Cultural Studies and Pathogenicity of *Pseudocercospora fuligena*, the Causal Agent of Black Leaf Mold of Tomato

G. L. HARTMAN, Plant Pathologist, Asian Vegetable Research and Development Center; S. C. CHEN, Assistant Plant Pathologist, Tainan District Agriculture Improvement Station, Tainan, Taiwan; and T. C. WANG, Associate Specialist, Asian Vegetable Research and Development Center, P.O. Box 42, Shanhua, Tainan, Taiwan 74199, Republic of China

ABSTRACT

Hartman, G. L., Chen, S. C., and Wang, T. C. 1991. Cultural studies and pathogenicity of *Pseudocercospora fuligena*, the causal agent of black leaf mold of tomato. Plant Dis. 75:1060-1063.

Pseudocercospora fuligena was studied in pure culture and was inoculated on tomato plants under controlled conditions. Germ tubes were observed most frequently from the tip and basal cells of conidia. Free moisture was not necessary for conidia to germinate, and some conidia germinated at 91% relative humidity. Conidia did not germinate at or below 84.5% relative humidity. The fungus grew slowly in culture on four media tested. The optimum temperature for mycelial growth was 26 C, whereas no growth was observed at 34 C. On tomato-oatmeal agar, 6.1×10^4 conidia per culture dish were produced after 3 wk, but conidia were not produced on potato-dextrose agar. Tomato plants inoculated with 5×10^3 conidia per milliliter had 96% leaf area infected 14 days after inoculation. In cross-inoculation experiments, isolates from *Solanum nigrum* infected tomato, and isolates from tomato infected *S. nigrum*.

Black leaf mold of tomato, also commonly referred to as Cercospora leaf mold, is caused by the fungus *Pseudocercospora fuligena* (Roldan) Deighton (syn. *Cercospora fuligena* Roldan). The disease and pathogen were first described from the Philippines in 1938 (12). The disease has also been reported to occur in Cambodia, India, Ivory Coast, Japan, Malaysia, Taiwan, Thailand, Solomon Islands, United States (Florida), and Vanuatu (1,3,5,6,8,9,12,15,16). Although the disease and pathogen appear to be widespread, there have been few detailed reports on the disease or on the biology of the pathogen.

The initial symptoms of black leaf mold appear as pale yellow to light green lesions 1–20 mm in diameter. Lesions on the lower leaf surface are initially covered with white mycelium that turns gray to black as the fungus sporulates (10,11, 13,16). Infected leaves wilt, dry with age, and often drop prematurely. Infection of petioles and stems was reported (11,13), but no reports have indicated the occurrence of infection or symptoms on fruits.

Black leaf mold develops under conditions of warm temperatures and high relative humidity. In Japan, the disease was found to be widely distributed and caused severe reductions in yield (16). Symptoms have been reported to occur from 4 to 35 days after inoculation (1,13,16). There are no reports on how the fungus survives in the field, but it was found that conidia could survive up to 6 mo on dried leaves stored in clay pots indoors (16).

Accepted for publication 26 April 1991 (submitted for electronic processing).

Conidiophores of *P. fuligena* are fasciculate, two to eight celled, and 20–210 × 3–6.5 μm in length and width, respectively (13). Yamada (16) cultured the fungus and reported that the pathogen grew poorly on artificial media but grew well on Fermi agar and sporulated well on tomato plant decoction agar. No other reports have indicated inducing sporulation of this fungus in pure culture. The objectives of our studies were to isolate and assess the growth and sporulation of the fungus in pure culture and inoculate tomato plants under controlled conditions.

MATERIALS AND METHODS

Identification, isolation, growth, and conidial morphology. Black leaf mold was observed for several seasons (1989-1991) at the Asian Vegetable Research and Development Center, Taiwan (AVRDC), and in farmers' tomato fields. Cultures of the fungus were established from leaf lesions of field-grown tomato plants in February and March 1990. Conidia from infected leaf lesions were directly streaked on 2% water agar (WA) medium and incubated at 28 C for 16–18 hr. Germinated conidia were individually transferred to 9-cm-diameter culture dishes of potato-dextrose agar (PDA) (4).

Tomato leaves of six herbarium specimens infected with black leaf mold (US 0436496-0436501) obtained from the National Fungus Collection in Beltsville, MD, were compared with our own collections of infected leaves. Several leaf lesions, 5–10 mm in diameter, were cut from infected tomato leaves and placed adaxial side down in a drop of cotton blue on a microscope slide. The leaf disks were removed 30–60 s later and observed under a light microscope (×250). The length, width, and the number of septations of 500 conidia were recorded.

Conidia obtained from washing leaf lesions were seeded on 2% WA in 9-cm-diameter culture dishes and incubated in the dark at 28 C for 13 hr. Every other hour, beginning at 3 hr, one dish was removed and flooded with 5–8 ml of a fixative (50 ml of formalin, 50 ml of acetic acid, and 900 ml of water). Germination and germ tube lengths from the tip, base, or from cells between the tip and base of 50 randomly selected conidia were recorded at each time interval. The experiment was repeated and the data combined to make two replications ($n = 100$). Data were analyzed by analysis of variance (ANOVA) and means were separated by least significant difference ($P \leq 0.05$).

Effect of media and temperature on fungal growth. Conidia from a single leaf lesion were smeared on WA. After 4–6 hr of incubation at room temperature, germinated conidia were individually transferred to PDA, V8, and carrot agar (CA) (4), and tomato-oatmeal agar (TOA) in 9-cm-diameter culture dishes. TOA was made by boiling 50 g of shredded tomato leaves and 15 g of oatmeal separately. The suspensions were sieved through two layers of cheesecloth, mixed before adding 25 g of agar per liter of water, and autoclaved at 121 C for 30 min. There were four dishes (replications) for each medium arranged in a completely random design. Cultures were incubated at 28 C in darkness. Colony diameter was measured after 7, 14, and 21 days. Data were analyzed for each date and means compared as previously described.

To test the effect of temperature, conidia were individually transferred to culture dishes containing either PDA or TOA. Cultures were incubated in the dark from 10 to 34 C at intervals of 4 C. There were three replications for PDA and four replications for TOA for each temperature. The diameter of colonies was measured after 7, 14, and 21 days. Data were analyzed as previously described. After 3 wk, the dishes were flooded with 10 ml of water and the colony surface brushed to release conidia. Conidia were counted with a hemacytometer.

Effect of relative humidity and heat treatment on conidial germination. Fifteen milliliters of the following saturated solutions was poured into culture dishes: $MgCl_2 \cdot 6H_2O$, $Ca(NO_3)_2 \cdot 4H_2O$, $(NH_4)_2SO_4$, KCl, KNO, KH_2PO_4, K_2SO_4, and H_2O, representing 32.5, 47, 80, 84.5, 91, 93.5, 96.5, and 100% relative humidity (RH), respectively (4). One drop of a conidial suspension, which was prepared by rinsing infected leaf lesions (approximately 1×10^2 conidia per milliliter), was placed on a glass slide and allowed to air dry (10–15 min). After air drying, the slides were placed inside culture dishes on top of 1-cm-high glass rings. All dishes were sealed with Parafilm and incubated at 28 C. There were three replications for each humidity level. Germination of conidia was recorded 3, 6, and 24 hr after incubation. Treatments where conidia did not germinate were not included in the ANOVA.

The effect of heat treatment on germination of conidia was tested by placing tomato leaf lesions (5–10 cm diameter) into 10 ml of distilled water in a test tube. After agitation for 1 min, aliquots of 1 ml of the conidial suspension were transferred to 24 test tubes and then incubated for 30, 45, 60, and 120 min in a 40-C water bath. In a similar experiment, aliquots of 1 ml were transferred to test tubes and placed in water baths at 40, 45, 50, and 55 C. The test tubes were incubated for 5, 10, and 15 min at the appropriate temperature. There were three replications for each temperature and time combination. At the end of each time period, a drop of the suspension was placed on a glass slide, air-dried, and then placed at 100% RH inside a 9-cm-diameter culture dish. Dishes were sealed with Parafilm and incubated at 28 C. Germination of conidia was recorded for both experiments after 24 hr. Data were

Fig. 1. Sporulation of *Pseudocercospora fuligena* on field-grown tomatoes in a noninoculated fresh market hybrid regional yield trial at the Asian Vegetable Research and Development Center in Taiwan.

analyzed and means separated as previously described.

Inoculation of plants. Two-week-old cultures grown on PDA were cut into 6-mm-diameter pieces, streaked on TOA, and incubated for 2 wk at 28 C. Ten milliliters of sterile water was poured on the colonies to dislodge conidia and the suspension was decanted through a 40-μm-mesh copper sieve. Concentration of the stock suspension was determined with a hemacytometer and adjusted to 0.5, 5, 50, 500, and 5,000 conidia per milliliter. Three-week-old tomato plants (breeding line CL 5915-153D_4-3-3-0) were atomized until runoff with each concentration or water, as a control, with a hand sprayer. Four plants (replications) were treated with each conidial concentration and plants were arranged in a completely random design inside a growth room set at 28 C, 98 ± 2% RH, and a light intensity of 49.3 $\mu E \cdot m^2 \cdot s$. Percent leaf area infected was assessed 14 days after inoculation by estimating on a plant-by-plant basis the area of leaves covered with lesions. All subsequent inoculations of tomato used the same breeding line and same growth room conditions unless otherwise stated.

In a growth room, 3-wk-old tomato plants were atomized with a conidial suspension of 500 conidia per milliliter. Five plants were removed after 1, 2, 4, and 10 days and placed in the greenhouse. Five plants were also inoculated in the greenhouse without incubating in the growth room. Percent leaf area infected was recorded 14 days after inoculation. Data from plant inoculations were analyzed and means separated as previously described.

A suspension of 500 conidia per milliliter was atomized until runoff on 3-wk-old tomato plants, seedlings of *Solanum nigrum* L. (transplanted from outdoors to pots), and 4-wk-old *Nicotiana benthamiana* Domin. There were three replications per plant species. Conidia of *P. fuligena* harvested from infected leaves of *S. nigrum* were diluted with water and then atomized on 3-wk-old tomato plants. Inoculated plants were placed inside a growth room at 28 C and 98 ± 2% RH. Pathogenicity was recorded after 2 wk.

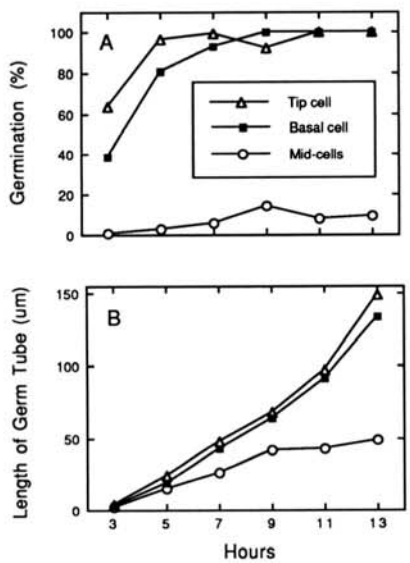

Fig. 2. (A) Percent germination and **(B)** germ tube lengths of *Pseudocercospora fuligena* conidia from tip, middle, and basal cells over a 13-hr period grown on water agar ($n = 100$ conidia).

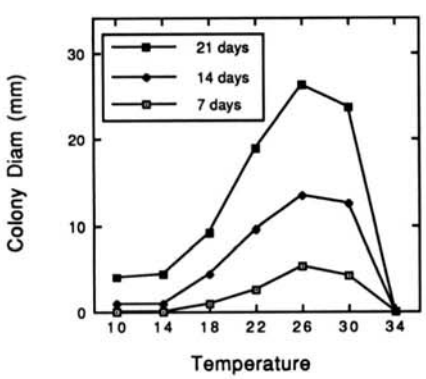

Fig. 3. The effect of temperature on growth of *Pseudocercospora fuligena* on tomato-oatmeal agar after 21, 14, and 7 days of incubation.

RESULTS

Identification, isolation, growth, and conidial morphology. Black leaf mold was observed throughout the year. Initial symptoms were observed on lower leaves causing some defoliation and advanced up the canopy to newly developed leaves. The most striking field sign was the abundant sporulation that occurred from lesions on both sides of the leaf (Fig. 1).

The pathogen was identified as *P. fuligena* using taxonomic keys and descriptions (2,6). One of the isolates was sent to Dr. Hsieh at Chung Hsing University, Taichung, Taiwan, who reconfirmed its identity as *P. fuligena*. Conidia were subhyaline to pale olivaceous, cylindrical to cylindrical obclavate, and straight to slightly curved. The tip cells were rounded with basal cells long obconic to long obconical truncate. The length of conidia was 9–137 (mean 85.1) × 3.5–6.1 μm (mean 4.7). The number of septations per conidia was 2–27 (mean 9.5). The hilum was unthickened. Some conidia were branched or forked at the tip cell. Conidia from the herbarium specimens were similar in shape and size to our own collection.

Conidia germinated most frequently from tip and basal cells, and germination was significantly less from cells between the tip and base (Fig. 2A). There was no significant difference between the frequency of germination from tip or basal cells after 5 hr. Germ tubes from tip and basal cells were significantly longer than germ tubes from the cells between the tip and base (Fig. 2B).

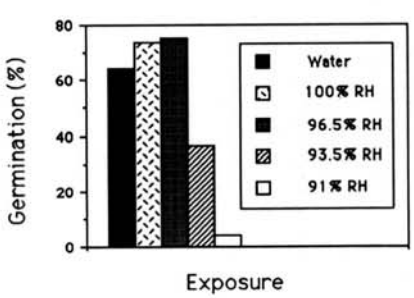

Fig. 4. Germination of *Pseudocercospora fuligena* conidia 24 hr after incubation in free water and different relative humidities (RH).

Effect of media and temperature on fungal growth. There was no significant difference in colony diameter on the four media tested. Colony diameter averaged 6, 13, and 22 mm (1.1 mm/day) at 7, 14, and 21 days, respectively.

The optimum temperature for growth was 26 C at each of the three sampling times (Fig. 3). There was no significant difference between colony diameter on PDA or on TOA and data were combined. No growth occurred at 34 C. There was no significant difference in colony diameter at 10, 14, or 34 C until 21 days. The average number of conidia produced on TOA was 6.1×10^4 per culture dish, but insufficient numbers of conidia were produced on PDA to make a valid estimate.

Effect of relative humidity and heat treatment on conidial germination. Forty-two percent of conidia germinated in free water after 6 hr. This was significantly higher than at lower RH where <6% of the conidia germinated. After 24 hr, germination at 96.5 and 100% RH and in free water was 74, 75, and 64%, respectively, which was significantly greater than 36 and 4% germination at 93.5 and 91% RH, respectively (Fig. 4). Germination did not occur at RH below 84.5% after 24 hr.

After 15 min of exposure at 40 C, more than 98% of the conidia germinated; however, germination was significantly reduced to 47 and 39% when kept for 30 and 45 min, respectively. Less than 1% of conidia germinated when exposed for 60 min and none germinated when exposed for 120 min. Conidia exposed to 50 or 55 C for 5, 10, or 15 min did not germinate. Conidia germinated at 40 C (98%) and at 45 C (84%) with no differences in germination attributable to exposure time.

Inoculation of plants. The lower inoculum concentrations had significantly less leaf area infected (<8%) than the 46 and 88% that occurred when 500 and 5,000 conidia per milliliter were inoculated on plants. Infection was greatest on plants that were kept at high humidities for a longer period of time. Plants inoculated and incubated in the greenhouse had 51% leaf area infected, which was not significantly different from the 54% leaf area infected that occurred on plants kept at high humidity

for 24 hr. Plants kept for 2 days had 87% leaf area infected, whereas those plants that were incubated longer had an average of 96% leaf area infected and were not significantly different from each other.

Conidia isolated from tomato infected *S. nigrum*, and conidia isolated from *S. nigrum* infected tomato. Lesion development and sporulation occurred on both plant species. No infection was observed on *N. benthamiana*.

DISCUSSION

Early reports of Cercospora leaf mold on tomato have described other species such as *C. canescens* Ellis & G. Martin (14). Chupp (2) considered these earlier reports of other *Cercospora* species that caused indistinct leaf lesions on tomatoes to be *P. fuligena*. Black leaf mold was first reported in the United States in 1974 (1), and based on earlier reports (3,5, 8,9,15,16), the disease occurs widely throughout Asia. Conidial sizes ranged from 13.3 to 170 × 2.5 to 6.5 μm (1,6,7,12,16). The conidial sizes we report are within these ranges, but we found up to 27 septations per conidia, which is greater than other reports of up to 15 septations (6,7,16). Conidia morphology varies, and more precise studies are needed to confirm if the variation is attributable to geographic location, host, or culture medium.

Black leaf mold and leaf mold, caused by *Fulvia fulva* (Cooke) Cif. (syn. *Cladosporium fulvum* Cooke), have similar symptoms. Leaf lesions of both turn pale-green to yellow on the upper leaf surface and both fungi sporulate most profusely on the lower leaf surface (13). Leaf mold appears olive-green to grayish purple in color whereas black leaf mold is initially white in color and turns gray to black with age (13). The two fungi can be readily distinguished under the microscope by morphology of conidia; however, because the field symptoms are similar, some past reports may have confused the two diseases. Further investigations are needed to determine the geographical ranges and the importance of these two diseases in areas of subtropical and tropical countries. In some cases, both diseases may have been reported, but no in-depth studies have been conducted. To reduce the possibility of confusing the common name of this disease with other leaf molds and spots on tomato, we suggest that the disease caused by *P. fuligena* be referred to as black leaf mold instead of Cercospora leaf mold or leaf mold.

Conidia of *P. fuligena* germinated after 3 hr on 2% WA at 28 C in our experiment and were reported to germinate in 5 hr on tomato leaves (10). In our studies, conidia did not germinate below 84.5% RH, but some germinated at 91% RH, indicating that disease could possibly develop without much, if any, dew period or free moisture. Under conditions at AVRDC, the disease is most prevalent in seasons without much rainfall and also can be severe in seasons of high rainfall when plants are grown under plastic cover or in the greenhouse.

The cross-inoculation studies using isolates from *S. nigrum* and tomato were successful in showing that *S. nigrum* was an alternative host. How important this host is in regard to the overseasoning of the fungus or to the epidemiology of the disease is not known. We know of no other reported hosts of *P. fuligena*.

Except for the variety Floradel (1), there are no other reported varieties with resistance to black leaf mold. Without fungicide control, we have observed 54–86% leaf area infected on several varieties and advanced breeding lines in replicated yield trials at AVRDC. Although there are no experimental reports showing the relationship of black leaf mold severity to yield loss, we feel that this disease may be more economically important than has been previously reported.

LITERATURE CITED

1. Blazquez, C. H., and Alfieri, S. A., Jr. 1974. Cercospora leaf mold of tomato. Phytopathology 64:443-445.
2. Chandrasrikul, A. 1962. A preliminary host list of plant diseases in Thailand. Thailand Dep. Agric. Tech. Bull. 6 pp.
3. Chupp, C. 1953. A monograph of the genus *Cercospora*. C. Chupp, Ithaca, NY. 667 pp.
4. Dhingra, O. D., and Sinclair, J. B. 1985. Basic plant pathology methods. CRC Press, Inc., Boca Raton, FL. 355 pp.
5. Govinou, H. C., and Thirumalachar, M. J. 1954. Notes on some Indian Cercosporae. Sydowia 8:221-230.
6. Hsieh, W. H., and Goh, T. K. 1990. *Cercospora* and similar fungi from Taiwan. Maw Chang Book Company, Taipei, Taiwan. 376 pp.
7. Jain, A. C. 1955. Cercospora leaf spot of tomato. Sci. Cult. 21:42-43.
8. Johnston, A. 1960. A supplement to a host list of plant diseases in Malaya. CMI Mycological Papers 77. 30 pp.
9. Johnston, A. 1963. Host list of plant diseases in the New Hebrides. FAO Plant Protect. Comm. South East Asia Pac. Reg. Tech. Doc. 27. 9 pp.
10. Magda, T. R., and Quebral, F. C. 1970. Effect of exposure period in moist chamber on *Cercospora fuligena* spore germination, penetration, infection and sporulation on tomato. Philipp. Phytopathol. 6:75-82.
11. Mohanty, U. N., and Mohanty, N. N. 1955. Cercospora leaf mold of tomato. Sci. Cult. 21:269-270.
12. Roldan, E. F. 1938. New or noteworthy lower fungi of the Philippines Islands, II. Philipp. J. Sci. 66:7-17.
13. Sherf, A. F., and Macnab, A. A. 1986. Cercospora leaf mold. Pages 668-669 in: Vegetable Diseases and Their Control. John Wiley & Sons, New York.
14. Solheim, W. G., and Stevens, F. L. 1931. Cercospora studies II. Some tropical Cercosporae. Mycologia 23:365-405.
15. Turner, G. J. 1971. Fungi and plant diseases in Sarawak. CMI Phytopathological Papers 13. 55 pp.
16. Yamada, S. 1951. New disease of tomato (*Lycopersicon esculentum* Mill.) caused by *Cercospora* sp. Ann. Phytopathol. Soc. Jpn. 15:13-18.