Anthracnose Development on Pepper Fruits Inoculated with *Colletotrichum gloeosporioides*

J. B. MANANDHAR, Research Fellow, International Rice Research Institute, P.O. Box 933, 1099 Manila, Philippines; G. L. HARTMAN, Research Plant Pathologist, USDA-ARS, University of Illinois, Urbana 61801; and T. C. WANG, Associate Specialist, Asian Vegetable Research and Development Center (AVRDC), Shanhua, Tainan 741, Taiwan, ROC

ABSTRACT

Manandhar, J. B., Hartman, G. L., and Wang, T. C. 1995. Anthracnose development on pepper fruits inoculated with *Colletotrichum gloeosporioides*. Plant Dis. 79:380-383.

Colletotrichum gloeosporioides caused anthracnose on pepper fruits of line PBC 510 when inoculated with a microdrop spore 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 incubated 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*.

Accepted for publication 15 December 1994.

MATERIALS AND METHODS

Sample preparation. Pepper fruits were detached from field- and screenhouse-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 ($20 \times 12 \times 6$ cm or $27 \times 18 \times 9$ 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 (10^3 conidia per drop). Boxes were covered with plastic bags and kept at 28 C, near 100% relative humidity, and a 12/12-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 screenhouse-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 screenhouse-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