Soil suppressiveness to clubroot disease of Chinese cabbage caused by \textit{Plasmodiophora brassicae}

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Abstract

Two types of soil, Haplic Andosols (HA-soil) and Low-humic Andosols (LA-soil), collected from Fukushima in Japan were used to study soil suppressiveness of clubroot disease of Chinese cabbage (\textit{Brassica oleraces}), caused by \textit{Plasmodiophora brassicae}. Relationships between disease index and concentration of resting spores in both soils, i.e. dose response curves, revealed that LA-soil was more suppressive to the disease than HA-soil. The disease index in LA-soil was significantly lower than that in sterilized LA-soil (SLA-soil) even at $10^6$ inoculum concentration, although there was no difference between HA-soil and sterilized HA-soil (SHA-soil) under severe disease pressure. The result suggests that biotic factors in LA-soil are responsible for disease suppression even at a high inoculum level of the pathogen. In addition, the disease index was lower in SLA-soil than in SHA-soil at all inoculum levels, suggesting that abiotic factors were also involved in the suppressiveness of LA-soil. These results indicate that the simultaneous effects of both biotic and abiotic factors are involved in the suppressiveness in LA-soil. The experiment with mixture of sterilized or non-sterilized LA-soils with infested HA-soil indicated that the suppressive factors, i.e. biotic plus abiotic factors, in LA-soil also function even in the soil mixture including HA-soil. The disease index was significantly higher in infested HA-soil diluted with SHA-soil than in infested HA-soil diluted with non-sterilized HA-soil at an inoculum level below estimated $10^3$ resting spores g$^{-1}$ soil. The results suggest that biotic factors play an important role in disease suppression even in conducive HA-soil infested with a low inoculum level of the pathogen. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Abiotic factor; Biotic factor; Clubroot; \textit{Plasmodiophora brassicae}; Suppressive soil

1. Introduction

Most crucifers, such as cabbage (\textit{Brassica oleraces} var. \textit{capitata} L.) and Chinese cabbage (\textit{B. rapa} var. \textit{pekinensis} L.), are continuously cropped in Japan because of limited availability land for agriculture. The clubroot disease caused by \textit{Plasmodiophora brassicae} Woron., a soil-borne fungal pathogen, results in a serious yield loss of crucifers, especially, under monoculture. Though chemical control is effective, the development of integrated control measures other than those that rely on chemicals alone are needed to promote agriculture with little environmental pollution.

Soil suppressiveness, where available, is an important component of integrated control. The disease incidence in such suppressive soils is also known to depend on abiotic factors, such as soil texture, pH and water content (Hamilton and Crete, 1978; Webster and Dixon, 1991a,b; Iwama et al., 1994).

Suppressive soils have been reported for many diseases (Scher and Baker, 1980; Schroth and Hancock, 1982; Kao and Ko, 1986; Lumsden et al., 1987; Ho et al., 1988; Amir and Alabouvette, 1993; Hoper et al., 1995; Serra-Wittling et al., 1996), but little has been published on those related to clubroot disease (Young et al., 1991; Worku and Gerhardson, 1996). Understanding the mechanisms of soil suppressiveness for
this disease should facilitate the development of soil assessment methods and, consequently, contribute to the promotion of integrated control, which may require only minimal use of chemicals.

Our purpose was to investigate soil suppressiveness for clubroot disease in Japan and to define factors, biotic or abiotic, associated with this phenomenon.

2. Materials and methods

2.1. Soil

Two types of soil, Haplic Andosols (HA-soil) and Low-humic Andosols (LA-soil), were collected from non-infested fields at Tohoku National Agricultural Experiment Station (TNAES), Fukushima, Japan and were sieved (5 mm) before use. Physical and chemical characteristics of these soils are shown in Table 1. For certain treatment the soils were autoclaved at 120°C for 1 h one week before use.

2.2. Plant cultivation

Thirteen seeds of Chinese cabbage (B. rapa var. pekinensis, cv. Shin-Azuma) per pot (115 mm diameter, 110 mm high) were sown and grown for about 35 days in a greenhouse. Four replicate pots per a treatment plot were placed at the corner of a container (330 mm × 330 mm) to avoid contamination between plots. Water or nutrients (N:20%, P2O5:12% and K2O:16%) were applied to the base of each pot when required. Soil temperature in the pots was maintained at ≥25°C by placing them on a thermostatically controlled mat.

2.3. Measurement of disease index

Roots of each Chinese cabbage plant were removed 29–39 days after sowing and washed to determine disease severity, which was classified into four categories (0–3) according to the following criteria; not clubbed: category 0; only lateral roots clubbed: category 1; less than half of tap root clubbed: category 2; more than half of tap root clubbed: category 3.

The disease index for each pot was calculated by a modification of the Dixon and Robinson (1986) formula as follows:

\[
\text{Disease index} = \left(1 \times n_1 + 2 \times n_2 + 3 \times n_3\right) / \left(3 \times N\right) \times 100
\]

\(N\): total number of plants; \(n_1\): number of plants classified as category 1; \(n_2\): number of plants classified as category 2; \(n_3\): number of plants classified as category 3.

Disease indices were examined statistically by the Tukey’s multiple range test (Sokal and Rohlf, 1995).

2.4. Dose response curves of two soils

Resting spores of P. brassicae were collected from clubbed roots of Chinese cabbage in the infested field at TNAES. The suspension of resting spores was prepared by the method of Takahashi and Yamaguchi (1987).

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Low-humic Andosols (LA-soil)</th>
<th>Haplic Andosols (HA-soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total C (%)</td>
<td>1.08</td>
<td>4.37</td>
</tr>
<tr>
<td>Total N (%)</td>
<td>0.11</td>
<td>0.28</td>
</tr>
<tr>
<td>C to N ratio</td>
<td>9.85</td>
<td>15.54</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td>1.86</td>
<td>7.52</td>
</tr>
<tr>
<td>Maximum water holding capacity (%)</td>
<td>68.6</td>
<td>68.1</td>
</tr>
<tr>
<td>Hydraulic conductivity (× 10^-3 cm S^-1)</td>
<td>2.69</td>
<td>2.05</td>
</tr>
<tr>
<td>pH (H2O)</td>
<td>6.53</td>
<td>6.01</td>
</tr>
<tr>
<td>Electric conductivity (mS cm^-1)</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>CEC (mequiv 100 g^-1 dry soil)</td>
<td>21.50</td>
<td>26.83</td>
</tr>
<tr>
<td>NO3-N (mg 100 g^-1 dry soil)</td>
<td>0.64</td>
<td>1.70</td>
</tr>
<tr>
<td>P2O5 (Bray no. 2) (mg 100 g^-1 dry soil)</td>
<td>22.25</td>
<td>14.97</td>
</tr>
<tr>
<td>Exchangeable base (extraction with N KCl)</td>
<td>0.62</td>
<td>0.29</td>
</tr>
<tr>
<td>K^+ (mequiv 100 g^-1 dry soil)</td>
<td>8.08</td>
<td>8.09</td>
</tr>
<tr>
<td>Ca^2+ (mequiv 100 g^-1 dry soil)</td>
<td>4.18</td>
<td>2.92</td>
</tr>
<tr>
<td>Mg^2+ (mequiv 100 g^-1 dry soil)</td>
<td>0.12</td>
<td>0.05</td>
</tr>
<tr>
<td>Na^+ (mequiv 100 g^-1 dry soil)</td>
<td>0.64</td>
<td>1.09</td>
</tr>
<tr>
<td>Phosphate absorption capacity (mg P2O5 100 g^-1 dry soil)</td>
<td>1860</td>
<td>1821</td>
</tr>
</tbody>
</table>
The soils, HA-soil and LA-soil, were sprayed with suspensions of resting spores (25 ml kg\(^{-1}\) soil) to provide a range from 0 to \(10^6\) resting spores g\(^{-1}\) soil and mixed thoroughly. The disease severity was determined 35 days after sowing.

### 2.5. Biotic and abiotic factors affecting soil suppressiveness

Non-sterilized soils (HA-soil and LA-soil) and sterilized soils (SHA-soil and SLA-soil) were adjusted to \(10^4\) and \(10^6\) resting spores g\(^{-1}\) soil to determine the influence of biotic or abiotic factors on soil suppressiveness. The disease index was calculated after plants were grown for 35 days.

### 2.6. Effect of soil mixing on disease severity

Infested HA-soil (IHA-soil) or infested LA-soil (ILA-soil) (\(10^5\) resting spores g\(^{-1}\) soil) was mixed with each of four soils, i.e. HA-soil, SHA-soil, LA-soil and SLA-soil, at the rate of 50:50 (w/w) or 10:90 (w/w) to study the effect of suppressive factors of each soil. The disease index was calculated 34 days after sowing.

### 2.7. Effects of soil dilution in two soils on the soil suppressiveness

Soils were diluted with soil to examine the effect of diluted biotic and abiotic factors on suppression of the disease development. IHA-soil (\(10^6\) resting spores g\(^{-1}\) soil) was diluted in a 10-fold series with non-infested HA-soil. IHA-soil was similarly diluted with SHA-soil. A 10-fold dilution series of ILA-soil (\(10^6\) resting spores g\(^{-1}\) soil) were also prepared using non-infested LA-soil. Plants were grown in pots for 29–39 days to determine disease indices since preliminary tests with plants for the same periods gave the same results. The experiments were replicated three times.

### 3. Results

#### 3.1. Dose response curves of two soils

The results, when assessed as the average of three experiments, indicated that there was a relationship between the concentration of resting spores in soil and the disease severity. The dose response curves were different for the two soils (Fig. 1). The disease index was 99 in HA-soil at \(10^6\) resting spores g\(^{-1}\) soil. The disease indices decreased with decreasing amount of inoculum, and were less than 10 below \(10^2\) resting spores g\(^{-1}\) soil. In contrast, the disease index in LA-soil was 62 even when the soil was adjusted to \(10^6\) resting spores g\(^{-1}\) soil, with no evidence of disease at \(10^2\) resting spores g\(^{-1}\) soil.

Disease indices at the same inoculum levels were always lower in LA-soil than in HA-soil, indicating that LA-soil was suppressive to the clubroot disease as compared to HA-soil.

#### 3.2. Biotic and abiotic factors affecting soil suppressiveness

At the concentration of \(10^4\) resting spores g\(^{-1}\) soil, the disease index was significantly lower in LA-soil (disease index: 1) than in HA-soil (60), and the disease indices in SHA-soil (98) and SLA-soil (27) were higher than those in their respective non-sterilized soils (HA-soil and LA-soil) (Fig. 2).

At the concentration of \(10^6\) resting spores g\(^{-1}\) soil, the disease index was significantly lower in LA-soil (38) than in HA-soil (97), again demonstrating the

![Fig. 1. Relationship between the disease index of clubroot disease and the concentration of resting spores in LA-soil and HA-soil. Bars show SEM.](image)

![Fig. 2. Effects of sterilization on the disease index of clubroot disease in LA-soil and HA-soil. Means followed by the same letter are not significantly different (\(P > 0.05\)) according to Tukey’s multiple range test at each resting spore concentration. Bars show SEM.](image)
suppressiveness of LA-soil (Fig. 2). Autoclaving, however, nullified its effect at this spore concentration, since no significant difference was observed between the indices of SLA-soil (93) and SHA-soil (100) at this inoculum concentration (Fig. 2).

3.3. Effect of soil mixing

When the soils were mixed at a ratio of 50:50 (Fig. 3a), the disease in IHA-soil (disease index: 90) was not reduced by mixing with HA-soil (91) or SHA-soil (93), though the inoculum levels were maintained at $5 \times 10^4$ g$^{-1}$ soil. On the other hand, the disease severity in IHA-soil (90) was reduced when mixed with LA-soil (50) although not reduced by mixing with SLA-soil (85).

When ILA-soil (18) was mixed with LA-soil or SLA-soil, the disease index remained low (10 and 7, respectively). On the other hand, the disease index in ILA-soil was increased by mixing with HA-soil (57) or SHA-soil (81) despite the reduction in the inoculum level.

The mixing of soils at the rate of 10:90 (Fig. 3b) reduced resting spore concentration to $10^4$ g$^{-1}$ soil. The disease index in IHA-soil was reduced by mixing with LA-soil (6) or SLA-soil (16). When IHA-soil was mixed with HA-soil or SHA-soil, the disease index was lower in HA-soil mixture (60) than in SHA-soil mixture (98).

When ILA-soil (18) was mixed with LA-soil or SLA-soil, the disease index remained low (0 and 7, respectively). On the other hand, when it was mixed with HA-soil or SHA-soil, the disease index became significantly higher (74 and 100, respectively) in spite of the decreased spore concentration due to soil mixing.

3.4. Effect of soil dilution

The dose response curves of HA-soil and LA-soil estimated by the soil dilution method were similar to those obtained in the inoculation test (Fig. 1), i.e., LA-soil was more suppressive to the clubroot disease than HA-soil (Fig. 4).

Whereas HA-soil diluted with SHA-soil was more conducive to the disease than that diluted with non-sterilized HA-soil at $10^{-2}$ to $10^{-4}$ dilution.

4. Discussion

In this experiment, the effect of biotic and abiotic factors on soil suppressiveness were examined by comparing the suppressive LA-soil, with the conducive HA-soil, collected from Fukushima in Japan.

The disease indices of non-sterilized soil were consistently lower than those of sterilized soil. This result suggests that biotic factors affect the suppressiveness of both soils. In this experiment, biotic factors could
Table 2
Biotic and abiotic factors involved in the suppression of clubroot disease

<table>
<thead>
<tr>
<th>Soil</th>
<th>Suppressive factors</th>
<th>Values used</th>
<th>Difference in disease indices between soils</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$10^{4b}$</td>
</tr>
<tr>
<td>LA-soil</td>
<td>biotic and abiotic factors</td>
<td>[HA]-[LA]</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>biotic factors</td>
<td>[SLA]-[LA]</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>abiotic factors</td>
<td>[SHA]-[SLA]</td>
<td>71</td>
</tr>
<tr>
<td>HA-soil</td>
<td>biotic factors</td>
<td>[SHA]-[HA]</td>
<td>38</td>
</tr>
</tbody>
</table>

$^{a}$ [HA], [LA], [SHA] and [SLA] disease indices of the respective soils as shown in Fig. 2.

$^{b}$ Concentration of resting spores (g$^{-1}$ soil).

$^{c}$ S = sterilized by autoclaving.

not be excluded completely because it was a greenhouse experiment. However, the fact that the soil becomes conducive after an autoclave treatment shows the involvement of biotic factors.

In addition, suppressiveness still remained higher in LA-soil even after autoclaving, indicating that abiotic factors are also critical in the suppressiveness of LA-soil. The result that the disease index in SLA-soil was significantly less than that in HA-soil at a low inoculum level also supports the conclusion that abiotic factors were involved in the suppressiveness of LA-soil.

Little is known about clubroot suppressive soils (Worku and Gerhardson, 1996). Involvement of abiotic factors in soil suppressiveness has been suggested. Suppressive soil was reported to be characterized by good drainage, high gas diffusivity and high CO$_2$ concentration in the soil (Osozawa et al., 1994). Though the infection by *P. brassicae* is influenced by boron (Webster and Dixon, 1991b), boron in both LA-soil and HA-soil was not detected in our experiment. The incidence of clubroot was reported to be dependent on the source of lime and soil pH (Hamilton and Crete, 1978; Webster and Dixon, 1991a). Young et al. (1991) suggested that suppression of clubroot disease was more dependent on phenolic compounds (gentisic acid) than pH, concentration of Ca, Mg or humic acids. There were differences in several abiotic characteristics, such as organic matter, NO$_3$–N content and pH values between LA-soil and HA-soil. However no differences were observed in other characteristics, such as Ca content (Table 1). LA-soil had a slightly higher pH and less organic matter than HA-soil. The disease severity in HA-soil decreased but was still higher than that in LA-soil in an experiment where the pH value of HA-soil was adjusted to that of LA-soil (data not shown). This result implies that abiotic factors other than pH may be associated with soil suppressiveness independently and are more likely to be involved in combination although pH was associated with suppressiveness in LA-soil.

The difference in disease indices between the two soils appeared to relate to the biotic and abiotic factors associated with the suppressive LA-soil (Table 2). These results indicate that biotic factors seem to be effective mainly at $10^4$ resting spore g$^{-1}$ soil and abiotic factors seem to be evident at a lower inoculum level ($10^4$ resting spore g$^{-1}$ soil) in LA-soil. The results suggest the need to assess suppressive factors at various inoculum concentrations, since not only biotic but also abiotic factors play an important role in the suppressiveness in LA-soil and their effects appear to vary with different inoculum concentrations.

It was noteworthy that biotic factors were as important as suppressive factors even in the conducive HA-soil, under low disease pressure. In addition, the disease index of HA-soil mixed with SHA-soil was higher than that of the same soil mixed with HA-soil and the dose response curve of HA-soil diluted with SHA-soil was higher than that of the same soil diluted with non-sterilized HA-soil. Biotic factors, therefore, may be associated with the suppressiveness of soil regardless of soil types although the extent of the suppression by biotic factors may vary among soil types.

The dose response curve used in the soil dilution method reflected the results obtained with the inoculation method. This result indicates that the soil dilution method is useful for assessment of soil suppressiveness in infested fields because the dose response curve of naturally-infested soil can not be obtained by an inoculation test.

Our study showed that the suppressiveness in LA-soil from Fukushima in Japan included both biotic and abiotic factors and that the biotic factors remained effective even at high inoculum concentrations. Biotic factors play an important role in disease suppression and are evident even at low inoculum levels in the conducive soil from Fukushima.

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References


