Anthracnose Development on Pepper Fruits Inoculated with Colletotrichum gloeosporioides

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ABSTRACT

Colletotrichum gloeosporioides caused anthracnose on pepper fruits of line PBC 510 when inoculated with a microdrop sporule suspension on immature fruits one-half the normal size up to fully mature ripe red fruits. Incidence of anthracnose was greater on inoculated purple and ripe red fruits than on fruits at other developmental stages. Cuticle and exocarp thicknesses varied by fruit maturity. Disease incidence differed among eight pepper lines based on the number of days to fruit lesion development. Over 50% of the fruits in lines PBC 452, PBC 454, and PBC 595 had lesions less than 5 days after inoculation, whereas it took 6 days for fruits in three lines (PBC 365, PBC 371, and PBC 518), 8 days for fruits in line PBC 370, and 11 days for fruits in line PBC 495. Fruits of PBC 595 had the largest lesions, while fruits of PBC 518 had the smallest lesions. Conidial production was lowest on fruit lesions of PBC 495 and highest on fruit lesions of PBC 595. Disease incidence was correlated to cuticle and exocarp thicknesses. Cuticle thickness was significantly negatively correlated to conidial production (r = -0.45) and lesion expansion (r = -0.46). C. gloeosporioides infected more fruits of var. Szechwan 90714 in a given period than did C. capsici, whether or not fruits were chloroform-dipped. Anthracnose was detected more on inoculated fruits that were chloroform-dipped than water-dipped prior to inoculation.

Anthracnose of pepper (Capsicum annuum L.), caused by Colletotrichum spp., results in losses of marketable fruits when production occurs in moist environments. Among several species of Colletotrichum (6), C. gloeosporioides (Penz.) Penz. & Sacc. in Penz. and C. capsici (Syd.) E.J. Butler & Bisby are the most frequently cited causal agents of pepper anthracnose. Reports from Korea described two C. gloeosporioides strains causing disease on green and ripe red pepper fruits (12,18).

To evaluate pepper lines for disease resistance, conidial suspensions of Colletotrichum spp. have been used to spray-inoculate fruits (2), dip detached fruits in suspension (15), and pinprick fruits with inoculum (7,10,12,18). Some of these methods do not resemble factors that occur during field infection. During an epidemic, infection, sporulation, and dissemination are important factors in disease progression (3,8). To initiate the infection process, dissolution of the appressorial wall of Colletotrichum spp. and the host cuticle has been documented in several crops (17,20). Fruit characteristics like exocarp thickness that were shown to vary among paprika lines (4) may also affect the infection process. In another host-pathogen interaction, infection was shown to be controlled by chemical stimuli (11). The objectives of our study were to evaluate disease incidence, lesion diameter, and sporulation on pepper fruits of varying development stages, and to examine the relationship of disease development to cuticle or exocarp thickness on detached ripe red pepper fruits using microdrop inoculation with C. gloeosporioides.

MATERIALS AND METHODS
Sample preparation. Pepper fruits were detached from field- and greenhouse-grown plants at the Asian Vegetable Research and Development Center (AVRDC), Shanhua, Tainan, Taiwan. Fruits were separated from their pedicels using scissors, washed in running tap water, wiped dry with paper towels, placed securely in plastic boxes (20 × 12 × 6 cm or 27 × 18 × 9 cm) containing galvanized iron mesh screen and 50 ml of distilled water on the bottom. Fruits were dot-marked with a marking pen near the equatorial region. Conidia obtained from a 5-day-old nonteleomorphic C. gloeosporioides culture (conidia 11.1-18.5 × 2.7-5.0 μm) grown on potato-dextrose agar (PDA) at 28°C under continuous fluorescent light were harvested by adding 5-10 ml of sterilized distilled water to culture dishes and gently swirling the liquid to dislodge conidia. Fruits were spot-inoculated adjacent to dot-marks with a 10-μl drop (10³ conidia per drop). Boxes were covered with plastic bags and kept at 28°C, near 100% relative humidity, and a 12-h light–dark cycle. Plastic bags were removed from boxes 48 h after inoculation, and fruits were incubated for an additional 3 days.

Infection on fruits of different development stages. Five ripe red fruits of eight AVRDC pepper lines, PBC 365, PBC 370, PBC 371, PBC 452 (var. Cajun 2), PBC 454, PBC 495 (var. Perennial HDV), PBC 518 (var. PSP 11), and PBC 595 (var. Yuak) were harvested from field-grown plants. Five fruits each of one-half normal size, three-quarters normal size, immature green, purple, and ripe red of line PBC 510 were harvested from greenhouse-grown plants. From each fruit, 10 × 3 mm sections of pericarp were extracted from near the blossom end, midfruit, and pedicel end. These were freehand sectioned with a sharp razor blade and were immediately stained in a saturated solution of Sudan IV in 70% ethanol for 20 min, rinsed in 50% ethanol, and mounted on a drop of glycerin. Five cross sections were measured under a compound microscope for each stained cuticle and exocarp from the three fruit regions (9). The bright red stain overlying the epidermis was measured as the cuticle composed of cutin and associated waxes (13). Bright red stain from the cuticle to the contiguous few layers of cells underlying the epidermis was measured as the exocarp. Cuticle and exocarp thicknesses were averaged per section and per fruit for each fruit developmental stage and pepper line.

Pepper fruits one-half normal size, three-quarters normal size, immature green, purple, and ripe red of line PBC 510 (Long Fruit) were detached from greenhouse-grown plants, washed, spot-inoculated, and incubated as previously described. Samples consisted of 50 fruits of each development stage per three replicates. Fungal infection was considered positive when a characteristic grayish, sunken lesion developed within the spot-inoculated area based on observations made 7 and 12 days after inoculation (DAI). The experiment was repeated once.

Varietal response to disease development. Ripe red fruits of lines PBC 365, PBC 370, PBC 371, PBC 452, PBC 454, PBC 495, PBC 518, and PBC 595 from field-grown plants were selected, washed, spot-inoculated, and incubated as previously described. There were 105 fruits in a box for PBC 494, PBC 518, PBC
Fruits were wiped dry with paper towels each time after harvesting conidia to avoid secondary lesion development before being reincubated. Four counts of conidia were taken per 1 ml for each pepper line and replicate. These counts were averaged and multiplied by the total volume of the suspension. Conidial production was accumulated daily and converted to the number of conidia per lesion per day.

Enhanced disease development as affected by chloroform. Red pepper fruits of var. Szechwan (approximately 15 cm long × 1.5 cm diameter at mid-point) were harvested from the field, separated from their pedicles, washed, wiped dry, and dipped (five fruits at a time) from blossom end to pedicle end in either chloroform or water for 3 s. Fruits were placed in plastic boxes (27 × 18 × 9 cm), dot-marked, spot-inoculated with 10 μl of 10^6 conidia per milliliter of either C. capsici or C. gloeosporioides near the dot-marks, and incubated as previously described. Plastic coverings were removed 48 h after inoculation, and fruits were incubated at the same conditions for 7 and 16 DAI for chloroform-dipped and water-dipped fruits, respectively. Disease incidence was recorded daily from 2 to 7 DAI for both chloroform-dipped and water-dipped fruits, and continued every other day from 8 to 16 DAI for water-dipped fruits. Grayish discoloration coupled with thick hyphal growth on the chloroform-dipped fruits and pin-head sink development coupled with grayish orange discoloration around the sink were rated positive for infection and discarded after each recording to avoid background contamination. The experiment was repeated once.

Data analysis. Data from repeated experiments were analyzed to determine if they could be combined (22) using JMP (21). Data were analyzed by ANOVA and means were separated by Duncan’s multiple range test (DMRT) (P = 0.01).

RESULTS

Infection on fruits of different development stages. Line PBC 510 fruits at all test stages developed characteristic anthracnose lesions. Ripe red and purple fruits had significantly (P = 0.01) higher disease incidence than did less developed fruits (Table 1). Cuticles were thicker in fruits of half normal size (13 μm), intermediate (12 μm) in ripe red fruits, and thinnest (11 μm) in fruits of three-quarters normal size, immature green, and purple stages. Exocarps were thickest in fruits of three-quarters normal size (60 μm) and thinner in fruits of immature green, purple, ripe red, and one-half normal size (Table 1).

Varietal response to disease development. PBC 452 had the highest disease incidence (9% at 2 DAI and 61% at 4 DAI) of all lines, while PBC 454 was significantly higher (6% at 2 DAI and 36% at 4 DAI) than the rest of the lines at 2 and 4 DAI (Fig. 1). After 6 and 8 DAI, test lines were grouped in three categories based on mean separation using DMRT (P = 0.05): high disease incidence in PBC 452, PBC 595, and PBC 454; medium disease incidence in PBC 365, PBC 371, and PBC 518; and low incidence in PBC 370 and PBC 495. After 8, 12, 14, and 16 DAI, PBC 495 had the least disease incidence, followed by PBC 370. Days to 50% fruit infection was longest for PBC 495 (10.5 days) and shortest for PBC 452 (3.6 days) (Table 2).

The rate of lesion expansion on PBC 595 was significantly (P = 0.01) highest (5.4 mm per day), while the rate of 2.9 mm per day for PBC 518 was significantly lowest (Table 2). Lines PBC 365, PBC 370, PBC 371, PBC 452, PBC 454, and PBC 495 were not significantly different. Thicker cuticles (17 μm) occurred in ripe red fruits of lines PBC 370, PBC 371, PBC 452, PBC 454, and PBC 495. Thicker exocarps (108 μm) occurred in fruits of PBC 454, followed by PBC 365 (96 μm) (Table 2). Conidial production was significantly positively correlated with cuticle thickness at 2, 4, and 6 DAI (r = 0.55, 0.33, and 0.26, respectively) but significantly negatively correlated at 8, 10, 12, 14, and 16 DAI (r = –0.26, –0.34, –0.37, –0.38, and –0.39, respectively).

Table 1. Disease incidence on pepper fruits of varying development stages of line PBC 510 at 12 days after inoculation with Colletotrichum gloeosporioides and corresponding cuticle and exocarp thicknesses of fruits

<table>
<thead>
<tr>
<th>Development stage</th>
<th>Disease incidence (%)</th>
<th>Cuticle (μm)</th>
<th>Exocarp (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/2 Normal size</td>
<td>73 b^</td>
<td>12.8 a</td>
<td>45 b</td>
</tr>
<tr>
<td>3/4 Normal size</td>
<td>55 b</td>
<td>10.8 c</td>
<td>60 a</td>
</tr>
<tr>
<td>Immature green</td>
<td>66 b</td>
<td>10.9 c</td>
<td>57 a</td>
</tr>
<tr>
<td>Purple</td>
<td>95 a</td>
<td>10.8 c</td>
<td>49 b</td>
</tr>
<tr>
<td>Ripe red</td>
<td>90 a</td>
<td>12.2 b</td>
<td>47 b</td>
</tr>
</tbody>
</table>

^Numbers followed by the same letters are not significantly different using Duncan’s multiple range test (P = 0.01).
Conidial production was significantly negatively correlated for overall data with cuticle thickness ($r = -0.45$). Lesion expansion was significantly negatively correlated with exocarp thickness ($r = -0.46$) (Table 3).

Sporulation of *C. gloeosporioides* on PBC 595 was significantly ($P = 0.01$) greater than PBC 452, PBC 454, PBC 365, PBC 371, PBC 370, and PBC 495 (Fig. 2). Fewer conidia per lesion per day at all DALD occurred on PBC 495.

**Enhanced disease development as affected by chloroform.** Disease incidence was significantly higher at 4 to 16 DA1 on fruits inoculated with *C. gloeosporioides* than with *C. capsici* when either chloroform-or water-dipped (Fig. 3). Disease incidence on chloroform-dipped fruits of var. Szechwan 90714 was significantly ($P = 0.01$) higher than incidence on water-dipped fruits at 2–7 DA1 for both *C. capsici* and *C. gloeosporioides*. Disease incidence for chloroform-dipped fruits was more than 50% 2 and 5 DA1 for *C. gloeosporioides* and for *C. capsici*, respectively (Fig. 3). On water-dipped fruits, disease incidence on *C. gloeosporioides*-inoculated fruits was more than 50% 8 DA1 and 92% 16 DA1, whereas on *C. capsici* inoculated fruits had only 21% at 16 DA1.

**DISCUSSION**

*C. gloeosporioides* produces splashborne conidia disseminated by wind-driven rains (14). Conidia germinate to form appressoria that adhere to the host epicuticular wax and/or cuticle. Once attached, the infection hyphae that form from the appressoria pierce the cuticle of the epidermal cell layer. The trophic hyphae which form from the infection hyphae interact with the exocarp and host tissues. The microdrop inoculation technique used in our study on detached pepper fruits imitates these components of the infection cycle. Inoculation methods such as pinprick bypass some of these events, and trophic hyphae are formed in the meso- or endocarp directly from the germinating conidia, resulting in faster disease development. Data from pinpricking fruits was not correlated with the disease incidence on fresh or 15-day incubated fruits of field-grown pepper plants (J. B. Manandhar, unpublished). Fruit anthracnose that developed after the microdrop inoculation varied among eight test lines, indicating the potential use of this technique to screen for resistance.

Different strains of *C. gloeosporioides* were reported to cause anthracnose on green and ripe red pepper fruits (12,18). One report indicated that red fruit leachates of pepper stimulated appressorial formation (5), while another report showed the same effect for green fruit leachates (1). Several reports have shown that mature fruits exude more nutrients from within tissues (1,5,11,18). In our study, *C. gloeosporioides* caused anthracnose on all ages of pepper fruits. Lesion size and conidial production of *C. orbiculare* (Berk. & Mont.) Arx (= *C. lagenarium* (Pass.) Ellis & Halst.) were reported to differ on cucumber cultivars (25). Dilatary resistance of pepper lines to anthracnose has not been reported. Based on our procedure, pepper lines may be evaluated on the basis of the time it takes for 50% of the fruits to become infected after inoculation. Field confirmation of resistance would also be needed to allow for conidial production and its contribution to secondary spread. The incidence and/or severity of disease may be reduced if pepper genotypes have smaller lesions, which allow for less conidial production and are components of resistance that may reduce the rate of epidemic development (19).

The differences in thickness of pepper fruit exocarp change during fruit development (4). Variation in cuticle and exocarp thicknesses of fruits of varying development stages in line PBC 510 and the infection process on fruit of eight pepper lines were positively correlated at 2–6 days after inoculation, indicating susceptibility, and were negatively correlated at 8–16 days after inoculation, indicating resistance on or in the cuticle. Cuticle thickness correlated with less

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**Table 2. Number of days to 50% fruit symptoms and rate of lesion expansion per day on detached fruits inoculated with *Colletotrichum gloeosporioides* and corresponding cuticle and exocarp thicknesses of ripe red fruits of eight pepper lines**

<table>
<thead>
<tr>
<th>Line</th>
<th>50% Fruit incidence (days)</th>
<th>Lesion expansion (mm per day)</th>
<th>Cuticle (μm)</th>
<th>Exocarp (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBC 365</td>
<td>5.3 c–c’</td>
<td>3.9 b</td>
<td>16 b</td>
<td>96 b</td>
</tr>
<tr>
<td>PBC 370</td>
<td>8.8 b</td>
<td>3.9 b</td>
<td>17 a</td>
<td>50 c</td>
</tr>
<tr>
<td>PBC 371</td>
<td>5.8 c</td>
<td>4.2 b</td>
<td>17 a</td>
<td>48 c</td>
</tr>
<tr>
<td>PBC 452</td>
<td>3.6 f</td>
<td>4.5 b</td>
<td>17 a</td>
<td>66 d</td>
</tr>
<tr>
<td>PBC 454</td>
<td>4.6 c–f</td>
<td>4.4 b</td>
<td>17 a</td>
<td>108 a</td>
</tr>
<tr>
<td>PBC 495</td>
<td>10.5 a</td>
<td>3.9 b</td>
<td>16 b</td>
<td>74 c</td>
</tr>
<tr>
<td>PBC 518</td>
<td>5.5 cd</td>
<td>2.9 c</td>
<td>14 c</td>
<td>76 c</td>
</tr>
<tr>
<td>PBC 595</td>
<td>4.7 c–f</td>
<td>5.4 a</td>
<td>13 d</td>
<td>74 c</td>
</tr>
</tbody>
</table>

Numbers followed by the same letters are not significantly different using Duncan’s multiple range test ($P = 0.01$).

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**Table 3. Correlations of fruit characteristics of pepper to lesion expansion and conidial production of *Colletotrichum gloeosporioides* inoculated on eight pepper lines**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Exocarp thickness</th>
<th>Lesion expansion</th>
<th>Conidial production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cuticle thickness</td>
<td>-0.13</td>
<td>-0.03</td>
<td>-0.45***</td>
</tr>
<tr>
<td>Exocarp thickness</td>
<td></td>
<td>-0.46**</td>
<td>-0.06</td>
</tr>
<tr>
<td>Lesion expansion</td>
<td></td>
<td></td>
<td>0.69***</td>
</tr>
</tbody>
</table>

*** = Significant at $P = 0.01$.

**Fig. 2. Number of *Colletotrichum gloeosporioides* conidia on fruit lesions of eight pepper lines after the lesions had expanded to 5 mm in diameter under continuous fluorescent light at 26 ± 3 C.**

**Fig. 3. Percent ripe red fruits of var. Szechwan 90714 with anthracnose lesions that had been chloroform- or water-dipped before inoculation with conidia of *Colletotrichum capsici* and *C. gloeosporioides*.**
conidial production \((r = -0.45)\) and lesion expansion \((r = -0.46)\). Cuticle or exocarp thickness alone does not adequately explain the response to infection, as other factors, including age, could be involved. In another study, it was shown that cuticles of bean hypocotyls that were more than 3 wk old were a barrier to infection by *Rhizoctonia solani* Kühn (2).

**Glemmerella cingulata** (Stoneman) Spauld. & H. Schrenk was reported to be less pathogenic than *C. capsici* on surface-intact (spot-inoculated) pepper fruits at 50–60 days (1). Appressoria of both fungi remained quiescent on green fruits and developed progressive lesions only after fruits turned ripe red (1). In our studies, the *C. gloeosporioides* strain (teleomorph not found) infected more fruits in a given period than did *C. capsici*, whether or not fruits were treated with chloroform. In addition, more fruits were infected by both fungi when fruits were chloroform-dipped. The occurrence of *C. capsici* may be based more on opportunistic effects, such as bruises and injuries caused by weather or pre- or postharvest handling or in storage (24).

A wax layer is the first line of defense against pathogenic invasion (23), which is believed to be absent on fruits dipped in chloroform. The differences in the number of diseased fruits after being chloroform- and water-dipped may be attributed to the removal of the wax layer and may affect cutin, pectin, and cellulose components of the cuticle, causing more permeability of nutrients from the epidermis (23). Dips of 3–5 s in chloroform might have affected the underlying cutin layer, causing leakage of nutrients from the host epidermal cells. No histological studies on inoculated chloroform-treated fruits were made. Based on microscopic observations of the microdrop, conidia formed both appressoria and mycelia abundantly (J. B. Manandhar, *unpublished*). Removal of epi cuticular wax and the cutin layer from the fruit surface needs to be studied in order to understand the infection process of *Colletotrichum* sp. on pepper fruits.

**LITERATURE CITED**


