Seasonal effect on infection with *Colletotrichum horii* causing anthracnose in persimmon twigs and the relation between its prevalence on twigs and occurrence in fruit

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Abstract

We inoculated persimmon with *Colletotrichum horii*, causing anthracnose on the young twigs from the third week in April to the second week in June. The anthracnose lesions occurred in late April, and did not occur on lignified twigs. In addition, we inoculated the fruits with high and low levels of inoculation using twigs with lesions. In the high inoculation group, the occurrence of diseased fruits was as high as 41.6%. The results indicated that the application of fungicides should start after late April, and also that twigs with lesions should be removed and controlled using the application of fungicides.

Key words: inoculation efficiency, *C. horii*, pot plants, bearing shoot

Introduction

Japanese persimmon (*Diospyros kaki* L.) is cultivated as a major economic fruit crop in several countries, including Japan, China, and Korea. The harvest period is broad, and runs from late July to early December, by production in greenhouses and fields and using several cultivars in Nara Prefecture, which produced the second largest amount of persimmon in Japan in 2016, next to Wakayama Prefecture.

Anthracnose disease caused by *Colletotrichum horii* is the most severe disease that affects persimmon production. Previously, the anthracnose pathogen was named *Gloeosporium kaki*, placed in synonymy with *C. gloeosporioides*. Recently, however, it was identified to be *C. horii*, based on molecular and morphological characteristics (Weir and Johnston, 2010).

Anthracnose occurs mainly on the fruit and young twigs of the tree, with little effects on leaves. The primary infection source is lesions on twigs. Fruits usually begin to show damage in July. The symptoms in fruits are small dark brown or black spots on their surface, and these gradually expand into round or oval lesions, and are occasionally slightly depressed (Xie et al., 2010). The fruits soften, and pale orange conidial masses are produced in the lesions when the humidity is high. Diseased fruits typically fall from the tree and/or ferment from August to September. Young twigs are susceptible to anthracnose disease. New shoots and young twigs usually develop symptoms of small, round, dark brown spots, which expand into brown oval- or irregular-shaped lesions. In some cases, the fungi cause twig blight. Defoliation occurs if lesions develop at the base of the petiole.

There are no detailed reports on the seasonal effects on the infection efficiency of *Colletotrichum horii* in twigs, nor on the effects of the number of twigs with lesions on the occurrence of anthracnose in fruits though those are the important information to determine when to apply the fungicides and how to control it.

We investigated the inoculation efficiency of *C. horii* against the twigs in different periods and the occurrence of anthracnose in fruits by inoculation using different numbers of twigs with lesions.

Materials and Methods

*Plant materials, inoculum preparation, and inoculation on persimmon twigs*

*Colletotrichum horii*, isolated from lesions on persimmon
fruit in Nara Prefecture in 2012, was inoculated onto twigs of the persimmon cultivar ‘Fuyu’ planted in 25 L pots. The trees were five to six years old in 2015. The inoculation periods were divided into the third and fourth week in April, first to fourth week in May, and first and second week in June.

*C. horii* isolate was cultured in 200 ml Erlenmeyer flasks, each containing 100 ml of potato sucrose broth (200 g of sliced potato, 20 g of sucrose, and 1 L of distilled water) on a rotary shaker at 120 rpm at 25°C for 10 days. Cultures were passed through sterile cheesecloth to collect the conidia. The conidia were suspended in sterile water and adjusted to $5 \times 10^5$ conidia/ml.

Three to six bearing shoots, with 15 to 50 twigs in potted plants received 10 ml of the conidial suspension using a hand-held sprayer at 18:00 h twice per each inoculation period, and were covered overnight with vinyl bags to maintain 100% humidity. In the third week of May, inoculation was conducted only once. Anthracnose symptoms on the twigs were recorded, and we confirmed infection with the pathogen about one month after inoculation.

### Inoculation by twigs with lesions

For inoculations, six artificially infected plants were placed in the center row of twelve healthy plants on July 9, 2014 (Fig. 1). The infected plants were prepared by spraying with conidial suspension ($10^5$ conidia/ml). The number of twigs with lesions in six infected plants were 0, 6, and 27 in the control, low inoculation treatment, and high inoculation treatment, respectively. Anthracnose symptoms on the fruits, black round or oval lesions, were recorded, and infection with the pathogen was confirmed every week.

### Results and Discussion

Symptoms were not observed after inoculation treatment for the third week in April, in which the average temperature was 15°C (Table 1; Fig. 2). After that, the average temperatures were more than 19°C, and the symptoms were confirmed every time, except for the fourth week in May and the second week in June, in which all the twigs inoculated were lignified. The occurrence rate of lesions in twigs gradually increased from the third week in April to the second week in May.

As stated, *C. horii* infection was not confirmed after

![Fig. 1. Design of location of the persimmon pots for survey of fruit anthracnose disease and pots with lesion in twigs and fruits removed](image)

<table>
<thead>
<tr>
<th>Period of inoculation of <em>C. horii</em></th>
<th>Percentage of diseased fruiting branches</th>
<th>Number of Bearing Shoot</th>
</tr>
</thead>
<tbody>
<tr>
<td>The 3rd week in April (April 21 and 23)</td>
<td>0.0 (0/35)$^a$</td>
<td>5</td>
</tr>
<tr>
<td>The 4th week in April (April 28 and 30)</td>
<td>8.9 (4/46)</td>
<td>6</td>
</tr>
<tr>
<td>The 1st week in May (May 6 and 8)</td>
<td>46.4 (22/50)</td>
<td>6</td>
</tr>
<tr>
<td>The 2nd week in May (May 13 and 15)</td>
<td>66.1 (19/31)</td>
<td>5</td>
</tr>
<tr>
<td>The 3rd week in May (May 18)</td>
<td>47.1 (8/17)</td>
<td>5</td>
</tr>
<tr>
<td>The 4th week in May (May 25 and 26)</td>
<td>0.0 (0/35)</td>
<td>6</td>
</tr>
<tr>
<td>The 1st week in June (June 3 and 4)</td>
<td>58.3 (7/12)</td>
<td>3</td>
</tr>
<tr>
<td>The 2nd week in June (June 15 and 17)</td>
<td>0.0 (0/34)</td>
<td>5</td>
</tr>
</tbody>
</table>

$^a$ Number of diseased branches/number of fruiting branches.

All the branches were lignified in the 4th week in May and in the 2nd week in June.
inoculation in the third week of April, suggesting that the temperature affected the efficiency of the infection. This results is in agreement with Zhang and Hu (2004), who reported that the disease occurred at 17°C and 23°C, but not at 15°C, according to a pathogenicity test.

In addition, lignification seemed to suppress the C. horii infection even when the average temperature was over 20°C. Lignification of the twigs mainly started in late May, and most twigs were lignified in mid-June. However, the water sprout was not lignified even after mid-June, and was infected with C. horii in the second week in June (data not shown). Therefore, the removal of the water sprout seems to be an important measure to reduce the infection source for fruits.

Lesions in fruits were observed on August 8 for the first time in both the high and low inoculation treatments (data not shown). The occurrence of lesions on fruits in the high inoculation group was three to four times higher than that in the low inoculation group on September 2 and October 8 (Table 2). In the control, the one and only lesion on a fruit was confirmed on October 8.

The number of twigs with lesions directly related to the occurrence of lesions in fruits. The occurrence of the disease climbed after rainfall in late August (Fig. 3). In the high inoculation treatment, the occurrence rate of the disease in fruits was as high as 41.6 %, indicating that lesions on the twigs are an important infection source for fruits.

Therefore, the removal of diseased twigs, as well as the application of fungicides seems to be important to reduce production loss.

In Nara Prefecture, in the dormant period and late May, the application of lime sulfur or thiram and thiophanate-methyl or dizinc bis is conducted, mainly in the fields with a lot of visible lesions on twigs. After that, the application of fungicides, such as azoxystrobin, mancozeb, tebuconazole, difenoconazole, dithianon, and kresoxim-methyl continues until early October. The timing of the fungicide application should be decided based on the temperature and cumulative precipitation. The application of fungicides was conducted from late April to early October because the average temperature could exceed 20°C in this period, which was considered to be suitable with respect to our result of the inoculation experiment.

Several fungicides were evaluated for rainfastness and anthracnose inhibition efficacy. The control efficacy of azoxystrobin and kresoxim-methyl significantly decreased when the cumulative precipitation exceeded 100 mm. Thus, the reapplication of fungicides is needed to maintain a high control efficacy according to the amount of cumulative precipitation. In contrast, dithianon showed a higher protective value and waterproof activity than those of azoxystrobin and

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of twigs with lesions</th>
<th>Percentage of diseased fruit</th>
<th>Number of surveyed fruits</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>9/2</td>
<td>10/8</td>
</tr>
<tr>
<td>High inoculation</td>
<td>27</td>
<td>18.8 (19)</td>
<td>41.6 (42)</td>
</tr>
<tr>
<td>Low inoculation</td>
<td>6</td>
<td>6 (5)</td>
<td>11.9 (10)</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0 (0)</td>
<td>3.8 (1)</td>
</tr>
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</table>

*a Number of diseased fruits
kresoxim-methyl (Ide and Tashiro, 2001) and maintained a high control efficacy when the cumulative precipitation was more than 300 mm (Ide et al., 2001).

To our knowledge, this is the first report to evaluate the seasonal effect on the occurrence of lesions on twigs and the risk level of the diseased twigs to be an infection source in persimmon. In conclusion, this study could assist to decide the timing of the application of fungicides, and explain the importance of removing diseased twigs from persimmon.

References
Ide, Y. and N. Tashiro (2001) Business annual report of the Saga Fruit Tree Experiment Station. 278-279. (in Japanese)