vesicles and arbuscules of VAM fungi were fre-

Table 1. The pH and electric conductivity (EC) of soils treated with charcoal (Experiment 1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH (H$_2$O)</th>
<th>EC ($\mu$S/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (without charcoal)</td>
<td>6.4</td>
<td>14</td>
</tr>
<tr>
<td>Rice husk charcoal</td>
<td>6.5</td>
<td>33</td>
</tr>
<tr>
<td>Western spruce bark charcoal</td>
<td>7.0</td>
<td>180</td>
</tr>
<tr>
<td>Citrus juice sediment charcoal</td>
<td>6.6</td>
<td>105</td>
</tr>
</tbody>
</table>
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Quently observed on/in citrus roots sampled from the charcoal-treated plots (Fig. 1). The SEM photomicrographs also indicated that in the charcoal-treated plot there were many sites where VAM fungi infected and penetrated into the root (Figs. 2 and 3).

Discussion

In Japan, it has long been known that charcoal is a very effective soil conditioner which promotes plant growth. Charcoal application may result in improving physical properties of soil, its fertility, and biological conditions. The present experiment indicated that citrus growth and VAM development in the root were stimulated by applying charcoal to soil. This stimulation of citrus growth by charcoal is attributed to an increase in the percentage of VAM infection in the roots. Ogawa (1987) also reported that the enhanced colonization by symbiotic microorganisms, such as Rhizobium and VAM fungi, by charcoal application, invigorated soybean plants.

Table 2. Effect of charcoal application on the growth of satsuma mandarin trees (Experiment 1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total fresh weight (g)</th>
<th>Root fresh weight (g)</th>
<th>Shoot fresh weight (g)</th>
<th>The length of roots observed on the glass plate of root box (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>713±55</td>
<td>284±4</td>
<td>102±19</td>
<td>3.1±0.0 8.4±0.2 12.3±2.0</td>
</tr>
<tr>
<td>Rice husk charcoal</td>
<td>1123±111</td>
<td>507±34</td>
<td>177±28</td>
<td>5.7±0.3 10.1±0.2 18.3±1.1</td>
</tr>
<tr>
<td>Western spruce bark charcoal</td>
<td>1091±50</td>
<td>425±21</td>
<td>250±26</td>
<td>2.7±0.1 10.4±1.0 17.1±0.4</td>
</tr>
<tr>
<td>Citrus juice sediment charcoal</td>
<td>1150±39</td>
<td>511±37</td>
<td>208±19</td>
<td>3.5±0.7 10.7±0.8 19.4±0.3</td>
</tr>
</tbody>
</table>

2 Mean±standard error (SE). (n=3)

Table 3. Effect of charcoal application on vesicular-arbuscular mycorrhizal (VAM) development and leaf phosphorus (P) concentration in satsuma mandarin trees (Experiment 1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>VAM infection (%)</th>
<th>Leaf P (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.4±1.3</td>
<td>0.190±0.013</td>
</tr>
<tr>
<td>Rice husk charcoal</td>
<td>41.5±2.5</td>
<td>0.244±0.004</td>
</tr>
<tr>
<td>Western spruce bark charcoal</td>
<td>11.2±0.2</td>
<td>0.188±0.025</td>
</tr>
<tr>
<td>Citrus juice sediment charcoal</td>
<td>12.0±1.2</td>
<td>0.219±0.037</td>
</tr>
</tbody>
</table>

2 Mean±SE. (n=3)

Table 4. Effect of soil management on VAM development in Citrus iyo trees (Experiment 2).

<table>
<thead>
<tr>
<th>Kind of soil management</th>
<th>VAM infection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clean culture</td>
<td>3.6a</td>
</tr>
<tr>
<td>Abandoned culture</td>
<td>7.3b</td>
</tr>
<tr>
<td>Sod culture</td>
<td>16.9c</td>
</tr>
<tr>
<td>Charcoal (rice husk) application</td>
<td>52.0d</td>
</tr>
</tbody>
</table>

2 Duncan's multiple range test at 5% level.

Fig. 1. Photomicrograph of VAM fungal structures in Citrus iyo roots stained with typan blue. a: fungal hyphae (×100), b: vesicle (×150), c: arbuscle (×600).
The increased VAM infections by charcoal application may be because charcoal absorbs many kinds of toxic substances and agrochemicals which inhibit root growth and microbial activity. It has also been shown that some agrochemicals inhibit the germination of VAM spores (Kobayashi, 1988; Ogawa, 1987). The growth inhibition of VAM fungi by fungicides such as thiophanate methyl, benomyl, iprodione, and copper fungicides is severe. In the case of herbicides, Kobayashi (1988) showed that the germination of Gigaspora margarita spores was severely repressed by 48 ppm paraquat dichloride or 410 ppm N-(phosphonomethyl) glycine. In our experiment, the percentage of VAM infection in the herbicide-treated clean culture plot is lower than that of the abandoned plot.

The pH value of water extracts from charcoal was high (Ishii and Kadoya, 1990), indicating that charcoal ameliorated soil acidity. Generally, soil pH is low in citrus orchards in Japan, so that the percentage of VAM infection in the root is low and the number of VAM spores in the soil is small (Ishii et al., 1989b, 1992a). By neutralizing soil acidity, charcoal may be improving the growth and development of VAM fungi.

There are very few reports on VAM development in citrus trees grown in Japan. When roots of satsuma mandarin and Citrus iyo trees from 24 orchards in Ehime prefecture (in southwestern Japan) were observed for VAM infections, they were not extensive except for an orchard with good soil conditions which produced 9–10 t satsuma mandarin fruit per 10a every year (Ishii et al., 1989b). Numerous VAM spores in the soil and VAM-infected plants are generally observed in woodlands and non-cultivated fields. This indicates that there are many factors which restrict...
the existence and growth of VAM fungi in orchards because of our present soil management practices, such as the usage of agrochemicals and chemical fertilizers. The average annual amount of P applied is about 20 kg per 10a. That P, especially soluble P, is detrimental to VAM development in citrus roots was reported earlier (Antunes and Cardoso, 1991; Graham and Timmer, 1985). Several kinds of VAM fungi, however, live in our soil in spite of many malpractices in our present soil management (Ishii et al., 1992a). We suggested that VAM formation in citrus roots could be effectively increased through application of charcoal to the soil or introduction of a sod culture system. In particular, the application of charcoal is very effective for VAM development. Contrarily, an excess of charcoal inhibits citrus growth (Ishii and Kadoya, 1990). This inhibition by an excessive application of charcoal might be concerned with an increment of soil pH value. Therefore, an appropriate amount of charcoal to be applied is less than 2 t per 10a (this is approximately equivalent to 2% charcoal, Table 2). Furthermore, such an effect of charcoal may be strengthened by mixing charcoal and soil.

VAM fungi develop well in citrus orchards where Bahia grass is used for sod (Ishii et al., 1993). We have also indicated that the intensity of VAM formation on some weeds grown in citrus orchards was higher than that on citrus trees (Ishii et al., 1989a). However, sod culture in commercial citrus orchards has been unsuccessful in Japan; most citrus growers believe that a clean culture is best for the production of high-quality fruits. Thus, our soil management system must be re-evaluated.

The prevailing cultural system in which large quantities of agrochemical and chemical fertilizers are used, should be thoroughly revamped so that a cultural system which maintains beneficial soil microorganisms is adopted. In conclusion, any application of charcoal to the soil is a practical method to improve soil properties and to foster the development of symbiotic microorganisms including VAM fungi.

Literature Cited


炭施用がカンキツの樹体生長およびVA菌根形成に及ぼす影響

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摘 要

炭施用がカンキツの樹体生長およびVA菌根形成に及ぼす影響を調査した。イネもみがら、ベイガサキ枝あるいはカンキツジュースから作られた炭で処理した土壌を用いて、ルートボックスにウンシュウミカン（カラダチ台）樹を移植し、これに*Glomus fasciculatum*の菌根を接種した。その結果、ボックスのガラス面に観察される根の伸長は、いずれの炭施用区においても対照（炭無施用）区より旺盛であった。全生体重、下降部重および新梢重も炭施用区で増大した。VA菌根形成は対照区よりも炭施用区で良好であり、特にイネもみがら炭ではその感染率が41.5％と著しく高く、また葉内のリン含量も増加した。一方、宮内イヨカン園における炭（イネもみがら）施用区、バピアグラス草生区、放任区および慣行裸地（除草剤年3回使用）区のVA菌根形成を比較調査したところ、VA菌根菌の感染率は炭施用区（52.0％）、バピアグラス草生区（16.9％）、放任区（7.3％）、慣行裸地区（3.6％）の順であった。