Host Specificity of Nigerian and North American Isolates of Corynespora cassiicola

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ABSTRACT

Isolates of *Corynespora cassiicola* from Nigeria, southern U.S., and western Mexico were indistinguishable on the basis of conidial size, shape and growth in culture. Optimum temp for radial growth was 28 C. Development of aerial mycelium was sparse under fluorescent light, but was copious in the dark. One group of isolates was highly virulent on tomato and eggplant, and moderately virulent on sesame and cotton. These isolates came from tomato, *Calopogonium mucunoides* and *Synedrella nodiflora* in Nigeria, and from cucumber and soybean grown in the southern U.S. Another group of isolates from two common Nigerian weeds, *Aspilia africana* and a *Lepistemon* sp., were highly virulent on cotton. Such weed hosts probably serve as sources of primary inoculum on tomato and cotton. One Nigerian isolate from papaya attacked only papaya. A second cucumber isolate was specific to cucumber. Other soybean isolates from southern U.S. and Mexico were highly virulent on soybean, sesame, eggplant, and cotton. Although the species has a very wide host range, host-specific strains appear to be numerous and quite varied in the range of their specificity.

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Additional key words: host range, tomato, cucumber, soybean, cotton.

Corynespora cassiicola (Berk. & Curt.) Wei incites target spot, a serious disease of tomato (*Lycopersicon esculentum* Mill.) in southern Nigeria (1). The fungus was first reported on tomato by Deighton (9) from Sierra Leone and subsequently by Mohanty and Mohanty (14) in India, by Simmonds (19) in Queensland, Australia, by Bailey (2) in Nigeria, and by Blazquez (5) in Florida, U.S.A. *C. cassiicola* attacks a large number of other hosts, primarily in tropical and subtropical areas. In the southern U.S. it incites foliage diseases of cucumber (*Cucumis sativus* L.) (4), soybean [*Glycine max* (L.) Merr.] (15), cotton (both *Gossypium barbadense* L. and *G. hirsutum* L.) (12), sesame (*Sesamum indicum* L.) (22), and cowpea [*Vigna unguiculata* (L.) Walp.] (15), as well as a number of other plants (7, 20). It has been reported on cowpea in Japan and on soybean in China (23). *C. cassiicola* is the incitant of the "greasy spot" disease of papaya (*Carica papaya* L.) in the Caribbean area (3, 13), and in the Ivory Coast of Africa it has been reported on a number of vegetables by Boisson and Renard (6). Recently, it was reported in Nigeria as a foliar pathogen of tobacco (*Nicotiana tabacum* L.) (11). It has been found to incite a stem and root rot of soybean in Nebraska, U.S.A. (8), and Ontario, Canada (18). Before the development of resistant varieties (23), *Corynespora* was a limiting factor in glasshouse production of cucumbers in Europe.

The present studies were undertaken to determine what hosts of the fungus might serve as sources of primary inoculum for target spot of tomato and other economic plants in southern Nigeria, and to compare the morphology and pathogenicity of isolates of the fungus from different geographic areas and from different hosts. An abstract of a paper on this work has already appeared (16).

MATERIALS AND METHODS.—Single-spore isolations of *C. cassiicola* were made from foliar lesions on 20 plant species from various locations around the world, as listed in Table 1.

The length and greatest width of more than 100 conidia were measured for each of the Nigerian isolates, and for isolates M1, M2, Mex1, and Mex5. These conidia were obtained from 5- to 7-day-old potato-dextrose agar (PDA) cultures. To study the effect of temp on radial growth of all the isolates, 5-mm diam disks from PDA cultures were placed in the center of petri plates containing 20 ml of PDA. The plates were incubated in the dark over a range of 4-36 C. The cultural characteristics of the isolates were also compared both in continuous light (cool-white fluorescent, 2,700 lx) and in the dark at 21-23 C.

For the pathogenicity studies, inoculum was obtained from 7-day-old PDA cultures in which maximum sporulation had been induced either by exposure to continuous cool-white fluorescent light (ca. 2,700 lx) or by a combination of scraping and fluorescent illumination (17). Inoculum was prepared by washing conidia from the cultures. After it had been filtered through a single layer of cheesecloth, the inoculum level was adjusted to ca. 50,000 spores/ml of water containing Tween 80 (one drop/100 ml spore suspension).

Eight crop plants from which isolates had been obtained were used in the cross-inoculation studies: Ife No. 1 tomato; Puna Solo papaya; Marketer cucumber; Acme, Chippewa, Hawkeye and Merit soybeans; Deltapine 16, Stoneville 7A, and Stoneville 20 cottons; Margo sesame; Black Beauty eggplant; and Iron cowpea. Plants were usually inoculated in the 3- to 6-leaf stage, although in some instances up to the 10-leaf stage. The plants were sprayed with an atomizer containing the spore suspension until the upper and lower leaf surfaces, as well as the petioles and stems, were uniformly wet. Plants sprayed with water and Tween 80 served as controls. All plants were then incubated in a mist-

chamber at 20-23 C for 24 h (tomato) or 48 h (all other plants), and subsequently transferred to a greenhouse bench at 21-23 C. Reisolations were made for every isolate from each variety of plant showing symptoms. The level of pathogenicity was evaluated on the basis of the extent of blighting or spotting of the leaves, as well as the extent of stem lesions. The inoculation experiments were repeated.

RESULTS.—The isolates were very similar in morphological and cultural characters. Within isolates, the conidia were extremely variable in size, ranging from 60 to 250 × 5 to 13 μm, with an average of 167 × 8.3 μm. The minimum temp for measurable growth on PDA was 12 C. Growth rate increased with increasing temp; 28 C was optimum for all isolates. At 32 and 36 C, growth was much slower, and colonies had irregular edges. All

TABLE 1. Code numbers of isolates of *Corynespora cassiicola* with their collection location and original host

Isolate no.[a]	Original host	
N1	*Solanum melongena* L.	Eggplant
N2	*Luffa cylindrica* (L.) M.J. Roem.	Vegetable sponge
N3	*Ficus exasperata* Vahl	
N4	*Calopogonium mucunoides* Desv.	
N5	*Triumfetta rhomboidea* Jacq.	
N6	*Synedrella nodiflora* (L.) Gaertn.	Nodeweed
N7	*Aspilia africana* (Pers.) C.D. Adams	
N8	*Solanum torvum* Swartz	
N9	*Manihot glaziovii* Muell.-Arg.	Ceara rubber
N10	*Dioscorea bulbifera* L.	Aerial yam
N11	*Dioscorea esculenta* (Lour.) Burk.	Lesser yam
N12	*Colocasia esculenta* (L.) Schott	Cocoyam
N13	*Lepistemon* sp.	
N14	*Vigna unguiculata* (L.) Walp.	Cowpea
N15	*Lycopersicon esculentum* Mill.	Tomato
N16	*Cucumis sativus* L.	Cucumber
N17	*Carica papaya* L.	Papaya
M1	*Glycine max* (L.) Merr.	Soybean
M2	*Glycine max* (L.) Merr.	Soybean
M3	*Gossypium hirsutum* L.	Cotton
M4	*Sesamum indicum* L.	Sesame
Fla1	*Cucumis sativus* L.	Cucumber
Fla2	*Cucumis sativus* L.	Cucumber
Mex1	*Glycine max* (L.) Merr.	Soybean
Mex2	*Glycine max* (L.) Merr.	Soybean
Mex3	*Glycine max* (L.) Merr.	Soybean
Mex4	*Glycine max* (L.) Merr.	Soybean
Mex5	*Glycine max* (L.) Merr.	Soybean

[a]N isolates from Ile-Ife, Nigeria; M isolates from Stoneville, Mississippi; Fla isolates from Florida; and Mex isolates from Culiacan in western Mexico.

TABLE 2. Groupings of isolates of *Corynespora cassiicola* from Nigeria, southern USA, and Mexico on the basis of virulence

		Disease rating on indicated hosts[a]							
Group	Isolates	Tomato	Papaya	Cucumber	Soybean	Sesame	Eggplant	Cotton	Cowpea
1	N1, N4, N6, N15, M1, Fla2	+++	+	+[b]	+	++	+++	++	+[c]
2	N17	−	+++	−	−	−	−	−	−
3	Fla1	−	−	++	−	−	−	−	−
4	M2, M3, M4, Mex1-5	+	+	−	+++	+++	+++	+++	+
5	N7, N13	−	−	−	−	+	+	+++	−
6	N14, N16	−	−	−	−	−	+	++	−
7	N2, N3, N5, N8, N9, N11, N12	−	−	−	−	−	+	+	−
8	N10	−	−	−	−	−	−	+	−

[a]Disease rating scale: − = nonpathogenic (no visible symptoms on leaves or stems); + = weakly virulent (few-to-many pinpoint lesions or a few 1-2 mm diam. foliar lesions); ++ = moderately virulent (lesions 4-10 mm in diam., some coalescing but no blighting); and +++ = highly virulent (lesions spreading to form large areas of necrotic tissue and giving a blighting of leaves and stems).

[b]Many pinpoint lesions with Fla2, fewer with other isolates.

[c]Fewer lesions than with group 4.

colonies were initially whitish-grey. Under continuous fluorescent illumination at 21-23 C, the colonies gradually became greenish-black and aerial mycelium became sparse. Under continuous darkness, the colonies of all isolates except N17 remained grey and produced a thick weft of aerial mycelium. Although sporulation was best under continuous light, there was considerable variation between isolates.

On the basis of host reaction in the cross-inoculation tests, the isolates could be grouped as indicated in Table 2. On tomato, isolates in group 1 (i.e., N1, N4, N6, N15, M1, and Fla2) produced large irregular coalescent brown foliar lesions and elongated brown stem lesions within 36 h, and most of the plants were killed by 96 h. The isolates in group 4 (i.e., M2, M3, M4, and Mex1-5) incited pinpoint foliar lesions and no stem lesions—a weakly virulent reaction. All other isolates produced no visible symptoms on tomato.

The papaya isolate N17 constituting group 2 was highly virulent on papaya only. Large, coalescing foliar lesions developed in 3 days. Within 5 days stems and petioles, as well as the leaf blades, became shriveled and desiccated, and most plants were dead. Groups 1 and 4 gave only a few small nonspreading foliar lesions on papaya. All others were nonpathogenic.

On cucumber N1, N4, N6, N15, and M1 gave only a few minute foliar lesions. Although Fla1 and Fla2 both incited many lesions, those produced by Fla2 remained pinpoint-size even after 14 days, and those incited by Fla1 enlarged to 6-9 mm in 5 days with heavily infected leaves dying by 14 days. All other isolates produced no visible symptoms.

The group 4 isolates were highly virulent on soybean. These incited many coalescent foliar spots, each surrounded by a broad yellow halo. Some minute lesions were also formed on the stems and petioles. Isolates in group 1 gave only a few minute spots on soybean leaves. The four cultivars of soybean were similar in disease reaction.

The group 4 isolates were also highly virulent on sesame, giving large coalescing leaf lesions within 3 days after inoculation. These lesions had a tan center with a darker brown border. Within the same time period, elongated, rust-colored lesions appeared on the stems. Plants were heavily blighted and many died within 5 days. Group 1 isolates incited large lesions, but they did not produce a general blighting of the plants. Group 7 isolates were weakly virulent, and all others were nonpathogenic on sesame.

Both groups 1 and 4 were highly virulent on eggplant. Reactions of plants were similar to those of sesame. Isolates in groups 5, 6, and 7 produced a few small lesions on eggplant. Groups 2, 3, and 8 gave no symptoms.

The cotton cultivars Stoneville 7A and Stoneville 20 were highly susceptible to isolates in groups 4 and 5. In these varieties there was a rapid coalescing of the lesions, which blighted large portions of the leaf blades, and many plants died within 7 days. Isolates in groups 1 and 6 were moderately virulent on the same two cultivars, giving lesions ca. 8 mm in diam, with white centers and narrow red borders. Groups 7 and 8 were weakly virulent; they gave a few, nonspreading spots. Groups 2 and 3 were nonpathogenic. On Deltapine 16, all pathogenic isolates produced only pinpoint lesions.

On cowpea leaves inoculated with isolates in group 4, many pinpoint red spots appeared within 4 days. Group 1 isolates produced a smaller number of the same type of lesion. These lesions remained pinpoint in size for up to 4 wk. All other isolates were nonpathogenic.

DISCUSSION.—The conidia of the isolates studied were indistinguishable in both size and morphology. When cultures were grown in the dark, all except isolate N17 produced a thick mat of aerial mycelium. Even under continuous light, which generally favors sporulation, the poorly sporulating isolates produced considerable aerial mycelium. With this variability among isolates of what appears to be one species, the value of the amount of aerial mycelium as a basis for distinguishing between *C. cassiicola* and *C. homaliicola,* as suggested by Ellis (10), is questionable.

The results of the cross-inoculations indicate possible sources of primary inoculum for some of the economic hosts and also lead to a consideration of the relationship between these and previously described strains of the fungus.

Isolates N4 and N6, two of the most virulent on tomato, were isolated from *Calopogonium mucunoides* and *Synedrella nodiflora*. These two plants, the first a cover or forage crop, and the other a weed, are common throughout the year in southern Nigeria and thus are likely sources of inoculum for the target spot of tomato. *Aspilia africana* and the *Lepistemon* species, from which the isolates most virulent on cotton (N7 and N13) were obtained, are potential sources of inoculum on susceptible cotton varieties in the same area.

Although direct comparisons between isolates used in the present study and those in earlier reports were not made, some tentative conclusions as to the number of strains or races presently known are of interest. Olive et al. (15) described race 1 as causing severe infection on cowpea and only small spotting on soybean, and race 2 as giving few-to-many very small spots on cowpea and light spotting on soybean. Spencer and Walters (21) obtained similar results, although their race 2 isolates were more virulent on soybean than had been described earlier. Their cotton and sesame isolates were similar to the soybean strain. Stone and Jones (22) found that isolates from sesame and soybean were pathogenic on both of those hosts, but gave only small lesions on cowpea—reactions most like those of the race 2 of Olive et al. (15) and Spencer and Walters (21). Jones (12) may also have been working with isolates similar to race 2, because he found no difference between isolates from soybean, sesame, cotton, and cowpea in cross-inoculations, but cowpea leaves showed only small lesions, which increased little in size.

None of the isolates in the present study could be assigned to race 1, because none was highly virulent on cowpea. The isolates in group 4 were similar in pathogenicity to race 2 described above; i.e., highly virulent on soybean, cotton and sesame, but weakly virulent on cowpea. Several other isolates, or groups of isolates, appeared to be distinct strains or races, i.e., group 1 was highly virulent on tomato and eggplant, group 2 (N17) was highly virulent on papaya, group 3 (Fla1) was moderately virulent on cucumber, and group 5

was highly virulent on cotton but not on soybean, sesame, or eggplant. Groups 6, 7, and 8 resemble group 5 most closely, but their status is not clear at present.

The higher optimum temp for growth in culture of all isolates in the present study would appear to distinguish them from soybean root and stem rot strains reported by Boosalis and Hamilton (8) and Seaman et al. (18).

It appears that *C. cassiicola* may be only a secondary invader, or an invader of senescent tissue, and may not be a true pathogen. Several of the isolates in the present study were pathogenic on the host from which they had been isolated, i.e., N1 from eggplant, N15 from tomato, M2 and Mex1-5 from soybean, M3 from cotton, M4 from sesame, and Fla1 from cucumber. Others were nonpathogenic or weakly virulent on their original host, i.e., N14 from cowpea, N16 and Fla2 from cucumber, and M1 from soybean.

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