Epidemiological Research on Botrytis Diseases of Tulip Plants Caused by B. tulipae and B. cinerea

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Abstract

We investigated the relationship between B. tulipae- and B. cinerea-induced Botrytis disease of tulip plants and the conidial densities in the air of an experimental field at Ibaraki University as well as of a tulip orchard in Kasumigaura Park (Tuchiura city, Ibaraki) in 2003. For two years prior to this investigation, the park was the focus of research, while the experimental field was cultivated with tulips for the first time. The dispersal of B. cinerea spores commenced in the early stages of tulip growth, while that of B. tulipae began just before flowering. For both Botrytis species, the number of dispersed spores increased with tulip plant growth. Differences in the development of Botrytis disease (formation of blight lesions) were observed between the two research sites. Numerous blight lesions formed on the leaves throughout the growth period of plants grown in the park. However, in the experimental field, relatively few lesions were observed and these occurred in the latter periods of growth. At both research sites, only B. tulipae was isolated from these lesions. Unlike in 2001 and 2002, when B. cinerea was isolated from the lesions, this fungus was not detected at either of the research sites in 2003.

INTRODUCTION

Botrytis tulipae and B. cinerea are fungi that cause “tulip fire” and “gray mold”, respectively, in tulip plants. B. tulipae induces lesion formation on the leaves, flowers and bulbs of the tulip. On leaves, infection appears as minute lesions or spots that are yellow to light brown in color. As these lesions enlarge, they become slightly depressed and turn gray with a brown border. The casual agent of gray mold, B. cinerea, induces lesions on the leaves, stems and flowers. Water-soaked lesions initially appear at the top and the edge of tulip leaves, and subsequently become larger and necrotic (Nishimura, 1988). The lesions caused by B. cinerea are very difficult to distinguish from those caused by B. tulipae.

In this study, we investigated the relationship between the density of Botrytis spores in the air and the ratio of lesions caused by B. tulipae and B. cinerea on tulip leaves. In 2003, a survey was carried out at an experimental farm of Ibaraki University as well as a tulip orchard in Kasumigaura Park. For two years prior to this study, Kasumigaura Park had been the focus of this research.

MATERIALS AND METHODS

Density of Botrytis Spores in Air

Surveys were performed at two research sites, Kasumigaura Park and an experimental farm of Ibaraki University between March and May 2003. The density of Botrytis conidia in the air above both fields was monitored by placing 2 spore traps in each field at 9:00 a.m. every Wednesday and leaving them in place for 24 hours. Spores caught in the traps were then counted using a microscope. Each spore trap consisted of a Petri dish lined with two-sided tape, and the number of conidia in the air was determined by counting the spores of B. tulipae and B. cinerea trapped on the two-sided tape (approximately 17 cm²). B. tulipae and B. cinerea conidia were distinguished by size; B.
cinerea conidia are typically 8-14 x 6-9 µm, while those of B. tulipae are typically 16-10 x 10-13 µm, (Nishimura, 1988; Hosono et al., 2002). The number of B. cinerea conidia was confirmed by the number of colonies formed on Kerssies medium (Kerssies, 1990), which were also attached to the spores traps.

**Isolation and Identification of Botrytis from Leaf Lesions**

Leaves closest to the ground were not used in order to avoid lesions derived from sclerotia in the soil. B. tulipae and B. cinerea were isolated from lesions using standard procedures, and the ratio of B. tulipae and B. cinerea was calculated. Isolates were identified based on sclerotia formation on Potato sucrose agar medium (PSA) and pathogenicity. Isolates that formed small black sesame-like sclerotia on PSA were regarded as B. tulipae. The remaining isolates were then inoculated onto cucumber leaves, and those forming lesions were regarded as B. cinerea.

**RESULTS AND DISCUSSION**

**Density of Botrytis Spores in the Air**

In Kasumigaura Park, dispersal of B. cinerea spores commenced towards the end of March, when tulip plants were in the early stages of growth. Dispersal of B. tulipae spores started just before flowering. For both fungal species, the number of dispersed spores increased as tulip growth increased. When compared with the results of 2002, there were no remarkable differences for B. cinerea, but the number of dispersed B. tulipae spores was greatly decreased (Fig. 1).

The environmental factors that may influence the number of dispersed B. tulipae conidia (temperature, wind speeds, precipitation, moisture content, amount of insulation) were compared between 2002 and 2003, and a difference was observed in temperature. Temperatures were higher in 2002 than in 2003 from the middle of March to early April (Fig. 2). The growth rate of B. tulipae is highest at 22°C, while there are no differences in the growth rate of B. cinerea grown at 18°C to 25°C (Hirakawa et al., 2003). These data suggest that relatively high temperatures during the early stages of tulip growth promoted enlargement of the lesions appearing on the first leaves of the tulip plant, which then increased the numbers of dispersed conidia seen in the latter periods of growth.

We also compared the density of Botrytis spores seen in the air at Kasumigaura Park with that at the experimental farm of Ibaraki University. Although Kasumigaura Park had been the focus of research for the previous two years, the experimental farm was being cultivated with tulips for the first time. Although the cultivation experiences were different for the two research sites, the results were almost the same for B. cinerea. However, for B. tulipae, the spore density at Kasumigaura Park was greater than that at the experimental farm (Fig. 1). This may be due to differences in the pathogenicities of B. cinerea and B. tulipae. B. cinerea is a well-known necrotrophic fungus that infects many plants in addition to tulip. Therefore, certain amounts of B. cinerea spores are likely to be in the air at all times, irrespective of when tulip plants are cultivated. However, B. tulipae is a host specific fungus, and thus B. tulipae spore density depends on the cultivation experience and growth of the host tulip plant.

**Ratio of B. tulipae and B. cinerea Isolated from Tulip Fire Lesions**

The formation of lesions on tulip leaves at different research sites was found to be different. The numerous lesions observed at Kasumigaura Park occurred throughout the growth period. However, at the Ibaraki University experimental farm, fewer lesions were observed and these occurred in the latter periods of growth. All isolates obtained from lesions at both research sites were B. tulipae. Although B. cinerea spores were present in the air at both research sites in 2003, B. cinerea was not isolated from lesions in 2003, which is in contrast to the 2002 results. This suggests that various factors (Hirakawa et al., 2003; Hosono et al., 2002) affect the process of B. cinerea infection. Lesions were observed on the tulip leaves as B. tulipae spores began to disperse at Kasumigaura Park,
where tulip plants had been cultivated every year. At the experimental farm, where tulips were cultivated for the first time, no lesions were observed until May 1\(^{st}\) (Table 1), despite the fact that almost the same number of spores as detected at Kasumigaura Park were present in the air (Fig. 1). This suggests that lesion formation induced by \textit{B. tulipae} during the middle stages of tulip growth is not related directly to the presence of airborne spores.

Morikawa (2002) showed that \textit{B. tulipae} sclerotia can induce primary lesions in tulip plants growing from tulip bulbs buried in soil containing \textit{B. tulipae} sclerotia. The main difference between the two research sites was cultivation experience with tulip plants, and thus it appears that sclerotia in the soil influence lesion formation on all leaves, not only leaves closest to the ground.

**Literature Cited**


**Tables**

Table 1. Formation of \textit{Botrytis} lesions on tulip plants.

<table>
<thead>
<tr>
<th>Place</th>
<th>Mar 12(^{th})</th>
<th>Mar 19(^{th})</th>
<th>Mar 26(^{th})</th>
<th>Apr 2(^{nd})</th>
<th>Apr 9(^{th})</th>
<th>Apr 16(^{th})</th>
<th>Apr 23(^{rd})</th>
<th>May 1(^{st})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kasumigaura Park</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Experimental Farm</td>
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</tbody>
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\(^{z}\): Not observed, +: Observed.
Fig. 1. Density of dispersed *B. cinerea* and *B. tulipae* spores in the air during cultivation period. A: Kasumigaura Park (2003), B: Ibraki University experimental farm (2003), C: Kasumigaura Park (2002).
Fig. 2. Mean temperatures during tulip growth period (Meteorological data from Ibaraki University experimental farm).