Inhibition of Conidial Germination and Germ Tube Growth of Cercospora canescens by Cowpea Leaf Diffusates

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ABSTRACT

Germination of conidia and germ tube growth of *Cercospora* spp. were observed in Nigeria to be inhibited on surfaces of young leaves, but not on older leaves, of cowpea (*Vigna unguiculata* 'Prima') which is susceptible to *C. canescens*. *C. canescens* conidia could not be distinguished from other *Cercospora* spp. on field-grown leaves since conidial morphology is not the sole taxonomic criterion. Germination of conidia and germ-tube growth of *C. canescens* were inhibited by diffusates from apical (but not basal) leaves of Prima, and by diffusates from apical and basal leaves of 'Lalita', resistant to *C. canescens*. Addition of nutrient broth to diffusates increased germination percentage and germ tube growth, but did not nullify inhibitory effects of the diffusates.

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Additional key words: Cercospora leaf spot, disease resistance.

Cercospora leaf spot of cowpea (*Vigna unguiculata* (L.) Walp.), caused by *Cercospora canescens* Ell. & Martin, causes severe losses in the humid tropics (6). Schneider (6) observed in Nigeria that leaf spots appeared only on leaves of susceptible cultivars which subtended flowering peduncles, but not on younger leaves which bore no reproductive structures in their axils. This was the case, even though inoculum was present and conditions were favorable for disease development.

This study was conducted to determine the basis for the 'leaf age' effect. We present information on the inhibition of germination and germ tube growth of conidia of *C. canescens* by leaf diffusates from two cowpea cultivars.

MATERIALS AND METHODS.—A young, fully expanded trifoliolate leaf from an apical branch, and an old leaf showing symptoms of Cercospora leaf spot from a basal branch, were collected from each of twenty 45-day-old field-grown cowpea (cultivar 'Prima') plants near Ibadan, Nigeria. The leaves were cut in rectangular sections to fit on a microscope slide, boiled in 70% ethanol for 30 minutes, stained with cotton blue in lactophenol, and the upper surface was observed microscopically (10). Twenty plants each of cultivars 'Prima' and 'Lalita', susceptible and resistant to *C. canescens*, respectively, were grown in a greenhouse chamber (18-30 C) with supplemental fluorescent light until the pod-filling stage (approx. 45 days). Fifteen to 20 g of leaves were harvested from each cultivar and segregated into: (i) trifoliolate leaves from basal branches which subtended peduncles bearing pods, and (ii) fully expanded apical trifoliolate leaves from branches which bore no reproductive structures in their axils. Each group was weighed separately and leached by shaking in 250 ml deionized water in 1-liter flasks for 30 minutes at 20 C on a reciprocating shaker (30 cycles/min). The leachates were reduced in vacuo (< 45 C) to a volume which corresponded to half the amount of water required to wet the leaves to run-off (approximately 2 μliter/cm^2). This value was determined by weighing leaves of a known leaf area before and after dipping in water. All diffusates were stored frozen (−5 C) until needed.

Cercospora canescens conidia were produced and aseptically harvested as described by Schneider et al. (7) after 3 days of incubation at 25 C, washed twice with deionized water by low-speed centrifugation (800 *g*) and adjusted to a final concentration of 10,000 conidia per ml in either deionized water or 0.1% Difco nutrient broth. Conidial suspensions were used immediately.

Leaf diffusates were thawed by filtration (0.22 μm). Drops (0.02 ml) were placed on sterile glass microscope slides in 9-cm diameter culture dish moisture chambers. Equal volumes of the conidial suspensions either in sterile, deionized water (nonamended) or in sterile 0.1% Difco nutrient broth (amended) were mixed with a drop of diffusate using a sterile glass needle. Controls were conidial suspensions mixed with equal volumes of sterile deionized water or nutrient broth. There were three replications for each treatment. The moisture chambers

TABLE 1. The effect of diffusates, with or without 0.1% nutrient broth, from cowpea (*Vigna unguiculata*) basal (with subtended reproductive structures) or apical (without reproductive structures) leaves of cultivars 'Prima' [susceptible, (S)] or 'Lalita' [resistant (R)] on germination of conidia and germ-tube length of *Cercospora canescens*

Source of diffusates			
Cultivar	Leaf location	Conidial germination (%)[b]	Germ tube length (μm)[c]
		Diffusates alone[a]	
Prima (S)	Apical	47.7 x[d]	27.8 vw
Prima (S)	Basal	93.2 z	57.9 y
Lalita (R)	Apical	25.6 v	18.9 v
Lalita (R)	Basal	51.1 x	25.9 v
Control (water)		72.0 y	29.1 wx
		Diffusates amended with 0.1% nutrient broth[a]	
Prima (S)	Apical	69.1 y	54.0 y
Prima (S)	Basal	100.0 z	91.0 z
Lalita (R)	Apical	36.7 w	36.3 wx
Lalita (R)	Basal	71.3 y	38.0 x
Control (water)		97.3 z	56.4 y

[a]Mixed 1:1 with conidial suspension either in sterile deionized water or nutrient broth.
[b]Mean based on 25 conidia in each of three replications (after 9 hours at 25 C).
[c]Mean total length of all germ tubes produced from 30 conidia in each of three replications.
[d]Means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

were kept under constant light at 25 C for 9 hours. Mean germination percentages were determined from twenty-five ×40 microscope fields (10-20 conidia per field) for each replication. A conidium was considered germinated if the length of at least one germ tube equaled the width of the conidium. Total germ tube length for each of 30 germinated conidia per replication was measured with an ocular micrometer.

RESULTS AND DISCUSSION.—The cleared and stained leaves from field-grown plants in Nigeria showed that germination of conidia and germ tube growth of the *Cercospora* spp. present on young leaves were completely inhibited. Practically all the conidia had germinated on the older, diseased leaves which resulted in extensive epiphytic hyphal development. Conidia of *C. canescens* could not be distinguished from other *Cercospora* spp. since conidial morphology is not the sole taxonomic criterion. Many of the conidia were probably washed off the leaf surfaces during the clearing process, but it was obvious from the several hundred conidia observed that germination was inhibited on the younger leaves.

Percentage conidial germination was significantly greater ($P = 0.05$) in diffusates from basal leaves than from apical leaves of both cultivars with or without nutrient broth (Table 1). Conidial germination and germ tube growth on nonamended leachates from basal leaves of 'Prima' were significantly greater than the nonamended control, but not significantly different from the amended control. Germination, but not germ tube length, was significantly less in nonamended and amended leachates from apical 'Prima' leaves than from the corresponding controls. Germ tube length in amended leachates from basal 'Prima' leaves was significantly greater than the amended control (Table 1). Germination and germ tube length were significantly less in amended and nonamended leachates from basal and apical leaves of 'Lalita' than in the corresponding controls.

Leaf exudates are known to contain carbohydrates, amino and organic acids, and other compounds (4). Deverall and Wood (2) and others (1, 8) suggested that increased leaf exudation conditions the host for susceptibility by making nutrients available to pathogens on the leaf surface. Preformed fungal toxicants have also been found in leaf exudates and were implicated in resistance to *C. beticola* on sugar beet (3).

Our results suggest that resistance to *C. canescens* in cowpea is due, in part, to the presence of one or more preformed fungal toxins on young leaves of the susceptible variety and young and old leaves of the resistant cultivar. Since the addition of nutrient broth to these diffusates did not nullify the inhibitory effect, we conclude that the absence of nutrients on the leaf surface is not a primary determinant of resistance in this host-parasite interaction. A reduction in germination percentage or hyphal growth would be important in conferring resistance since *Cercospora* spp. are known to penetrate leaf tissue only through stomata (5, 6, 9), thus necessitating epiphytic hyphal growth until stomata are encountered.

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