Production of Conidia by *Cercospora kikuchii* in Culture

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ABSTRACT


Few attempts to induce in vitro sporulation of *Cercospora* spp. have been successful (1,4-8). Workers have subjected isolates to manipulations of nutrition, light, and temperature (2,6-8,11) with only occasional success. Calpouzos and Stallknecht (2) concluded that the erratic sporulation of *B. beticola* in culture partially was due to the nature of the medium and partially to other factors. Nagel (8) obtained conidia of 12 species in culture by conidial transfers from host plants. To maintain sporulation, it was necessary that conidia be transferred to fresh media at 3- to 6-day intervals. Two of the six sporulating isolates representing six species were avirulent. Goode and Brown (4) made 1,500 conidial suspensions in 250 ml of distilled water, macerated 5 min in a Waring Blendor, filtered on four layers of cheesecloth, and squeezed to extract all of the filtrate. The volume of the filtrate was brought to 1 liter. Jones (5) used the selective subculturing method of Calpouzos (1) and obtained a sporulating isolate of *C. kikuchii* which was avirulent. Best results were obtained when the fungus was cultured on media prepared from host tissues. Nagel (8) obtained sporulation of *C. beticola* on a sugar beet leaf agar. Diachun and Valleeau (3) reported sporulation of *C. nicotianae* on tobacco leaf agar.

This study of in vitro production of conidia by *Cercospora kikuchii* was conducted to obtain a supply of conidia sufficient for screening soybean cultivars and breeding lines for a source of resistance to *Cercospora* leaf blight.

MATERIALS AND METHODS

Twenty isolates of *C. kikuchii* were obtained from spores picked from a drawn capillary tube from leaves, seeds, and stems of soybeans that had been kept in a moisture chamber 3-4 days at 23–27 C. Spores were placed on Difco potato dextrose agar and incubated at 24 C.

Various media were evaluated for ability to support sporulation. Several standard laboratory media (Difco potato dextrose agar, Difco lima bean agar, Difco bean pod agar, V-8 juice agar, and cornmeal agar) were tested. Plant decotion agars were prepared from leaves of carrot and soybean and from aboveground parts of alfalfa, corn, cotton, and wheat. Leaf decoction agar from immature soybeans yielded abundant conidiophores. The fungus sporulated sparsely on SSPA in continuous darkness, but sporulated profusely when exposed to several light regimes. Sporulation was most abundant in cultures exposed 8 hr/day to illumination from Gro-Lux lamps at room temperature. More spores were produced on cultures grown from spore transfers than from those grown from mycelial transfers. Transfer of spores by tapping the bottom of an inverted petri plate containing a sporulating culture over a plate of fresh SSPA medium produced the maximum numbers of spores per plate.

Additional key words: Glycine max.
RESULTS

Isolates of C. kikuchii maintained on PDA showed only a typical dense mat of mycelium with a reddish-purple pigment in the medium surrounding the colonies. Similar types of growth were observed on other standard laboratory media. Only vegetative growth of the fungus occurred on media prepared from plant material other than soybean. Although no spores were produced on soybean leaf decoction agar, a moderate number of conidiophores developed within the colonies. Colonies produced on SSPA were composed primarily of conidiophores. Sporulation was as profuse on autoclaved as on steamed media. Cultures incubated in darkness on SSPA produced no spores. Minimal sporulation occurred on cultures exposed to NRL and on those exposed 10 or 100 sec/day to long- or short-wave length UV radiation. Abundant spore production occurred in cultures incubated in NRL plus 8 hr/day exposure to Gro-Lux lamps. Mycelium transferred with forceps produced small, dense colonies with a few conidia on the periphery. Transfer of mycelial plugs resulted in a slight increase in colony size and production of proportionately more conidia. Streaking a spore suspension on the medium resulted in colonies with slight vegetative growth and more conidia than those of the previous methods. Maximum number of spores per area of culture was obtained by tapping the bottom of an inverted plate of a sporulating culture over a plate of fresh medium. Small colonies developed uniformly over the medium of each plate, and these produced numerous conidia within 7 days.

Ten to 14 days after inoculation of soybean plants with a spore suspension of C. kikuchii, reddish-purple, angular-to-irregular lesions developed on both upper and lower surfaces of the leaves. The lesions varied from pinpoint spots to areas up to 1 cm in diameter. Later, reddish-purple, sunken lesions one to several millimeters long developed on the stems. Lesions appeared on the new leaves as they developed.

DISCUSSION

In artificial culture, Cercospora kikuchii sporulates most abundantly under conditions that resemble those under which it sporulates in nature. Sporulation was not observed on carrot leaf decoction agar as reported by Kilpatrick and Johnson (6) nor on V-8 juice agar as reported by Roy and Abney (9,10). Abundant sporulation of C. kikuchii occurred in cultures on SSPA exposed to alternating dark and light periods and 8 hr/day to Gro-Lux lamps, but it did not occur in continuous darkness. Kilpatrick and Johnson (6) observed that cultures exposed to light sporulated more abundantly than did those in total darkness. Trione and Leach (12) concluded that near-ultraviolet radiation enhanced sporulation of some fungi. Sparse spore production by NRL and NRL + 10 or 100 sec/day of long or short wavelengths of UV radiation and abundant sporulation with Gro-Lux lamps indicate that the lamps provide sufficient UV radiation for spore production.

Transfer of spores rather than mycelium resulted in cultures that produced more conidia. Nagel (8) and Calpouzos (1) obtained similar results with other Cercospora spp. Goode and Brown (4) postulated that the ability of some Cercospora spp. to sporulate for a few generations in artificial culture indicates that those isolates have a genetic component for sporulation. They suggested that the problem of maintaining sporulating Cercospora isolates may be explained by a genetic model based on heterokaryosis. Twenty of our isolates of the fungus sporulated on SSPA and we have maintained two sporulating isolates for up to 2 yr. Apparently there is a nutritional factor in senescent soybean plants that promotes sporulation of C. kikuchii.

LITERATURE CITED