Production of Conidia by *Cercospora kikuchii* in Culture

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ABSTRACT

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Only vegetative growth of *Cercospora kikuchii* occurred on standard laboratory media, on agar media prepared from decoctions of carrot leaves, and on agar media prepared from immature and senescent tissues of alfalfa, corn, cotton, and wheat. Leaf decoction agar from immature soybeans yielded few conidiophores, but agar media prepared from senescent soybean plants (SSPA) 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.

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 *C. 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 isolations of *C. citrullina* and obtained two which were pathogenic, sporulated on potato-dextrose agar, and remained stable for 2 yr.

Kilpatrick and Johnson (6) reported that carrot leaf decoction agar effectively induced sporulation of *Cercospora kikuchii* (Matsumoto & Tomoyasu) Chupp and observed that the cultures exposed to daylight sporulated more abundantly than did those exposed to continuous darkness. Roy and Abney (9) reported erratic spore production of *C. kikuchii* on V-8 juice agar. 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 Valleau (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 with 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 decoction agars were prepared from leaves of carrot and soybean and from aboveground parts of alfalfa, corn, cotton, and wheat plants according to the method of Kilpatrick and Johnson (6) modified by allowing the plants to dry on a greenhouse bench and by using 100 g of plant material per liter of media. Other media, which will be called "senescent plant agars" were prepared from field-collected senescent plant tissue of alfalfa, corn, cotton, and wheat. The senescent plant materials were ground to a powder in a Wyley mill. One hundred grams of the material was suspended 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 L with distilled water that contained 20 g of agar. The media were sterilized either by steaming for 40 min or by autoclaving at 104 kPa (15 psi) at 121 C for 20 min. Approximately 25 ml of media was poured into plastic petri plates. Cultures were placed in a dark incubator at 28 C, and observed for sporulation after 7 days.

Cultures were incubated under various lighting conditions to determine whether light affected sporulation. Mycelial plugs (6 mm in diameter, cut with a cork borer) were transferred to senescent soybean plant agar (SSPA) in culture plates and incubated at 28 C in darkness, normal room light (NRL) at 23–28 C, exposed for 10 or 100 sec/day to long- or short-wavelength ultraviolet (UV) radiation, and NRL + 8 hr/day exposure to three GTE Sylvania 20T12 Gro-Lux lamps 61 cm above cultures. Plastic culture plates were used because glass lids would filter out UV radiation.

Inoculum virulence was tested by inoculating soybean plants in the fully developed unifoliolate leaf stage grown in 45 × 60 cm flats filled with a sterilized mixture of sand, peat, vermiculite, and soil (1:1:1:1, v/v). Inoculum was prepared from spores of 6-day-old cultures produced on SSPA. Ten milliliters of 0.25% Tween-20 solution was added to each plate and the surface of the cultures was rubbed gently with the fingers to detach the spores. Each plate was rinsed twice with an additional 10 ml of the Tween-20 solution, and the spore suspension was filtered through a Millipore filter. Spores were washed from the filter with 0.25% Tween-20 solution and concentrated to 25,000 spores/ml. Spore suspensions were atomized onto soybean plants to run-off. Plants were placed in a mist chamber for 4 days at 22–31 C and then placed on a greenhouse bench to allow symptom development.

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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.

Four techniques were used to transfer the fungus to SSPA for spore production. Cultures were 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.*

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