Production of Cercosporin and Colonization of Soybean Seed Coats by *Cercospora kikuchii*

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ABSTRACT

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Isolates of *Cercospora kikuchii* produced cercosporin in culture and soybean seed coats. *C. kikuchii* isolates recovered from purple-stained soybean seeds were divided into three groups based on colony coloration on potato-dextrose agar (PDA). Ckcp isolates produced purple coloration, Ckcy isolates a yellow background due to lipids, and Ckcc isolates red cercosporin. Among 72 isolates from 72 seeds studied, 75% were Ckcp, 13% Ckcy, and 12% Ckcc. One isolate representing each group was studied further. The Ckcp isolate produced significantly higher amounts of cercosporin on PDA than either the Ckcy or Ckcc isolates. Production of cercosporin by the three isolates in seed coats was in the order of Ckcc > Ckcp > Ckcy. All isolates inoculated on soybean seeds in culture caused purple stain on the seed coats. The extent of purple stain on seed coats coincided with the extent of colonization by *C. kikuchii*. A positive relationship ($r^2 = 0.96$) was found between the extent of purple seed coat stain and the amount of cercosporin per 100 μg of seed coat tissue in situ. Extracts from *C. kikuchii* grown on PDA contained cercosporin, isocercosporin, and lipids. However, lipids were not detected from soybean seed coats inoculated with *C. kikuchii* or naturally infected purple-stained seed coats, suggesting that the pathogen produced lipids only in culture and that lipids were not involved in seed colonization. This is the first report on the estimation of cercosporin from *Cercospora* in plant tissues with reference to pathogenesis. The greater occurrence of isolates producing high amounts of cercosporin, and the correlation between cercosporin production and the extent of purple stain or *C. kikuchii* colonization, indicated a role for cercosporin in facilitating soybean seed coat tissue colonization by *C. kikuchii*.

Cercospora kikuchii (T. Matsumoto & Tomoyasu) M.W. Gardner causes purple seed stain of soybean (*Glycine max* (L.) Merr.) (9,13). Purple seed stain is one of the most common seedborne fungal diseases of soybeans and is associated with reduced yields and seed size (16,29). Cercosporin was first detected in *C. kikuchii* mycelia and in purple-stained soybean seed coats infected by *C. kikuchii* (11). Cercosporin is a red photoreactive polyketide, which produces superoxide (O_2^-) and singlet ($^1O_2$) oxygen in the presence of light and oxygen (6). These active oxygen species cause peroxidation of cell and organelle membrane lipids, resulting in membrane permeability, ion leakage, and cell death (3,5,12,23). Isocercosporin, an optical isomer of cercosporin, was isolated from *Cercospora* in culture (30). However, its role in pathogenicity was not studied. Cercosporin production by *C. kikuchii* in culture was related directly to pathogenicity on soybean leaves and a requirement for symptom development (24). Cercosporin accumulation in purple-stained soybean seed coats can provide a competitive advantage to *C. kikuchii* in colonizing seed coats (28).

The soybean seed coat is covered by a cuticle and consists of four distinct layers—epidermis, mesodermis, endodermis, and hypodermis. Most seedborne fungal pathogens of soybean colonize the mesodermis (27). *Cercospora beticola* Sacc.-infected or cercosporin-injured beet (*Beta vulgaris* L.) leaf tissue showed damage to cell and organelle membranes (22,23). Since hyphae of *C. beticola* grew through intercellular spaces and entered leaf cells late in the disease process, a possible role for cercosporin in facilitating pathogen colonization was suggested (23). Several lipids and related compounds were produced by *Cercospora* spp. in culture (1). A mixture of long-chain fatty acids and triglycerides termed "*C. beticola* toxin" or "yellow toxin" (1,2,19), which caused necrotic lesions on beet leaves (23), was reported from *C. beticola* in culture.

We report on 1) the occurrence of three distinct pigment-producing isolates of *C. kikuchii* from purple-stained soybean seeds; 2) a comparison of cercosporin accumulation by these isolates in culture and in soybean seed coats; and 3) the relationship between the area of purple stain and extent of *C. kikuchii* colonization, and the amount of cercosporin in soybean seed coats. The possible role of cercosporin in facilitating soybean seed coat tissue colonization by *C. kikuchii* is discussed.

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MATERIALS AND METHODS

Recovery of *C. kikuchii* isolates from purple-stained seeds. Soybean seeds (Illinois Foundation Seeds, Inc., Tolono) of cv. Hack, without or with purple stain, stored for 1 yr in the dark at 4 C, were used. Seeds were surface sterilized for 5 min in 0.5% NaOCl and washed three times in sterile deionized distilled water. Seeds without or with purple stain were placed on acidified (pH 5.0) potato-dextrose agar (PDA) at three seeds per 9-cm culture plate in 24 replicates. The plates were incubated under fluorescent light (140 $\mu E \cdot m^{-2} \cdot s^{-1}$) for 15 days at room temperature (RT) (24 ± 2 C). The fungi associated with each seed were identified, and the mean percent occurrence of morphologically distinct *C. kikuchii* isolates was calculated. The isolates recovered were divided into three groups based on colony color: Ckcp (purple), Ckcy (yellow), and Ckcc (red).

Cercosporin accumulation on PDA by three isolates of *C. kikuchii*. Blocks (4 mm diameter) from the margin of 5-day-old PDA cultures of one isolate from each of the three groups based on colony coloration were transferred separately onto 9-cm-diameter culture plates containing 20 ml of PDA (pH 7.0) and incubated under 16-hr light (140 $\mu E \cdot m^{-2} \cdot s^{-1}$) at RT. Sampling was done within a 1- to 2-cm radius from the point of inoculation rather than advancing margins (8). Each sample consisted of five mycelial plugs (3 mm diameter) per plate after 21, 24, 27, 30, or 33 days. The samples were extracted separately with 5 ml of 5 N KOH, and the absorption was recorded at 480 nm (visible absorption maximum in alkaline pH) using a Hitachi (U 2000) spectrophotometer. The amount of cercosporin was calculated using the formula: [(absorbance at 480 nm/23,300) 534] μg, where 23,300 equaled the molar extinction coefficient (ϵ) at 480 nm and 534 equaled the molecular weight of cercosporin (30).

The experiments were performed in a completely randomized design with nine replicates. The combined data were analyzed using ANOVA, and means were separated by least significant difference (LSD) at $P = 0.0001$ (17).

Cercosporin production on seed coats by three isolates of *C. kikuchii*. Asymptomatic seeds were surface sterilized with 0.5% NaOCl for 5 min, washed three times with sterile deionized distilled water, and placed on PDA containing 40 g/ml 2,4-dichlorophenoxyacetic acid (2,4-D) to inhibit germination. Seeds were incubated for 3 days at RT, and all seeds with fungal growth were discarded. Four fungus-free seeds per plate were transferred to PDA containing 40 μg/ml of 2,4-D. Mycelial pieces 1- to 2-mm-diameter were cut from the margins of 8-day-old PDA cultures of isolates Ckcp, Ckcy, or Ckcc and inoculated onto seeds separately (10). Controls consisted of noninoculated asymptomatic seeds. The experiment was done in a completely randomized design, with four seeds per plate in four replications. Two sets of experiments were conducted, one incubated under 16 hr of fluorescent light, and the other in the dark. All the experiments were conducted twice. Seed coats were removed after 3, 5, 8, or 11 days, and each seed coat was extracted separately with 10 ml of glacial acetic acid in the dark for 48 hr at 24 C (11). Methylene chloride (15 ml) was added to each extract and washed with deionized distilled water to remove water-soluble impurities and acetic acid. The methylene chloride was allowed to evaporate in the dark at 24 C. The residues were redissolved in 1 ml of absolute ethanol, and absorption by the samples was determined at 473 nm (maximum absorption at acidic pH). The cercosporin content of the seed coat then was determined using the formula [(473 nm/23,600) 534] 1,000 μg (30).

Histopathology of purple-stained seed coats. Twenty seeds with purple seed stain or asymptomatic seeds (controls) were soaked in boiling deionized distilled water for 3 min. The softened endodermis was peeled off to expose the mesodermis, which was stained with aniline blue and observed for the presence of hyphae using a stereo microscope.

Cercosporin accumulation in purple-stained seeds in situ. Symptomatic seeds were separated into five groups based on the extent of purple-stained area on the seed coats: 5, 25, 50, 75, 90, or 100% (Fig. 1). The extent of purple stain was measured from the hilar region, and the percentage of purple stain per seed was calculated. Asymptomatic seeds served as controls. Seeds of each group were longitudinally split into halves using a razor blade, and the seed coats were separated, weighed, and extracted with 10 ml of glacial acetic acid in the dark for 48 hr at RT. Cercosporin in glacial acetic acid was extracted into methylene chloride as described above. The residues were redissolved in 1 ml of absolute ethanol, and absorption was recorded at 473 nm. Each sample consisted of extracts from five seed coats weighing 100 mg; the amounts were determined as described above. The experiment was arranged in a completely randomized design with nine replications, and done twice. A linear regression was drawn using natural logs of independent (extent of purple stain) and dependent (cercosporin content of 100 mg of seed coats) variables based on the equation $LN(Y) = \alpha + LN(X)$.

Survey for lipids from *C. kikuchii* grown on PDA, soybean seeds in culture, and purple-stained seeds, in situ. Mycelia of *C. kikuchii* grown on PDA were air-dried at RT and then ground to a powder by mortar and pestle. The mycelial powder was extracted with diethyl ether, and the ether was evaporated to dryness in vacuum at less than 24 C. The seed coats of purple-stained seeds and of seeds inoculated with *C. kikuchii* were extracted with glacial acetic acid, and the acid was evaporated in vacuum at less than 24 C. The crude extracts were resolved by thin-layer chromatograph (silica gel G) sheet type K301R with fluorescent indicator (Distillation Products Industries [Kodak], Rochester, NY). The sheets were sprayed with 2% phosphoric acid in water and dried for 3 hr at 80 C prior to chromatography. The residuals were spotted in ethanol, and the chromatograms were developed with an ethyl acetate:benzene (2:3) solvent system. The lipid fraction eluted as a yellow, oily liquid, which was placed as a thin film between two NaCl IR windows and subjected to infrared spectrometry (IR/32 spectrometer, IBM Instruments Inc., Danbury, CT) (15).

Change from purple to red color of cercosporin in vitro. To simulate the change in cercosporin from purple to red, which occurred when extracting it with glacial acetic acid either from purple-stained seed coats or from isolate Ckcp grown in culture, and to determine possible chemical reactions to account for the change, the following experiments were designed. Equal amounts of purified cercosporin (25) were dissolved in either 2 ml of glacial acetic acid, 2 ml of 85% phosphoric acid, or 1 ml of glacial acetic acid, each followed by 1 ml of 85% phosphoric acid (v/v), or 1 ml of 85% phosphoric acid followed by glacial acetic acid. Changes in visible spectra were recorded using a Hitachi (U 2000) spectrophotometer.

RESULTS

C. kikuchii was recovered from purple-stained seeds but not from asymptomatic seeds. Three different color-producing types of *C. kikuchii* isolates were recovered on PDA from 72 purple-stained seeds. The Ckcp isolate produced the greatest amount of cercosporin; the Ckcy isolate produced less cercosporin and excess yellow lipids; and the Ckcc isolate produced the least amount of cercosporin. The mean percent occurrence of *C. kikuchii* isolates from purple-stained seeds was 75% for isolate Ckcp, 15% for Ckcy, and 10% for Ckcc. The characteristic pigmentation of isolates Ckcp, Ckcy, and Ckcc was obvious after 10 days in PDA.

The accumulation of cercosporin on PDA by isolate Ckcp was significantly ($P = 0.0001$) higher than that of isolates Ckcy or Ckcc, but not between isolates Ckcy and Ckcc. Cercosporin accumulation increased in all the isolates with increased incubation (Fig. 2). Isolate

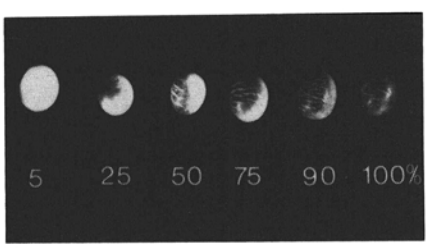

Fig. 1. Soybean seeds infected by *Cercospora kikuchii* showing increasing percentages of purple stain.

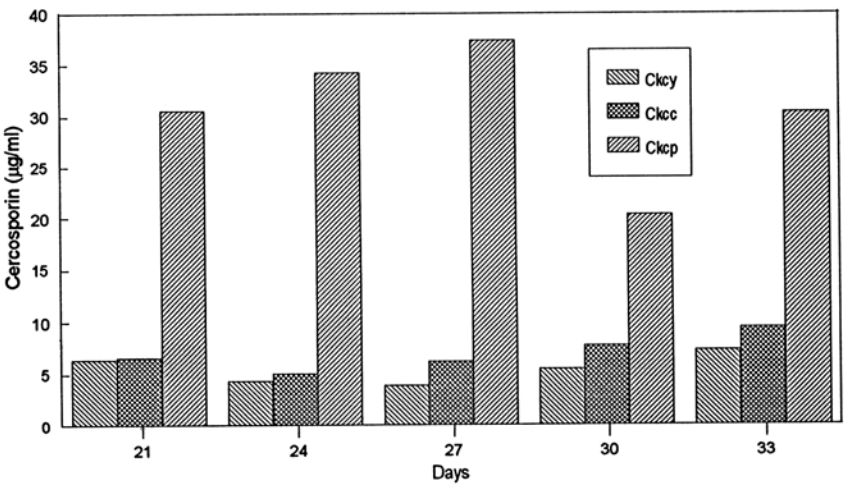

Fig. 2. Accumulation of cercosporin (μg/ml) in 3-mm-diameter mycelial plugs of isolates Ckcy, Ckcc, and Ckcp of *Cercospora kikuchii* on potato-dextrose agar (pH 7) at 24 ± 2 C. Mean of two experiments.

Ckcp showed greatest cercosporin accumulation at 27 days after inoculation.

All isolates produced a purple stain on soybean seed coats, irrespective of pigments produced in culture. The accumulation of cercosporin in seed coats increased with time after inoculation. Seeds inoculated with isolate Ckcp and incubated under light showed a significant ($P = 0.0001$) increase in cercosporin accumulation compared to those incubated in the dark (Fig. 3). Seed coats inoculated with Ckcy or Ckcc showed only a slight increase in the cercosporin accumulation when incubated in the dark. Isolate Ckcp incubated only under light, and Ckcy incubated under light and in the dark, resulted in significantly ($P = 0.0001$) greater amounts of cercosporin accumulation compared to Ckcc.

Mycelia of *C. kikuchii* were observed in the funiculus and intercellularly among the hourglass cells of the seed coat mesodermis (Fig. 4A and B). The gross anatomy of symptomatic, infected seeds differed little from that of asymptomatic seeds. Hyphae were sparse near the hilar region but abundant in the mesodermis. The margin of purple stain always coincided with the presence of hyphal tips.

Seeds with 5, 25, 50, 75, 90, or 100% of the seed coat with purple stain (Fig. 1) contained 1.7, 9.0, 19.9, 27.7, or 78.6 μg of cercosporin, respectively, in their seed coats. Cercosporin was not detected in seed coats of asymptomatic seeds. Cercosporin content increased significantly ($P = 0.0001$) between 0, 5 to 25, 50, 75, and 100% purple-stained seed coats. A positive relationship ($r^2 = 0.96$) between the extent of purple seed stain and cercosporin content was recorded.

Scanning crude ether extracts of *C. kikuchii* mycelia containing cercosporin showed no interfering spectra at the wavelengths (visible region) used for estimating the quantity of cercosporin. Thin-layer chromatography of crude ether extracts of *C. kikuchii* mycelia grown on potato-dextrose broth showed the presence of cercosporin ($R_f = 0.34$), isocercosporin ($R_f = 0.71$), and lipids ($R_f = 0.83$) (Fig. 5). Cercosporin and isocercosporin were identified by their absorption spectra (30). *C. kikuchii* grown on soybean seed coats in vitro and from naturally infected purple-stained seed coats showed the presence of cercosporin and isocercosporin, but not lipids.

The infrared absorption spectrum of lipids isolated from *C. kikuchii* mycelia showed bands about 2,910 cm^{-1} and 2,876 cm^{-1} for methyl and methylene groups, respectively; 1,738 cm^{-1} for C=O carbonyl groups of methyl esters in long-chain fatty acids; 1,157 cm^{-1} for ester –C–O– bonding; rocking at 758 cm^{-1} for methylene groups of esters and long-chain fatty acids (Fig. 6); 1,456 cm^{-1} for CH_3–C and CH_2 deformation vibrations; and 1,460 cm^{-1} for hydrogen bending vibrations. Absorption stretch at about 3,052–2,995 cm^{-1} showed the presence of double bonds in the fatty acid(s) chains. Absorption between 1,100 and 1,200 cm^{-1}, and 1,700 and 1,800 cm^{-1} are typical of ester and alkane groups of triglycerides. Stretch at 1,745 cm^{-1} indicated the presence of ester carbonyl in the triglycerides. Absence of O–H stretching region at 3,340 cm^{-1} and C–O stretching at about 1,157 cm^{-1} are characteristic of triglycerides (14).

Cercosporin dissolved in glacial acetic acid showed absorption peaks at 470 and 563 nm, and in phosphoric acid at 490 and 590 nm. Cercosporin dissolved in acetic acid followed by phosphoric acid or vice versa showed absorption peaks at 479 and 579 nm (Fig. 7).

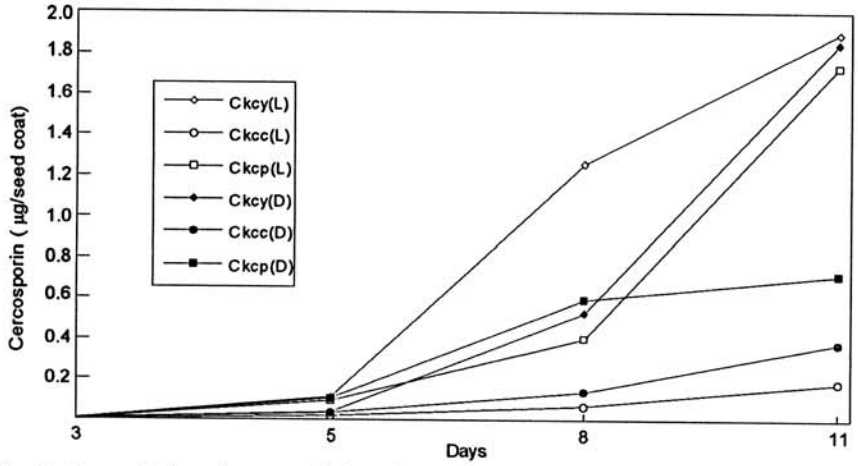

Fig. 3. Accumulation of cercosporin in soybean seed coats inoculated by isolates Ckcy, Ckcc, and Ckcp of *Cercospora kikuchii* and incubated in light (L) or dark (D). The points represent the mean of two experiments.

DISCUSSION

Purple stain on most seed coats infected by *C. kikuchii* radiates out from the funiculus (21). We showed that hyphal growth of *C. kikuchii* in seed coats followed a similar pattern, spreading from the hilar region through the mesoderm after entering through the funiculus and coinciding with the extent of purple stain. We did not observe tissue damage in the mesoderm under 450× magnification (R. K. Velicheti and J. B.

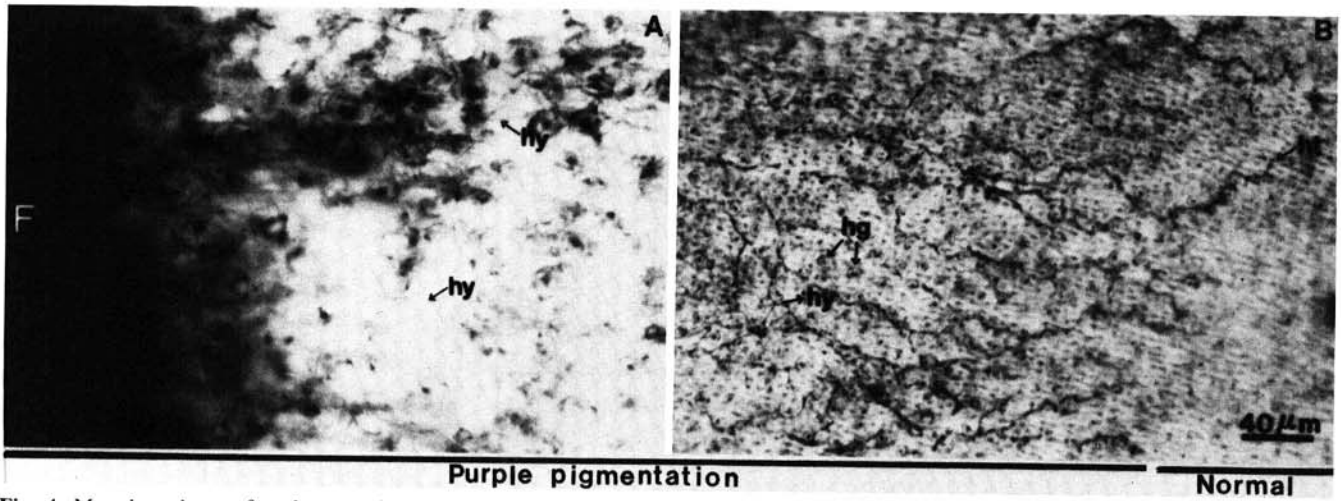

Fig. 4. Mesoderm layer of soybean seed coat showing **(A)** presence of *Cercospora kikuchii* in the funiculus and mesoderm, and **(B)** hyphal tips of *C. kikuchii* and the extent of purple stain. Funiculus (F), hourglass cells of the mesoderm (hg), hyphal tips (ht), and intercellular hyphae (hy).

Sinclair, *unpublished*). However, protein degradation occurred in *C. kikuchii*-infected soybean seed coats (26), suggesting damage at the cellular level analogous to cercosporin-injured and *C. beticola*-infected sugar beet leaves (22,23).

C. kikuchii has been isolated from soybean seeds lacking purple stain (8). Infection by isolates that do not produce cercosporin (10) or limited colonization may be responsible for lack of seed coat staining. However, none of the 72 isolates collected had white or hyaline hyphae. It may be that the extent of purple stain on any seed coat will vary depending on the stage of seed development at which infection takes place, since an increase in purple stain after seed maturity was never observed (R. K. Velicheti and J. B. Sinclair, *unpublished*). The percent purple stain on seed coats was correlated with the amount of cercosporin present in seed coat tissue. The increase in cercosporin production by *C. kikuchii* on seed coats was similar to the report showing a relationship between cercosporin production and mycelial biomass in culture (8). The relationship between the area of purple seed stain and cercosporin content may allow for the prediction of the amount of cercosporin in soybean seed coats.

The release of a toxin in low quantities during colonization may allow the fungus to infect tissues by damaging cell membranes, and toxin(s) accumulated later in pathogenesis could result in host cell death (18). Large quantities of cercosporin produced during colonization by the pathogen caused cell death and the formation of a hard seed coat, which is characteristic of seed infections by *C. kikuchii* (16). Hard seed coats may result in reduced seed size and development of characteristic cracks in seed coats of heavily infected seeds (20).

Cercosporin was always extracted from *C. kikuchii*-infected seed coats or mycelia grown in culture. Electrophoresis of *C. kikuchii*-infected seed coat extracts under nondenaturing conditions (26) showed no binding to seed coat proteins (R. K. Velicheti and J. B. Sinclair, *unpublished*). The R_f values and the mass spectral data of cercosporin extracted from *C. kikuchii*-infected seed coats were similar to those of cercosporin recovered from fungal cultures. The fact that cercosporin and its derivatives vary in color (11,30) may explain differences

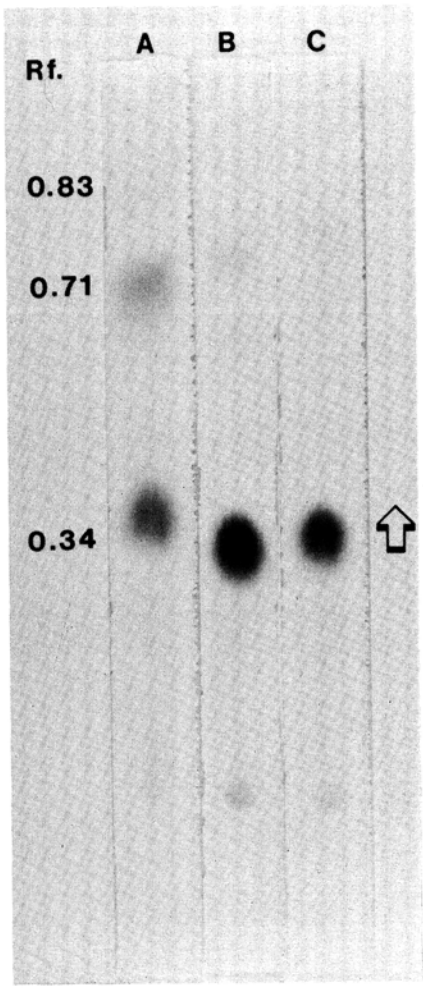

Fig. 5. Crude extracts of *Cercospora kikuchii* resolved on thin layer chromatograms. A = *C. kikuchii* isolate Ckcp grown on potato-dextrose agar; B = *C. kikuchii* grown on soybean seed coats in vitro; and C = purple-stained soybean seed coat collected from field-grown plants.

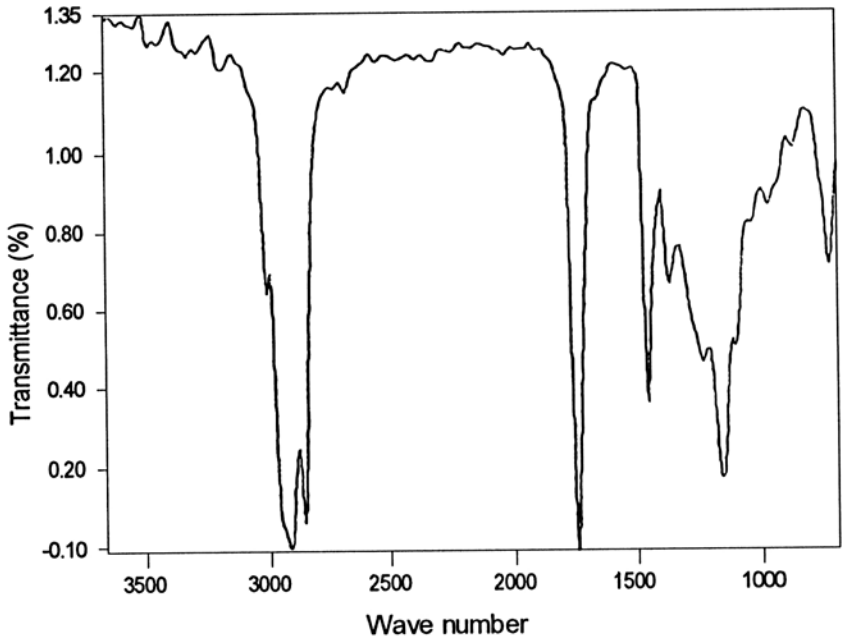

Fig. 6. Infrared spectra of lipids extracted from *Cercospora kikuchii* cultures grown on potato-dextrose agar.

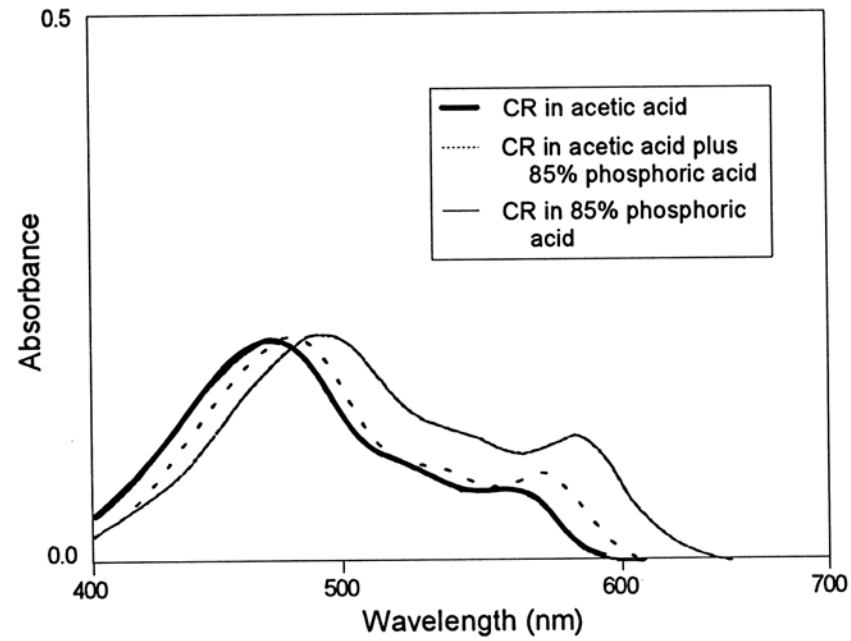

Fig. 7. Shifts in absorption spectra of cercosporin when dissolved in acetic acid (red), phosphoric acid (purple), and equal volumes of acetic and phosphoric acids (green).

in symptoms on soybean leaves, which are reddish purple, and those on soybean seed coats, which are deep purple (20). The change in the color and spectral properties of cercosporin suggested a possible chemical reaction. Since the conversion of nonanhydrocercosporin, which is purple (30), to red cercosporin was not possible under our experimental conditions, further studies of these derivatives are needed. Our isolates of *C. kikuchii* varied in the color of cercosporin produced in culture from purple to red. Of the 72 isolates studied, 75% produced purple and 25% red coloration under identical conditions. The production of cercosporin by isolates of *C. kikuchii* differed among isolates on PDA and in seed coats, showing that the amount of toxin produced in culture was unrelated to the amount produced in seed coats. In addition, the relatively high amounts present in purple-stained seeds confirmed that cercosporin could provide a competitive advantage to *C. kikuchii* against a range of fungi associated with soybean seeds (28).

Cercosporin production also is influenced by light. A 50-fold increase in cercosporin produced by *C. kikuchii* was recorded in cultures incubated under light (7). We did not find a similar increase in cercosporin production in seed coats inoculated with *C. kikuchii* and incubated under light. Cercosporin production in *C. beticola* cultures increased in the dark when the cultures were amended with ascorbic acid (2). The difference in cercosporin production by isolates Ckcy and Ckcc between light and dark incubation was not significant, suggesting the presence of undefined substances in soybean seed coats that may influence cercosporin production by the fungus. The decrease in cercosporin content from isolate Ckcp after 27 days could be due to a decrease in production or photodecay (2).

Lipid production by several *Cercospora* spp., including *C. kikuchii,* in culture has been shown (1,2,4,19). Lipids recovered from *C. beticola,* described as *C. beticola* toxin or yellow toxin, were toxic to beet leaves (2,19,23), but there has been no evidence of their production in plant tissues. All of our isolates of *C. kikuchii* grown on PDA showed evidence of lipid production, but lipids were not detected in soybean seed coats infected by *C. kikuchii.* Thus, lipids produced by *Cercospora* in culture were not related to colonization, and their role as a toxin in pathogenicity is doubtful.

Isolates representing the three groups produced purple stain when inoculated to soybean seeds. However, only isolate Ckcp produced purple colonies in PDA. The reason for this difference is not clear.

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LITERATURE CITED

1. Assante, G., Locci, R., Camarda, L., Merlini, L., and Nasini, G. 1977. Screening of genus *Cercospora* for secondary metabolites. Phytochemistry 16:243-247.
2. Balis, C., and Payne, M. G. 1971. Triglycerides and cercosporin from *Cercospora beticola*: Fungal growth and cercosporin production. Phytopathology 61:1477-1484.
3. Daub, M. E. 1982. Peroxidation of tobacco membrane lipids by the photosensitizing toxin, cercosporin. Plant Physiol. 69:1361-1364.
4. Daub, M. E. 1987. Resistance of fungi to the photosensitizing toxin, cercosporin. Phytopathology 77:1515-1520.
5. Daub, M. E., and Briggs, S. P. 1983. Changes in tobacco cell membrane composition and structure caused by cercosporin. Plant Physiol. 71:763-766.
6. Daub, M. E., and Hangarter, R. P. 1983. Light-induced production of singlet oxygen and superoxide by the fungal toxin, cercosporin. Plant Physiol. 73:855-857.
7. Ehrenshaft, M., and Upchurch, R. G. 1991. Isolation of light-enhanced cDNAs of *Cercospora kikuchii.* Appl. Environ. Microbiol. 57:2671-2676.
8. Jenns, A. E., Daub, M. E., and Upchurch, R. G. 1989. Regulation of cercosporin accumulation in culture by medium and temperature manipulation. Phytopathology 79:213-219.
9. Jones, J. P. 1959. Purple stain of soybean seeds incited by several *Cercospora* species. Phytopathology 49:430-432.
10. Kilpatrick, R. A., and Johnson, H. W. 1956. Purple stain of legume seeds caused by *Cercospora* species. Phytopathology 46:201-204.
11. Kuyama, S., and Tamura, T. 1957. Cercosporin. A pigment of *Cercospora kikuchii* Matsumoto et Tomoyasu. 1. Cultivation of fungus, isolation and purification of pigment. J. Am. Chem. Soc. 79:5725-5726.
12. Marci, F., and Vianello, A. 1979. Photodynamic activity of cercosporin on plant tissues. Plant Cell Environ. 2:267-271.
13. Murakishi, H. H. 1951. Purple seed stain of soybean. Phytopathology 41:305-318.
14. O'Connor, R. T. 1960. Fatty acids. Page 463 in: Fatty Acids, Their Chemistry, Properties, Production, and Uses. K. S. Markely, ed. I. Intersci. Publ., Inc., New York.
15. Roberts, R. M., Gilbert, J. C., Rodewald, L. B., and Wingrove, A. S. 1985. Modern Experimental Organic Chemistry. 4th ed. Saunders Golden Sunburst Ser., Saunders College Publishing, New York.
16. Roy, K. W., and Abney, T. S. 1976. Purple seed stain of soybeans. Phytopathology 66:1045-1049.
17. SAS Institute. 1985. SAS User's Guide: Statistics. Version 5 ed. SAS Institute, Cary, NC.
18. Scheffer, R. P., and Livingston, R. S. 1984. Host-selective toxins and their role in plant diseases. Science 223:17-21.
19. Schlosser, E. 1971. The *Cercospora beticola* toxin. Phytopathol. Mediterr. 10:154-158.
20. Sinclair, J. B., and Backman, P. A., eds. 1989. Compendium of Soybean Diseases. 3rd ed. American Phytopathological Society, St. Paul, MN.
21. Singh, T., and Sinclair, J. B. 1986. Further studies on the colonization of soybean seeds by *Cercospora kikuchii* and *Phomopsis* sp. Seed Sci. Technol. 14:71-77.
22. Steinkamp, M. P., Martin, S. S., Hoefert, L. L., and Ruppel, E. G. 1979. Ultrastructure of lesions produced by *Cercospora beticola* in leaves of *Beta vulgaris.* Physiol. Plant Pathol. 15:13-26.
23. Steinkamp, M. P., Martin, S. S., Hoefert, L. L., and Ruppel, E. G. 1981. Ultrastructure of lesions produced in leaves of *Beta vulgaris* by cercosporin, a toxin from *Cercospora beticola.* Phytopathology 71:1272-1281.
24. Upchurch, R. G., Walker, D. C., Rollins, J. A., Ehrenshaft, M., and Daub, M. E. 1991. Mutants of *Cercospora kikuchii* altered in cercosporin synthesis and pathogenicity. Appl. Environ. Microbiol. 57:2940-2945.
25. Velicheti, R. K., Bhandal, I. S., and Sinclair, J. B. 1991. A simple method for purification and detection of cercosporin. (Abstr.) Phytopathology 81:1238.
26. Velicheti, R. K., Kollipara, K. P., Sinclair, J. B., and Hymowitz, T. 1992. Selective degradation of proteins by *Cercospora kikuchii* and *Phomopsis longicolla* in soybean seed coats and cotyledons. Plant Dis. 76:779-782.
27. Velicheti, R. K., and Sinclair, J. B. 1991. Histopathology of soybean seeds colonised by *Fusarium oxysporum.* Seed Sci. Technol. 19:445-450.
28. Velicheti, R. K., and Sinclair, J. B. 1992. Reaction of seedborne soybean fungal pathogens to cercosporin. Seed Sci. Technol. 20:149-154.
29. Wilcox, J. R., and Abney, T. S. 1973. Effects of *Cercospora kikuchii* on soybeans. Phytopathology 63:796-797.
30. Yamazaki, S., and Ogawa, T. 1972. The chemistry and stereochemistry of cercosporin. Agric. Biol. Chem. 36:1707-1718.