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BRIEF COMMUNICATION

Culture conditions affect colonization of watermelon by *Colletotrichum orbiculare*

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Abstract

Area of lesions produced by *Colletotrichum orbiculare* on watermelon (*Citrullus lanatus*) seedlings was not affected by amount of culture medium on which the fungus was grown, and decreased as length of time the fungus was in culture increased. Internal integrity of *C. orbiculare* spores appeared degraded, and this was coincident with decreases in lesion area.

Additional key words: age, *Citrullus lanatus*, culture, length of incubation, medium, potato dextrose agar.

Spores of pathogenic fungi must be physiologically capable of germinating (Schisler *et al.* 1991, Leite and Nicholson 1993), and adhering to tissues (Sela-Buurlage *et al.* 1991) to be effective. The germinating spore must also be able to overcome host defenses (Nicholson *et al.* 1986). *Colletotrichum orbiculare* (Berk. & Mont.) Arx, a causal agent of anthracnose on watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), can cause reductions in yield (Amin and Ullasa 1981). Lesions on foliage reduces the canopy, affects photosynthesis, and exposes fruit to sunburn (Peregrine and Bin Ahmand 1983).

Age of spores affects germination (Griffin 1993). More vacuoles, and fewer glycogen deposits and lipid bodies, were found in aging spores of *C. graminicola* (Ces.) Wils. (Mims *et al.* 1995). Changes occurring with aging may cause depletion of stored reserves, and reduce the spore's ability to germinate and infect plant tissues (Weber and Hess 1974). Objectives of this study were to determine how culture conditions, *i.e.* amount of medium and age of the culture, affected colonization of watermelon by, and internal changes in, spores of *C. orbiculare*.

Watermelon, *Citrullus lanatus* (Thumb.) Matsum. & Nakai cv. Sugar Baby, seeds were planted in potting soil (Redi-Earth 3CF, *Grace Sierra*, Milpitas, USA) in 3.5 dm^3 pots. *C. orbiculare* (Race 6, ATCC #15471) was cultured on 10 or 20 cm^3 of potato dextrose agar (PDA; *Bacton Dickinson*, Cockeysville, USA) in plastic Petri dishes (100 × 15 mm; *VWR Scientific*, Willard, USA) under 12-h photoperiod (cool-white fluorescent tubes *Dura-Test*, Fairfield, USA; irradiance at 25 cm of 4.6 W m^{-2} and day/night temperature of 23 ± 0.5 °C. After 7-, 14- or 21-d of incubation cultures were flooded with 10 cm^3 of sterile deionized water, and spores were dislodged with a sterile camel hairbrush. Spore suspensions were adjusted to 10^6 spores cm^{-3} with water, and placed in atomizers. All surfaces of plants were sprayed at the expanded third-leaf stage until run-off with the spore suspensions.

Inoculated plants were placed in a mist chamber. After 1 h, misting (10 s per 30 min) was begun, and the cycle continued for 24 h. Plants were removed from the mist chamber and maintained in a greenhouse for 7 d at a time of year when day length exceeded 12 h. After 7 d cotyledons, and first- through fourth-true leaves, were removed from plants, scanned with an *Olympus* video system (*Hitschfel Instruments*, St. Louis, USA), and the percent of lesion area on cotyledons, and true leaves, was determined with *Optimas* software (*Meyer Instruments*, Houston, USA). The design was a randomized complete block with three plants in each of five replications. Data were analyzed with the General Linear Models procedures in *SAS* (ver. *6.12*, *SAS*

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Institute, Cary, USA).

Spores were stained with acridine orange (Yamamota and Uchida 1982). Ten fields, on 5 slides containing spores from each combination of PDA amount and length of incubation, were viewed with an *Olympus BX60* microscope using reflected-fluorescence with the WBV filter cube, or Nomarski differential interference contrast microscopy (*Olympus, Hitschfel Instruments*, St. Louis, USA). Images of typical spores, observed with an objective and ocular combination that provided 1000× magnification at the focal plane, were captured using *Optimas 5* software (*Optimas*, Bothell, USA). Spore condition was classified on the condition of the nucleus and distribution of DNA and RNA containing organelles.

C. orbiculare can infect when viable inoculum and appropriate environmental conditions are present, and the

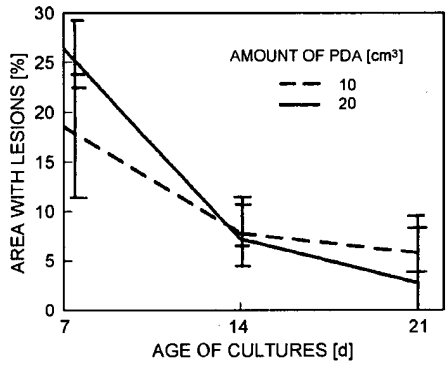

Fig. 1. Area with lesions on cotyledons and true leaves of plants inoculated with spores of *C. orbiculare* from 7-, 14-, or 21-d old cultures incubated on 10 or 20 cm^3 of PDA. *Bars* are confidence intervals.

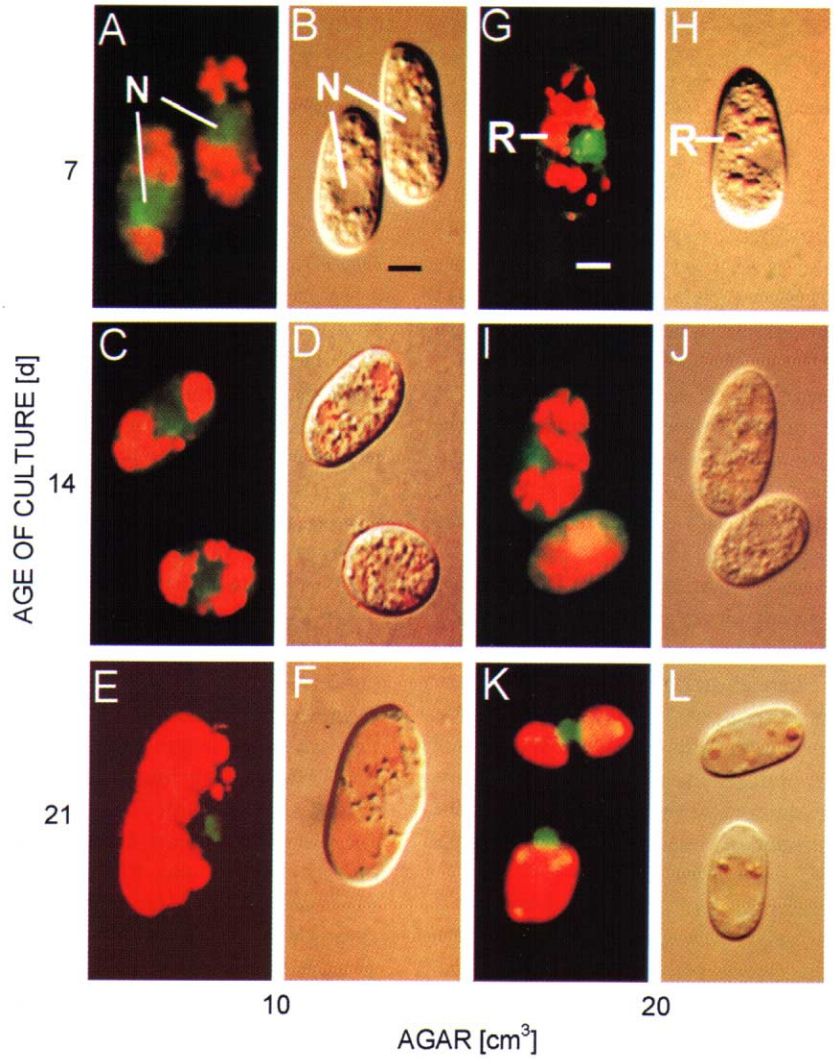

Fig. 2. Spores of *C. orbiculare* from 7-d- (*A, B, G, H*), 14-d- (*C, D, I, J*), and 21-d- (*E, F, K, L*) old cultures incubated on 10 cm^3 (*A - F*) or 20 cm^3 (*G - L*) of PDA visualized with reflected-fluorescence (*A, C, E, G, I, K*) or Nomarski differential interference contrast (*B, D, F, H, J, L*) microscopy. Green fluorescence indicates DNA and red, or red-orange, fluorescence indicates RNA. N - nucleus, R - RNA-containing organelle. *Bars* in *B* and *G* are for all images and equal 2 μm.

plant's ability to resist is reduced or absent. Susceptibility to infection, which is under genetic control (Van der Plank 1968, Goodman and Novacky 1994), changes as plants develop (Heath 1984). The area with lesions was more dependent on age of the culture than on amount of PDA on which spores were produced (Fig. 1). Approximately 40 % of the area with lesions was on cotyledons.

Changes of distribution of DNA and RNA in spores was observed (Fig. 2). Green fluorescing nuclei became smaller and/or dimmer in spores from cultures incubated for longer times. In spores from 21-d old cultures, on 10 cm^3 of PDA, the red-orange fluorescing RNA containing areas coalesced. For spores from 21-d old cultures, on 20 cm^3 of PDA, RNA containing areas were found in discrete red-orange fluorescing packets. Not all spores lost the ability to germinate and infect tissue. Russo and Pappelis (1984) found that non-germinated spores of *C. circinans*, lost dry mass over time, and they suggested that the dry mass was used by other spores. This suggests a way by which fungi conserve germplasm.

Spores of *C. orbiculare* are formed in a cirrus from an acervulus. As spores age their ability to infect may be reduced. The visible differences between spores from younger and older cultures indicated a degradation of the organelles, especially those containing RNA. Van Etten *et al.* (1983) described the importance of RNA to germination. If cellular integrity is disrupted there will likely be less capability to germinate even if RNA levels are not diminished. The reduced integrity of internal structure in spores accompanying length of incubation suggests that membranes had collapsed. This was coincident with decreases in area on tissues with lesions when inoculation was with spores from older cultures.

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