Conidial Germination and Appressorial Formation of *Colletotrichum capsici* and *C. gloeosporioides* Isolates from Pepper

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ABSTRACT

Manandhar, J. B., Hartman, G. L., and Wang, T. C. 1995. Conidial germination and appressorial formation of *Colletotrichum capsici* and *C. gloeosporioides* isolates from pepper. Plant Dis. 79:361-366.

Conidial germination and appressorial formation of *Colletotrichum capsici* and *C. gloeosporioides* were examined on pepper fruits and in association with some inorganic and organic compounds. Conidial germination and appressorial formation were greater on immature green or ripe red fruits of pepper (*Capsicum annuum*) variety Long Fruit than in water droplets on glass slides. Conidial germination was significantly ($P = 0.05$) higher for both fungi as concentrations of either sucrose or KCl increased. Appressorial formation for *C. capsici* was highest when sucrose was 10 mM and for *C. gloeosporioides* at 0.1 mM. Appressorial formation was reduced and mycelia formed for both fungi at higher sucrose concentrations, but not at 1-100 mM of KCl. Among six compounds tested for induced conidial germination and appressorial formation, $CaCl_2$ and sucrose caused a significant ($P = 0.05$) increase, KCl an intermediate one, and $AlCl_3$ was lowest. β-resorcilic acid did not stimulate conidial germination and appressorial formation, and Fe-resorcilic acid stimulated conidial germination only for *C. capsici*. Three test melanin biosynthesis inhibitors at 1 μg per milliliter stimulated conidial germination but varied in causing appressorial formation for both fungi. Appressorial formation of *C. capsici* was completely inhibited by tricyclazole and stimulated by fthalide and isoprothiolane; appressorial formation of *C. gloeosporioides* was completely inhibited and stimulated by isoprothiolane and tricyclazole, respectively, and only slightly inhibited by fthalide. Inorganic and organic compounds that affect conidial germination and appressorial formation may play a role in the preinfection process of *Colletotrichum* spp. on pepper fruits.

Colletotrichum capsici (Syd.) E. J. Butler & Bisby and *C. gloeosporioides* (Penz) Penz. & Sacc. in Penz. are the two main causal agents of pepper (*Capsicum annuum* L.) anthracnose in the hot humid tropics of Asia (15). Conidia of *Colletotrichum* spp. often do not germinate in situ because of the presence of germination-inhibitors in the spore matrix (13), but will germinate after being washed or rain-splash disseminated (14). In order for conidia to infect plants, there are pre- and post-infection phases associated with disease development on the plant surface (19,21). Conidia are dispersed along with water droplets on the plant surface and germinate to form adhesive appressoria at the end of germ tubes (7) by means of hemicellulose mucilage (11) to initiate direct penetration. Water droplets on the plant surface cause an increase in conductivity that leaches compounds such as free sugars, amino acids, minerals, and antifungal substances from the fruit (23,24). Formation of appressoria is a prerequisite for direct penetration of the host surface. Appressoria also may play an important role in survival (4). Conidial germination, appressorial formation, and the formation of infection hyphae are three independent processes that interact with stimulators or inhibitors present in plant exudates. Red pepper fruit exudates promoted higher conidial germination of *C. gloeosporioides* than did green fruit exudates (6). In another study (1), green fruit exudates stimulated the formation of appressoria of *C. capsici* and *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk (1), suggesting that conidial germination and appressorial formation depended upon the quality and quantity of nutrients in the leachates. Ca^{+2} and K^{+} are major cations in plant leachates (24), while aluminum was reported to inhibit calmodulin that promotes Ca-intake in *Colletotrichum* spp. (25). An intermediate iron-binding compound of anthranilic acid, 2,3-dihydroxybenzoic acid, exuded from banana fruits, stimulated conidial germination and appressorial formation of *C. musae* (Berk. & M. A. Curtis) Arx (22). Melanin biosynthesis inhibitor, a curative systemic fungicide, interfered with appressorial melanization and reduced or destabilized infection peg formation (8,10,26). Relative importance of these cations and organic compounds in conidial germination and appressorial formation of *C. capsici* and *C. gloeosporioides* is not known. The precise mechanism and physiological process of conidial germination and appressorial formation of *Colletotrichum* spp. are not well understood. The objective of this study was to compare conidial germination and appressorial formation of *C. capsici* and *C. gloeosporioides* in association with inorganic and organic compounds, and on pepper fruits.

MATERIALS AND METHODS

One isolate each of *C. capsici* and *C. gloeosporioides* was obtained from infected red fruits of a field-grown hot pepper (*Capsicum annuum* L.) breeding line PBC 595 from the Asian Vegetable Research and Development Center (AVRDC), Shanhua, Tainan, Taiwan in August 1992. Single conidial isolates of each species were obtained by picking conidia from sporulating acervuli and placing them in water. A 0.1-ml conidial suspension was placed on acidified (pH 5) 2% water agar and spread throughout the dish. Single germinating conidia were selected with the aid of a dissecting microscope. Isolates were maintained in acidified (pH 5) potato-dextrose agar at 28 C. These isolates were frequently re-isolated from inoculated detached pepper fruits to maintain their pathogenicity.

For all experiments, glassware was dipped in concentrated HCl acid for 3 h and rinsed two or more times in sterile distilled water. Nail polish diluted 1:1 with acetone was used to make each of three 5-mm inner diameter circles on sterile microscope slides. Conidial suspensions of *C. capsici* and *C. gloeosporioides* were harvested from 5-day-old potato-dextrose agar cultures grown under 12-h light per day at 28 C by brushing the colony surface with a sterile artist brush with 10 ml of sterile distilled water. Conidial suspensions were passed through two layers of cheesecloth, resuspended in 50 ml of sterilized distilled water, and centrifuged at 15,000 rpm for 10 min before discarding the supernatant. This was repeated three times, after which suspensions were adjusted to 10^5 conidia per milliliter using a hemacytometer. All test compounds were sterilized at double the test concentration. Ten microliters of a test compound and the conidial suspension of either *C. capsici* or *C. gloeosporioides* were

Accepted for publication 19 December 1994.