Effect of Constant and Diurnally Fluctuating Temperatures on Arbuscular Mycorrhizal Fungus Infection and the Growth of Infected Asparagus Seedlings

Yoh-ichi Matsubara and Takashi Harada
Faculty of Agriculture, Hokkaido University, Sapporo 060

Summary

Effect of constant and diurnally fluctuating temperatures on arbuscular mycorrhizal (AM) fungi [Glomus etunicatum (GE) and Gigaspora margarita (GM)] infection and the promotion of infected seedling growth through asparagus-AM fungus symbiosis were investigated.

1. At constant temperatures, spore germination rate became maximum at 25 Ž in GE, and at 25 ° and 30 °C in GM. Hyphal growth was enhanced at 20 °, 25 ° and 30 °C in GE, and at 25 °, 30 ° and 35 °C in GM.

2. Under constant bed soil of 20 °, 25 ° and 30 °C for 8 weeks after inoculation, there was no growth enhancement at 20 °C regardless of the AM fungus species; whereas at 25 ° and 30 °C, the GE- and GM-inoculated plants were taller than the noninoculated plants. At 30 °C, the GM-inoculated plants were taller than those inoculated with GE. At 25 °C, the GE-or GM-inoculated plants had the most shoots and storage roots than did those at other temperatures. The rate of AM fungus-infected portions in a whole root system (as percentage of the total portions infected with AM fungus in a whole root system) was maximum at 25 °C in GE-inoculated plants but at 25 ° and 30 °C in GM-inoculated ones.

3. Under the bed soil temperature conditions diurnally fluctuating in the range of 25 °C to 15 °C and 30 °C to 18 °C, 8 weeks after inoculation, the plants were taller and had more numerous shoots and storage roots in GE-inoculated plants than in GM-inoculated ones in a regime between 25 °C and 15 °C; the reverse was noted in a regime between 30 °C and 18 °C. Little difference in the rate of AM fungus-infected portions in a whole root system was recognized between the two temperature regimes in GE-inoculated plants, whereas in GM-inoculated ones, the rate became higher at the temperature fluctuated between 30 °C and 18 °C than it did at the temperature fluctuated between 25 °C and 15 °C.

Consequently, it seems that in asparagus, the optimum temperature range for infection and the plant growth enhancement through symbiosis differed between Glomus etunicatum and Gigaspora margarita.

Introduction

Infection of arbuscular mycorrhizal (AM) fungus promotes the growth of host plants mainly by enhancing phosphorus uptake through host-fungus symbiosis which was established by symbiotic organ formation in the roots. We previously reported that growth enhancement by symbiosis occurs in asparagus, and that such enhancement might be useful for growing vigorous seedlings and for shortening the seedling-raising period in asparagus cultivation (Matsubara et al., 1994).

To use AM fungus in raising asparagus seedlings, environmental conditions in the seedling-raising period are considered an important factor in inducing AM fungus infection in roots and enhancing growth. Environmental conditions, especially temperature, affect the AM fungus infection in
host roots and the plant growth promotion of the fungus (Hayman, 1974; Raju et al., 1990). However, the optimum temperatures for AM fungus infection in host roots and for the promotion of plant growth have not been established for AM fungus species.

In this study, we investigated the effect of constant and diurnally fluctuating temperatures on the AM fungus infection and on the promotion of asparagus seedling growth.

Materials and Methods

1. Effect of temperatures on spore germination and hyphal growth of AM fungus

Spores of AM fungus [Glomus etunicatum (GE) and Gigaspora margarita (GM)] were surface-sterilized in sodium hypochlorite solution (active chlorine, 1%) for 2 min, rinsed three times in sterile deionized water; then immersed 200 ppm streptomycin and 100 ppm gentamycin for 30 min, and rinsed again three times. The spores were transferred to a multiple well plate (well size: 22.1 mm in diameter and 17.6 mm in depth); each well contained 4 ml of sterile deionized water (pH 6.5). Spores were incubated at constant temperatures of 5°C, 10°C, 15°C, 20°C, 25°C, 30°C and 3°C in the dark. Five replications of 15 spores each were made at each temperature. After 2 weeks of incubation, spore germination rate and average length of main hyphae (excluding branched hyphae) of germinated spores were measured.

2. Effect of temperatures on AM fungus infection and infected seedling growth

AM fungus inoculation

Seeds of asparagus (Asparagus officinalis L., cv. MW500W) were germinated on moistened filter paper in a Petri dish (11 cm in diameter). Ten-day-old seedlings with radicles approx. 20-mm long were inoculated with spores of GE or GM at densities of 1000 spores per gram of inoculum (GE) and 100 spores per gram of inoculum (GM). The inoculum is a sticky mixture consisting of spores, water, and a medium containing powdered vermiculite in GE or peat-moss and zeolite in GM.

Sieved soil [pH 6.5-7.0 (H2O); available-P content, 58.8 mg/100 g dry soil] was autoclaved at 1.2 kg·cm⁻² and 121°C for one hour to eliminate other indigenous microbes, and packed in a plastic vat [46 cm×30 cm×16 cm (H)] into which the GE- or GM-inoculated plants were transplanted.

Growing seedlings

The GE or GM-inoculated plants were raised for 8 weeks in a growth chamber under: (1) constant bed soil temperatures at 20°C, 25°C and 30°C ± 0.5°C (12,000 lx, 13-hr daylength, 55-60% RH; air temperatures approximated bed soil temperatures); (2) diurnally fluctuating bed soil temperatures in 2 different ranges of 25°C ± 2°C to 15°C ± 2°C and 30°C ± 2°C to 18°C ± 2°C (natural light, approx. 13-hr daylength and 55-65% RH; air temperatures fluctuated approximately in the same ranges as bed soil temperatures in the greenhouse). Plants were watered adequately but not fertilized.

Evaluation of rate of AM fungus-infected portions in a whole root system

The rate of AM fungus-infected portions in a whole root system (abbreviated RFIPR is expressed as the percentage of total AM fungus-infected portions in a whole root system) was evaluated 8 weeks after inoculation by the method of Giovannetti and Mosse (1979).

Results

1. Effect of constant temperatures on spore germination and hyphal growth in AM fungus

The spore germination rate became maximum at 25°C in GE, and at 25°C and 30°C in GM (Fig. 1). Average length of the main hyphae was long at 20°C, 25°C and 30°C in GE, and at 25°C, 30°C and 35°C in GM in the dark. Five replications of 15 spores each were made at each temperature. After 2 weeks of incubation, spore germination rate and average length of main hyphae (excluding branched hyphae) of germinated spores were measured.

2. Effect of constant temperatures on AM fungus infection and infected seedling growth

Regardless of the species, no growth enhancement appeared at 20°C (Fig. 3), but at 25°C and 30°C, the GE- or GM-inoculated plants were taller than the noninoculated plants. At 25°C, plants infected by both species grew equally well but at 30°C, plants in GM-plots grew taller than did those in GE-plots. The maximum numbers of shoots and storage roots were attained at 25°C (Table 1). RFIPR reached maximum at 25°C in GE-inoculated plants, and at 25°C and 30°C in GM-inoculated ones. RFIPR was higher in GE-inoculated
plants than in GM-inoculated ones at 20 °C but the relation reversed at 30 °C. At 25 °C, the 2 fungal species showed the similar RFIPR.

3. Effect of diurnally fluctuating temperatures on AM fungus infection and infected seedling growth

Eight weeks after inoculation, at both temperature regimes, the GE-and GM-inoculated plants were taller than the noninoculated plants. At the fluctuating temperature between 25 °C and 15 °C, GE enhanced the plant growth more greatly than did GM, but in a fluctuation range of 30 °C to 18 °C, the degree of the efficacy reversed between the 2 fungus (Fig. 4). The growth of noninoculated plants differed little between the two temperature regimes (Table 2). Shoots and storage roots proliferated more in GE-inoculated plants than in GM-inoculated ones at the temperature fluctuated between 25 °C and 15 °C but the reverse occurred at that fluctuated between 30 °C and 18 °C.

In GE, RFIPR differed little between the two temperature regimes, whereas in GM, it was higher at the temperature fluctuated between 30 °C and 18 °C than at that fluctuated between 25 °C and 15 °C.

Discussion

It is reported that the optimum constant temperatures for spore germination are 20 °C–25 °C in *Glomus mosseae* (Schenck and Green, 1975), 28 °C in *Glomus intraradices* (Haugen and Smith, 1992), and 34 °C in both *Gigaspora coralloidea* and *Gigaspora heterogama* (Schenck, 1975). In this experiment, the optimum constant temperatures for spore
Germination are 25 °C in GE, but 25 ° and 30 °C in GM. Likewise, the optimum temperatures for hyphal growth in both the fungi coincided with those of spore germination. Spore germination and hyphal growth were promoted at high temperatures (30 ° and 35 °C) in GM, but not in GE. These results indicate that the optimum temperatures for spore germination and hyphal growth differ between the AM fungus species.

RFIPR was maximum at 25 °C in GE-inoculated plants, but at 25 ° and 30 °C in GM-inoculated ones. Hayman (1974), and Daniels and Bloom (1984) mentioned that at constant 10 ° and 15 °C, the number of arbuscules decreased resulting in low growth enhancement. Raju et al. (1990) reported that mineral uptake was not promoted in the infected plants kept at 20 °C. We observed no growth enhancement, a decrease in the number of arbuscules, and no promotion of mineral uptake in plants infected with GE or GM and kept at 20 °C (data not shown).

Takagi (1986) reported that the optimum diurnally fluctuating temperature for asparagus plant growth in field is the range of 24 °C to 15 °C. Our data show that growth enhancement was improved by the diurnally fluctuating temperatures. In our study, the levels of AM fungus infection and plant growth enhancement were similar with the 2 fluctuating temperature ranges of 25 ° to 15 ° and 30 ° to 18 °C in GE, but in GM they were higher in the latter temperature than in the former one. These results suggest that in GE, a range of the diurnally fluctuating temperature might not noticeably influence the AM fungus infection level and plant growth enhancement. In GM, a slightly higher temperature than the optimum temperature for asparagus growth is favorable for the infection and the promotion of plant growth. Schenck and Schroder (1974) reported that in soybean, the

<table>
<thead>
<tr>
<th>Bed soil temperature</th>
<th>AM fungus inoculation</th>
<th>No. of shoots</th>
<th>Diameter of shoots</th>
<th>Dry weight of shoots</th>
<th>No. of storage roots</th>
<th>Diameter of storage roots</th>
<th>Dry weight of roots</th>
<th>RFIPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>20°C</td>
<td>None</td>
<td>5.3 ± 0.8</td>
<td>0.88 ± 0.10</td>
<td>0.62</td>
<td>6.1 ± 2.6</td>
<td>1.85 ± 0.12</td>
<td>0.56</td>
<td>0</td>
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<td>GE</td>
<td>4.9 ± 0.8</td>
<td>1.12 ± 0.11</td>
<td>0.59</td>
<td>7.3 ± 1.8</td>
<td>1.88 ± 0.10</td>
<td>0.62</td>
<td>26.8 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>GM</td>
<td>5.0 ± 1.0</td>
<td>1.03 ± 0.17</td>
<td>0.63</td>
<td>8.8 ± 2.2</td>
<td>1.78 ± 0.10</td>
<td>0.68</td>
<td>20.2 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>25°C</td>
<td>None</td>
<td>6.8 ± 1.1</td>
<td>1.03 ± 0.11</td>
<td>0.95</td>
<td>6.7 ± 1.3</td>
<td>1.92 ± 0.17</td>
<td>0.65</td>
<td>0</td>
</tr>
<tr>
<td>GE</td>
<td>10.2 ± 1.2</td>
<td>1.21 ± 0.13</td>
<td>1.52</td>
<td>11.8 ± 1.5</td>
<td>2.01 ± 0.10</td>
<td>0.93</td>
<td>48.8 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>GM</td>
<td>9.5 ± 2.1</td>
<td>1.15 ± 0.10</td>
<td>1.63</td>
<td>10.0 ± 2.1</td>
<td>1.98 ± 0.12</td>
<td>0.89</td>
<td>54.0 ± 2.4</td>
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</tr>
<tr>
<td>30°C</td>
<td>None</td>
<td>6.2 ± 1.1</td>
<td>0.82 ± 0.10</td>
<td>0.60</td>
<td>5.5 ± 1.1</td>
<td>1.82 ± 0.11</td>
<td>0.51</td>
<td>0</td>
</tr>
<tr>
<td>GE</td>
<td>8.3 ± 0.8</td>
<td>0.95 ± 0.10</td>
<td>1.11</td>
<td>8.4 ± 1.3</td>
<td>1.91 ± 0.12</td>
<td>0.61</td>
<td>30.1 ± 2.2</td>
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</tr>
<tr>
<td>GM</td>
<td>6.5 ± 0.8</td>
<td>0.93 ± 0.15</td>
<td>0.95</td>
<td>8.5 ± 1.1</td>
<td>1.85 ± 0.11</td>
<td>0.68</td>
<td>50.2 ± 2.1</td>
<td></td>
</tr>
</tbody>
</table>

* Data were obtained from 10 plants at 8 weeks after inoculation.
* 55-60% RH, 12,000lx and 13-hr daylength. Air temperatures approximated to bed soil temperatures (±0.5°C).
* None, noninoculated; GE, inoculated with *Glomus etunicatum* (1000 spores/g inoculum); GM, inoculated with *Gigaspora margarita* (100 spores/g inoculum).
* Mean ± SE
* Rate of AM fungus-infected portions in a whole root system (evaluated by the gridline intersect method).

![Figure 4](image-url)
optimum temperature for rapid increase of dry weight of the AM fungus (species unknown)-infected plants was higher than that of the noninoculated plants. This fact agrees with our findings on asparagus plants establishing symbiosis with GM. From these findings, it is suggested that the optimum temperatures for AM fungus infection and the promotion of asparagus plant growth through symbiosis differ between AM fungus species.

### Literature Cited


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**Table 2.** Effect of diurnally fluctuating temperatures on AM fungus infection and infected seedling growth in *Asparagus officinalis* L. (cv. MW500W) .

<table>
<thead>
<tr>
<th>Bed soil temperature</th>
<th>AM fungus inoculation</th>
<th>No. of shoots</th>
<th>Diameter of shoots</th>
<th>Dry weight of shoots</th>
<th>No. of storage roots</th>
<th>Diameter of storage roots</th>
<th>Dry weight of roots</th>
<th>RFIPR</th>
</tr>
</thead>
<tbody>
<tr>
<td>25°C-15°C</td>
<td>None</td>
<td>4.2 ± 0.5</td>
<td>1.05 ± 0.12</td>
<td>0.60</td>
<td>8.5 ± 1.8</td>
<td>2.25 ± 0.11</td>
<td>0.81</td>
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<td></td>
<td>GE</td>
<td>7.2 ± 0.6</td>
<td>1.28 ± 0.11</td>
<td>1.27</td>
<td>15.2 ± 1.5</td>
<td>2.42 ± 0.15</td>
<td>1.42</td>
<td>30.2 ± 3.3</td>
</tr>
<tr>
<td></td>
<td>GM</td>
<td>5.8 ± 0.4</td>
<td>1.08 ± 0.11</td>
<td>1.02</td>
<td>10.7 ± 1.5</td>
<td>2.29 ± 0.13</td>
<td>1.08</td>
<td>35.5 ± 4.0</td>
</tr>
<tr>
<td>30°C-18°C</td>
<td>None</td>
<td>5.0 ± 0.6</td>
<td>1.07 ± 0.10</td>
<td>0.71</td>
<td>9.3 ± 1.3</td>
<td>2.18 ± 0.13</td>
<td>0.97</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>GE</td>
<td>6.8 ± 0.5</td>
<td>1.30 ± 0.11</td>
<td>1.34</td>
<td>13.5 ± 1.5</td>
<td>2.70 ± 0.10</td>
<td>1.51</td>
<td>35.2 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>GM</td>
<td>8.2 ± 0.6</td>
<td>1.41 ± 0.11</td>
<td>1.52</td>
<td>18.0 ± 1.4</td>
<td>2.51 ± 0.12</td>
<td>1.73</td>
<td>47.5 ± 3.1</td>
</tr>
</tbody>
</table>

Data were obtained from 10 plants at 8 weeks after inoculation.

Fluctuated diurnally in the range described (±2°C), 55-65% RH, natural light and daylength (approx. 13hr).

Air temperatures fluctuated approximately in the same ranges as bed soil temperatures.

None, noninoculated; GE, inoculated with *Glomus etunicatum* (1000 spores/g inoculum); GM, inoculated with *Gigaspora margarita* (100 spores/g inoculum).

Mean±SE

Rate of AM fungus-infected portions in a whole root system (evaluated by the gridline intersect method).
アスパラガス実生における arbuscular 菌根菌の感染および感染実生の生長に及ぼす恒温ならびに変温の影響

松原陽一・原田 隆
北海道大学農学部 060 札幌市北区北 9 条西 9 丁目

摘 要

アスパラガス (Asparagus officinalis L., cv. MW500W) 実生における arbuscular 菌根（AM）菌 [Glomus etunicatum (GE) および Gigaspora margarita (GM)] の感染および感染実生の生長に及ぼす温度の影響について調査した。

1. AM 菌胞子の発芽率は、GE では 25 ℃で最大となり、GM では 25 ℃および 30 ℃で最大となった。菌糸の伸長は、GE では 20 ℃、25 ℃および 30 ℃で良好であり、GM では 25 ℃、30 ℃および 35 ℃で良好であった。

2. 恒温条件（床土の温度: 20 ℃、25 ℃および 30 ℃）下における接種 8 週間後の結果についてみると次のとおりである。菌種にかかわらず 20 ℃区では生長促進効果が現れなかった。25 ℃および 30 ℃の両区においては、GE または GM 接種区の草丈は無接種区のそれより高くなり、また、30 ℃では GM 接種区で GE 接種区より草丈が高くなった。GE または GM 接種による茎数および貯蔵根数の増加は 25 ℃において最大となった。