# Detection and Characterization of Cercospora citrullina Isolates That Sporulate Readily in Culture

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ABSTRACT

*Cercospora citrullina,* like most species of *Cercospora,* has not produced conidia in artificial culture. Attempts to induce sporulation by subjecting mycelial isolates to various cultural and nutritional variables were unsuccessful. The genetic variability of *C. citrullina* was exploited by examining a large number of isolates which had been obtained by streaking conidia from lesions on watermelon, *Citrullis vulgaris.* Two pathogenic isolates were obtained that sporulated readily on potato-dextrose agar (PDA) under ordinary room conditions. The sporulating and pathogenicity characters remained stable throughout a 2-year study. Conidia could be produced in 72-96 hr on fresh PDA whether initiated from conidia or mycelia from stock cultures. Loss of the ability to sporulate in culture by *Cercospora* isolates may be explained on the basis of heterokaryosis. Phytopathology 60:1502-1503.

Most attempts, as with the present study, to induce *Cercospora* species to sporulate in artificial culture have been in relation to pathological studies. A few successful attempts have been reported (2, 8, 9, 11, 14). Isolates of most species of *Cercospora,* however, failed to sporulate when grown in artificial culture (10, 11, 14). Frequently, workers have subjected a single or a few isolates of a given species to various cultural manipulations, usually involving variables in nutrition, light, and temp (3, 9, 10, 11, 14, 15). The approaches have been primarily through trial or error, and successes have been sporadic. Recently, Calpouzos & Stallknecht (3) concluded that erratic sporulation of *C. beticola* in culture was due partially to the nature of the medium and partially to other factors not fully understood. Nagel (11) presented an excellent review of the sporulation problem with *Cercospora* spp. He was able to obtain conidia in culture with 12 species by conidial transfers from host plants. To maintain the sporulating habit, however, it was necessary that conidia be transferred at 3- to 6-day intervals to fresh media. In this way, sporulating cultures were maintained from 5 weeks to 3 months. He studied the pathogenicity of six sporulating isolates representing six species, and found two to be avirulent. Ryker (13) concluded that the loss of sporulating ability in culture was due to suppression by nonconidial variants.

Calpouzos (2), working with *C. musae,* secured sporulating strains through selective subculturing from tiny sporulating areas of cultures. After several transfers, a uniformly sporulating strain was secured. Jones (8) successfully used the method of Calpouzos with *C. kikuchii.* The sporulating strain developed by Jones, however, was avirulent to soybean, *Glycine max* (J. P. Jones, *personal communication*).

A study of conidial production by *C. citrullina* Cooke on artificial media was made to secure conidia when needed for inoculations. Watermelon, *Citrullus vulgaris* Schrad., and its relatives were to be screened for a source of resistance to *Cercospora* leaf spot.

MATERIALS AND METHODS.—Mycelial isolations of *C. citrullina* were obtained from infected areas on leaves of Charleston Gray watermelon. These isolates were subjected to a long series of variables in an attempt to obtain conidia. Various concn of potato-dextrose agar (PDA), cherry leaf agar, and watermelon leaf extract agar were tried over a range of temp and light conditions. Isolates were also exposed to ultraviolet light, grown in thick colonies, grown on water agar, and subjected to rapid transfer and to infection and reisolation cycles. The isolates grew well under various conditions, but no conidia were produced. After the unsuccessful attempts to produce conidia from mycelial isolates, this approach was abandoned.

Most workers have been impressed by the variability within *Cercospora* species. Consequently, a study was designed to exploit the genetic variability in *C. citrullina.* It was assumed that if enough isolates were examined, a pathogenic one could be found that would sporulate readily in artificial culture. The use of conidia as a source of isolates was considered important. The chances for finding the desired combination of genetic characters appeared greater when isolates were obtained by streaking conidia on artificial media rather than by starting with fragments of diseased host tissues containing mycelia. Fifteen hundred conidial isolates from 150 lesions were obtained from different watermelon varieties from various localities over a 3-month period. While observing sporulating lesions with the aid of a stereoscopic microscope, a bit of PDA adhering to the tip of a transfer needle was brought into contact with conidia and then streaked on PDA plates. The resulting isolates were selected for ability to sporulate, pathogenicity, and stability of these characters in culture.

RESULTS.—After approximately 72 hr of incubation, 10 of the conidial isolates were sporulating heavily. Subtransfers of conidia from the sporulating colonies again produced conidia within 72 hr. After approximately 96 hr, conidia were difficult to find on these cultures. A sporulating isolate was found in the first 200 obtained. But the study was continued so as to obtain some idea of the frequency of this trait in the population. Also, additional sporulating isolates were desired to provide a broader genetic base for the possibility of selection for both pathogenicity and stability in culture.

In pathogenicity tests, nine of the 10 isolates were

highly virulent to watermelon, and typical *Cercospora* leaf spot disease resulted after inoculation. One isolate was avirulent to watermelon.

After several conidial transfers, seven of the pathogenic isolates tended to become more mycelial, whereas two retained the sparsely vegetative, heavy sporulating habit. Consequently, further studies were conducted only with these two stable isolates. Dark mycelia of the two isolates would develop sporulating colonies when broken up and streaked over the surface of fresh PDA. Sporulation of the isolates was heavy under ordinary room conditions. Growth on agar following inoculation by a dilute conidial suspension was typified by formation of discrete, dark colonies 2-5 mm in diam. Inoculation with either dense conidial or mycelial suspensions resulted in more or less continuous growth and sporulation on the agar surface.

The pathogenicity and sporulating characteristics of the two isolates remained stable throughout a 2-year period while they were being used in host resistance studies.

DISCUSSION.—The two stable isolates were relatively simple to manipulate in culture, and none of the difficulties usually reported with artificial culture of *Cercospora* species were encountered. The critical factor was finding individuals in the population having the desired genetic components. *Cercospora citrullina* might be unique in the genus for possessing the genetic types described, but this seems unlikely.

The demonstrated ability of conidial isolates of some *Cercospora* species to sporulate for a few generations in artificial culture (11) certainly indicates that these isolates have the genetic components for sporulation. It is not known whether these genes are lost or merely cease to be expressed in succeeding generations. But, from a consideration of many papers, together with observation of the *C. citrullina* isolates in the present study, it is suggested that they are lost. The problem of maintaining sporulating *Cercospora* isolates may be explained by a genetic model based on heterokaryosis.

If the heterokaryosis explanation is correct, the failure of mycelial isolates of *C. citrullina* to form conidia in culture could be explained by the assumption that the isolates were homokaryotic for nuclei with vegetative genes. The seven conidial isolates that became progressively more vegetative after a few conidial transfers were heterokaryotic when isolated. The two stable isolates which produced sporulating colonies from either mycelial or conidial transfers were homokaryotic for nuclei containing genes for sporulation. No drift toward vegetative-type nuclei would take place in the stable sporulating isolates regardless of the selection pressures of cultural conditions. In such an isolate, however, a mutation for nonsporulation in a single nucleus would establish a heterokaryotic state which, under cultural conditions, would lead ultimately to a homokaryotic, nonsporulating culture.

Detailed studies of behavioral patterns and shifts in nuclear ratios reflecting environmental selection pressures have been reported for heterokaryotic fungi (1, 4, 5, 6, 7, 12). It is on this basis that heterokaryosis is postulated to explain the phenomena reported in the present study. Further genetical and cytological studies will be necessary for confirmation.

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