Semiselective Medium for *Colletotrichum gloeosporioides* and Occurrence of Three *Colletotrichum* spp. on Pepper Plants

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ABSTRACT

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Inhibition of mycelial growth of *Colletotrichum capsici* and *C. gloeosporioides* was significantly ($P = 0.01$) less than that of *Alternaria* sp. and *Fusarium* spp. when grown on a semiselective medium, *C. gloeosporioides* pepper isolate medium (CGPIM) containing one-quarter strength potato-dextrose agar amended with fenarimol and vinclozolin at 5 μg/ml each, chloramphenicol and erythromycin at 6.5 μg/ml each, iprodione at 15 μg/ml, neomycin sulfate at 20 μg/ml, and tetracycline hydrochloride at 25 μg/ml. Fenarimol enhanced the detection of *C. gloeosporioides* as cream-yellow sporulating colonies formed around infected and/or infested pepper (*Capsicum* spp.) seeds. When pepper seeds were placed on CGPIM and wet filter paper, *C. capsici* occurred at equal frequencies, but the frequency of *C. gloeosporioides* was significantly ($P = 0.01$) higher on CGPIM than on wet filter paper. *C. capsici* was detected on 14.5% of the seeds from var. LSU Sport, while *C. gloeosporioides* detection was less frequent. *C. gloeosporioides* was isolated from 30 and 1% of diseased fruits harvested and stored for 130 and 225 days, respectively. CGPIM and wet filter paper were equally effective in evaluating the occurrence of *C. capsici*, but the occurrence of *C. gloeosporioides* and *Glomerella cingulata* appressoria was significantly ($P = 0.01$) higher on CGPIM than on wet filter paper. *C. capsici* was recovered more frequently than either *C. gloeosporioides* or *G. cingulata* on inoculated leaves.

Colletotrichum capsici (Syd.) E.J. Butler & Bisby and *C. gloeosporioides* (Penz.) Penz. & Sacc. in Penz. are the most important *Colletotrichum* spp. reducing marketable fruit yields of pepper (*Capsicum annuum* L. and *Capsicum frutescens* L.) in the tropics and the subtropics (8,12). *Colletotrichum* spp. infect plants by germinating conidia deposited on plant parts by splashing rain (24). Conidia germinate to form appressoria, which facilitate penetration of host tissue or serve as survival units (15,16). Appressoria of *C. graminicola* (Ces.) G.W. Wils. were shown to remain dormant until higher temperatures occurred after their formation (21). The occurrence and viability of appressoria of *Colletotrichum* spp. has not been reported on pepper foliage.

Colletotrichum spp. are seedborne in crop plants. *C. capsici* (5–9,17,22) and *C. gloeosporioides* (syn. *C. piperatum* Ellis. & Everh. in Halst.) (5,8,22) occur either externally or internally in pepper seeds. Survival of mycelia and stromata in infected pepper seeds has been reported (22). Moist filter paper is commonly used to detect seedborne *Colletotrichum* spp. (9,12,18). In preliminary studies, *C. capsici* grew from pepper seeds and other plant parts when placed on wet filter paper, but slow-growing *C. gloeosporioides* was not detected (J. B. Manandhar, *unpublished*). Fast-growing organisms such as *Alternaria* and *Fusarium* spp. and bacteria often interfere with the detection of slow-growing organisms. Semiselective media, made by incorporating certain fungicides and antibiotics to inhibit growth of fungi and bacteria, were used to detect other *Colletotrichum* spp. (7,10). In addition, a selective medium differentiated two citrus strains of *C. gloeosporioides* (1,2). A semiselective medium to detect *C. gloeosporioides* and *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk in pepper seeds and other plant parts would be useful for monitoring fungal survival and studying host-pathogen interactions. The objectives of this study were to develop a semiselective medium to detect *C. capsici*, *C. gloeosporioides*, and *G. cingulata* on pepper leaf disks, petioles, and seeds, and to determine their occurrence on seeds and inoculated pepper leaves.

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MATERIALS AND METHODS

C. capsici (conidia 19.8–28.3 × 2.7–4.8 μm, mean 23.7 × 3.7 μm), a nonteleomorphic isolate of *C. gloeosporioides* (conidia 11.1–18.5 × 2.7–5.0 μm, mean 15.5 × 3.6 μm), and *G. cingulata* (formed glomerate perithecia on pepper plant parts, anamorph *Colletotrichum* sp., conidia 11.1–17.7 × 3.5–6.5 μm, mean 14.4 × 4.8 μm) were originally isolated from hot red pepper fruits of line PBC 595 grown during August 1992 at the Asian Vegetable Research and Development Center (AVRDC), Shanhua, Tainan, Taiwan. Single-conidial isolates of the *Colletotrichum* spp. were maintained on slants of acidified (pH 5) potato-dextrose agar (PDA) at 28 C. The semiselective medium consisted of a basal medium, one-quarter strength PDA (10 g of PDA and 15 g of agar in 1 L of water). To suppress bacterial growth, chloramphenicol and erythromycin at 6.5 μg/ml each, neomycin sulfate at 20 μg/ml, and tetracycline hydrochloride at 25 μg/ml were added when the basal medium cooled to 50 C after autoclaving. In addition, iprodione (Rovral 50W), 15 μg a.i./ml; fenarimol (Rubigan 11.76%E), 5 μg a.i./ml; and vinclozolin (Ronilan 50W), 5 μg a.i./ml, were added to suppress fungal growth other than *Colletotrichum* spp. Stock solutions of chloramphenicol, erythromycin, and tetracycline HCl were prepared in 10% methanol; iprodione, fenarimol, and vinclozoline in 20% methanol; and neomycin sulfate in distilled water. All were filter-sterilized through a 0.2-μm Nalgen filter. The basal media with combinations of fungicides and antibiotics were tested to develop the best semiselective medium, which was named *C. gloeosporioides* pepper isolate medium (CGPIM).

Inhibition of mycelial growth on fungicide-amended media. Three-millimeter-diameter disks from the margins of 3- to 5-day-old colonies on PDA of an isolate of an *Alternaria* sp., two isolates of *Fusarium* spp. (isolated from pepper seeds), and *C. capsici* and *C. gloeosporioides* (one isolate of each previously mentioned and five isolates of each from the AVRDC pepper isolate collection) were transferred to the middle of 9-cm-diameter plastic plates containing either basal medium and antibiotics (control) or basal medium amended with either iprodione, iprodione + fenarimol, or iprodione + fenarimol + vinclozolin. Inoculated plates were incubated under 12/12-h day-night cycles at 28 C. Three plates were used as replicates. The colony diameter was measured at 5 and 7 days. Percent inhibition of the mycelial growth was calculated: [(colony diameter of control − colony diameter of amended medium)/colony diameter of control] ×