Dry Powder and Extract of *Posidonia australis* Hook. F., a species of Seagrass, Stimulate the Germination of the Pathogen *Plasmodiophora brassicae* and Control Clubroot of Chinese Cabbage

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Summary

The germination of spores of *Plasmodiophora brassicae* was stimulated by the treatment with the dry powder and extract of *Posidonia australis*, a species of Seagrass, collected from the coast of southern Australia. The formation of clubroots in Chinese cabbage was prevented by a treatment with the dry powder and extract, attributed to the stimulation of the germination of *Plasmodiophora* spores, indicating that the preparations are useful to control clubroot.

Key Words: Chinese cabbage, clubroot, germination, *Plasmodiophora brassicae*, *Posidonia australis*.

Introduction

*Plasmodiophora brassicae* Wor., a parasite which causes the formation of clubroots, is one of the most economically harmful soil-borne pathogen of cultivated crucifers (Fujita, 1994). The yields of crucifer crops such as cabbage, Chinese cabbage, broccoli, cauliflower, tskena, turnip, and oil rapeseed have been reduced or completely lost by *Plasmodiophora*. The breeding of resistant cultivars, liming, crop rotation, and the application of fungicides are strategies recommended for controlling this disease, but all have limitations.

Throughout the life cycle of the pathogen, the resting spore and primary zoospore are the only stages that have been proven to be independent of host tissue. It has not been clearly confirmed yet whether or not the secondary zoospore is released outside of the host tissue (Suzuki et al., 1992). The resting spores of clubroot fungus are produced in the clubroots and can survive several years in the soil. On the other hand, primary zoospores, released after germination of the resting spores, are the primary source of infection has been established; they are short-lived without hosts and to be sensitive to fungicidal agents.

*Posidonia* leaves, accumulated on the beach, have been traditionally used as a fertilizer in Sicily, and as cattle fodder during the war (Augier, 1982). Cariello and Zanetti (1979) reported that the root extract of *Posidonia oceanica* in the Bay of Naples has a growth-stimulating activity for *Staphylococcus aureus* Rosenbach.

Posidonia australis* Hook. F. is distributed along the coast of southern Australia. Our preliminary experiment showed that the formation of the clubroot of Chinese cabbage was decreased when *Plasmodiophora*-infected soil was treated with the leaf powder of *Posidonia australis*.

We report that the dry powder and extract of *Posidonia* stimulated the germination of *Plasmodiophora* spores and showed a possible usefulness for the control of clubroot.

Materials and Methods


The spores of *Plasmodiophora* were collected from clubroots, according to the method of Williams (1966). Severely infested clubroots of Chinese cabbage ‘Kukai’ (Takii seed) were collected in August 1997 from Nobeyama field in Nagano Prefecture, Japan, washed in tap water and stored at −20 °C until use. The clubroots were crushed and ground with a mixer, and the homogenate filtered through a 50 μm nylon mesh; the filtrate, the suspension of the resting spores, was adjusted to the density of 1 × 10⁷ spores·mL⁻¹ with sterilized water.

Enough dry powdered seagrass of *Posidonia* was added to 1 × 10⁷ spores·mL⁻¹ suspension of *Plasmodiophora* spores to bring the mixture to a 0.5 or 5.0% *Posidonia* medium. The mixture was incubated on a rotary shaking at 25 °C and 60 rpm for 7 days in the dark; the germination rate of spores was then recorded.
staining of chitin. Germinated and ungerminated spores were counted under UV excitation with a fluorescent microscope (Olympus BHS-RFC). The stages of germination were defined as follows: 0 for the presence of developed cell wall, 1 for the presence of both cell wall and budding cave, 2 for the disappearance of cell wall and clear development of budding cave, and 3 for the disappearance of both cell wall and budding cave, and appearance of the dark red pigment (Fig. 1).

2. Effect of the dry Posidonia powder on the clubroot formation in Chinese cabbage

1) Experiment 1:
A potting mixture of loam soil sterilized at 121 °C for 30 min. and sand (1:1, v/v; pH 5.8), was sprayed with the diluted suspension of the Plasmodiophora spores (1.4 × 10^5 ml⁻¹), incubated for 7 days, and mixed above at a rate of 7 ml per 100 g soil (1 × 10^4 spores · g⁻¹ soil). Eight seeds of Chinese cabbage ‘Muso’ were sown per Jiffy pots filled with infected soil; seedlings were thinned to five plants per pot after 10 days and grown at 25 °C under a 16–hr photo period of 3000 lx. The plants were fertilized weekly the first two weeks after sowing with 15–15–18 (N:P₂O₅:K₂O) soluble fertilizer (4 g · liter⁻¹, 40 ml/pot). Sodium molybdate (0.25 g · liter⁻¹, 40 ml/pot) was applied at the time of the first fertilizing. On the 35th day after sowing, the plants were harvested and examined for the number, length, and fresh weight of leaves. After the soil was washed away from the roots, the weight and length of longest root and the clubroot severity were recorded. The degree of the formation of clubroots was classified on a scale of 0–3 according to Buczack and Moxham (1983): 0 for no clubroots, 1 for slight swelling on taproots and/or lateral roots, 2 for moderate swelling on taproots and/or lateral roots, and 3 for severe swelling on taproots and/or lateral roots.

2) Experiment 2:
The above potting mixture was sprayed with a dilute suspension of the fresh resting spores (1.4 × 10^6/ml) at the rate of 7 ml per 100 g soil (1 × 10^6 spores · g⁻¹ soil). The infected soil was brought to 2 and 5% with the dry Posidonia powder and seeded with Chinese cabbage ‘Muso’. In one treatment, the infected soil was amended with the dry Posidonia powder at planting day; in the other plot the soil was amended 7 days before planting. In the controls, no Posidonia powder was added on planting day or 7 days before planting. On the 79th day after planting, the twenty plants of each treatments were harvested, and the clubroot severity was examined. The degree of the clubroot severity was calculated by the following formula:
The degree of the clubroot severity =
\[
\frac{\text{Number of plants with each degree of clubroot} \times \text{degree numbers of clubroot}}{\text{Total number of plants} \times 3}
\]
3. Effect of the extract of dry Posidonia on in vitro germination of Plasmodiophora spores

4 kg of dry Posidonia were extracted with 40 liter of 90% methanol and concentrated up to 8.5 liter at 60°C. The concentrate was then partitioned with 1 liter of hexane (hexane fraction), and the aqueous–methanol fraction further concentrated to 1.8 liters. The entire residual concentrate was loaded on 400 ml of HP20 resin (Mitsubishi Chemical) and the adsorbed fraction was eluted with 800 ml of methanol. The eluate was concentrated and dried. Five ml of extract redissolved in methanol was column and eluted successively with 200 ml of the following solvent fractions: chloroform (fractions 1 to 3), 4:1(v/v) mixture of chloroform:ethyl acetate (fraction 4), 1:1(v/v) mixture of chloroform:ethyl acetate (fraction 5), 9:1(v/v) mixture of ethyl acetate:acetone (fraction 6), 1:1(v/v) mixture of ethyl acetate:acetone (fraction 7), acetone (fraction 8), 4:1(v/v) mixture of acetone:methanol (fraction 9), 1:1(v/v) mixture of acetone:methanol (fraction 10) and methanol (fraction 11). Each fraction was concentrated and examined for its effect on the germination of Plasmodiophora spores. The concentration of each extract was equivalent to 0.5% of the dry powder.

Results

1. Effect of the dry Posidonia powder on in vitro germination of Plasmodiophora spores

The stained cell wall layer of the resting spores showed intense light–blue fluorescence (germination stage 0 in Fig. 1). The germination stage of resting spores without any treatment remained 0 without exception, while most of the spores incubated in the root exudate solution of Chinese cabbage attained the stage 3 (Table 1). In the treatment with 0.5% and 5% dry Posidonia powder, most of the spores proceeded to the stage 2 or 3, whereas with 1 mM arylisothiocyanate and in a 1 mM solution of flusulfamide, most of the spores were in the stage 0.

2. Effect of the dry Posidonia powder on the clubroot formation in Chinese cabbage

1) Experiment 1:

The degree of formation of clubroots on the soil with 5% dry Posidonia powder was 0 (Table 2, Fig. 2a), but that on the soil without the dry powder was 3 (Table 2, Fig. 2b). The effects of the dry powder on the number of leaves and their length and weight were not clear (Table 2). However, the roots treated with the dry powder were longer and lighter than those without treatment. This experiment was replicated three times and yielded similar results.

2) Experiment 2:

The degree of the clubroot severity in the treatment with 5% dry Posidonia powder at planting was higher than in the control, whereas in soils amended 7 days before planting, less infection occurred than in the control (Table 3). No significant difference was observed between the treatment with 2% dry powder and the control.

<table>
<thead>
<tr>
<th>Table 1. Effect of Posidonia powder, root exudate of Chinese cabbage, arylisothiocyanate and flusulfamide on the germination of Plasmodiophora spores.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Not added</td>
</tr>
<tr>
<td>Root exudate solution</td>
</tr>
<tr>
<td>5% Posidonia powder</td>
</tr>
<tr>
<td>0.5% Posidonia powder</td>
</tr>
<tr>
<td>1 mM arylisothiocyanate</td>
</tr>
<tr>
<td>1 mM flusulfamide</td>
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</tbody>
</table>

†Stage of germination, see Fig. 1.

<table>
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<th>Table 2. Effect of Posidonia powder on growth and clubroot formation of Chinese cabbage seedlings.</th>
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</thead>
<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Not added</td>
</tr>
<tr>
<td>5% Posidonia powder</td>
</tr>
</tbody>
</table>

†Standard deviation of the mean (n=5).
3. Effect of the extract of Posidonia on the germination of Plasmodiophora

An apparent variation of frequency distributions of spores with different stages of germination was observed among the treatments with different fractions of extract from Posidonia (Fig. 3). In the treatment with fractions 3 and 4, about 40% of the spores reached the germination stage 2 or 3.

Discussion

We demonstrated that the dry powder and methanol extract of Posidonia stimulated germination of spores of Plasmodiophora brassicae and showed a possibility to control the formation of clubroots in Chinese cabbage. Treating the infected soil with 5% dry Posidonia powder 7 days before planting reduced significantly the clubroot formation in Chinese cabbage. However, the treatment at planting day resulted in greater clubroot formation than that of the control. This difference might be due to the nature of pathogen zoosporae. Suzuki et al. (1992) reported that the resting spores of the pathogen could survive several years in the soil, whereas newly germinated zoosporae are the primary source of infection but they are short-lived without hosts. The germination of resting spores of Plasmodiophora was also stimulated by treatments with the powder and extract of Posidonia; thus, the short-lived character of zoosporae might influence the severity of clubroot formation.

After treating the resting spores of Plasmodiophora with the root exudate of Chinese cabbage or with the dry powder or extract of Posidonia, the cell wall of the spore had disappeared, and budding cave was observed in the spore. These changes may precede and promote the germination of spores. Yuzawa and Tanaka (1979) who studied the structure of the resting spores of Plasmodiophora by scanning and transmission electron microscopy, found that the wall of the mature resting spore consisted of three layers, W1 (outer wall), W2 (middle wall), and W3 (inner wall). In addition, the thickened circular region of W2, surrounded by a ring of electron-dense material, was found to be the region of the

Table 3. Effect of Posidonia powder on the degree of clubroot severity in inoculated soil.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>At planting day</th>
<th>7 days before planting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not added</td>
<td>66.9</td>
<td>39.4</td>
</tr>
<tr>
<td>2% Posidonia powder</td>
<td>62.5</td>
<td>37.5</td>
</tr>
<tr>
<td>5% Posidonia powder</td>
<td>97.5</td>
<td>27.8</td>
</tr>
</tbody>
</table>

*The degree of the clubroot severity was calculated by following formula:
The degree of the clubroot severity = \[\frac{\sum (\text{The number of plant with each degree of clubroot} \times \text{degree number of clubroot})}{\text{Total number of plants} \times 3} \times 100\]
germination cave of the spore. Tanaka et al. (1985), using a transmission electron microscope, reported that the W2 layer became thinner or disappeared during the formation of the primary zoospore in Plasmiodiophora. Furthermore, Buckzack and Moxham (1983) showed that the wall of the resting spore consisted of five layers by transmission electron microscope; they induced chemical digestion of the resting spore. After incubation with KOH, chitinase digested the middle layers 3 and 4, indicating that chitin was a major component of these layers. Since the Fluorescent Brightener 28 is specific for chitin staining, these electron microscopic observations suggest that the root exudate of Chinese cabbage and the dry powder and extract of Posidonia used in this study stimulated the germination of resting spores of Plasmiodiophora through the dissolution of chitin.

Hooker et al. (1945) reported that arylisothiocyanate, a major component of mustard oil, stimulated the germination of resting spores of Plasmiodiophora brassicae, but it did not stimulate the germination of spores in our experiment. MacFarlane (1970) cast doubt on the results reported by Hooker et al. (1945) because of the lack of evidence that the flagellates counted were of Plasmiodiophora brassicae. Because of the contradiction and no clear cut results with arylisothiocyanate in this trial, we believe that further studies on chemical promotion of germination by this compound are necessary.

Flusulfamide at 1 mM, which was sufficient to prevent the formation of clubroots, did not stimulate significantly the germination of spores. However, Fujita (1994) showed flusulfamide inhibits the germination of resting spores and prevents the primary zoospore formation. Thus, the effects of Posidonia on the germination of resting spores and on clubroot formation are different from those of flusulfamide.

The components of the extract of Posidonia adsorbed on to HP20 and its partly purified fractions (fractions 3 and 4) stimulated the germination of spores of Plasmidiophora. Suzuki et al. (1992) also reported the existence of a germination-stimulating factor (GSF) in the root exudate of turnip (Brassica campestris L.) and lettuce (Lactuca sativa L.). These results suggest that the GSF is involved not only in crucifers but also in other kinds of plants. We are examining several chemicals to evaluate their germination-stimulating activity.

**Literature Cited**


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ポシドニア乾燥粉末および抽出物によるアブラナ科植物根こぶ病菌胞子の
発芽促進作用とハクサイ根こぶ病の防除

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摘 要

南オーストラリア海岸由来ポシドニア（Posidonia australis
Hook. F.）乾燥粉末および抽出物は根こぶ病菌（Plasmo-
diophora brassicae）胞子の発芽を促進した。この乾燥粉末およ
び抽出物は、おそらく発芽促進作用に基づいて、ハクサイ
における根こぶの形成を抑制し、根こぶ病の予防に有効であ
ることを示唆した。