Mycosphaerella musae and *Cercospora* "Non-Virulentum" from Sigatoka Leaf Spots Are Identical

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Mycosphaerella musae and *Cercospora* "Non-Virulentum" from Sigatoka Leaf Spots Are Identical.

ABSTRACT

Cercospora "non virulentum", commonly isolated from the early streak stage of Sigatoka leaf spots caused by *Mycosphaerella musicola* and *M. fijiensis*, is identical to *M. musae*. Both produce the same verruculose *Cercospora*-like conidia within 4 to 5 days on plain agar. No conidia are produced on banana leaves. Discharge of *M. musae* ascospores from massed leaf spots is more abundant from leaves infected with *M. musicola* than with *M. fijiensis*. Cultures of *Cercospora* "non-virulentum" and *M. musae* can cause spotting on young leaves of Cavendish varieties after inoculation but the incubation period exceeds 70 days. *M. musae* is an endophyte in banana leaves infected by Sigatoka pathogens in Central America, Mexico, Ecuador, Surinam and the Carribbean.

Comparaison des souches de *Mycosphaerella musae* et de *Cercospora* "non virulent" isolées sur des nécroses de Sigatoka.

RÉSUMÉ

Des souches de *Cercospora* "non virulentes", isolées habituellement lorsque les premières nécroses de Sigatoka, dues à *Mycosphaerella musicola* et *M. fijiensis*, apparaissent sur les feuilles, sont identiques à celles de *M. musae*. Les deux souches produisent les mêmes conidies verruqueuses après 4 à 5 jours de culture sur de l'agar pur. Aucune conidie n'est produite sur les feuilles de bananiers. La décharge d'ascospores de *M. musae* à partir de nécroses fusionnées entre elles est plus abondante quand les feuilles sont infectées avec *M. musicola* qu'avec *M. fijiensis*. Des cultures de souches non virulentes de *Cercospora* et de *M. musae* peuvent provoquer des lésions sur des jeunes feuilles de la variété Cavendish, mais la période d'incubation est supérieure à 70 jours. *M. musae* est un champignon endophyte des feuilles de bananiers infectées par les pathogènes responsable des deux cercosporioses en Amérique centrale, au Mexique, en Equateur, au Surinam et dans la Caraïbe.

Mycosphaerella musae y *Cercospora* "no virulenta" de Sigatoka son identicas.

RESUMEN

Cercospora "no virulenta", comunmente aislada de estadios tempranos de Sigatoka causada por *Mycosphaerella musicola* y *M. fijiensis*, es identica a *M. musae*. Ambas producen el mismo conidio entre 4 a 5 dias en agar. No se produjeron conidios en las hojas. Descargas de ascosporas de *M. musae* son mas abundantes en hojas infectadas con *M. musicola* que con *M. fijiensis*. Cultivos de *Cercospora* "no virulenta" y *M. musae* pueden causar esporulación en hojas jovenes de variedades Cavendish después de ser inoculadas, pero el período de incubación excede los 70 dias. *M. Musae* es un endofito en hojas de banano infectadas, con Sigatoka en América Central, Mexico, Ecuador, Surinam y el Caribe.

Fruits, vol. 49, n°3, p. 187-190

KEYWORDS
Musa, Mycosphaerella, Cercospora, blotches, conidia, lesions.

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PALABRAS CLAVES
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introduction

Previous studies showed that a variety of fungal flora is present in leaf spot caused by *Mycosphaerella musicola* (= *Pseudocercospora musae*) (STOVER, 1963, 1969). *M. musae* was found to be one of the most ubiquitous species (STOVER, 1969). A similar flora was found in leaf spots caused by *M. fijiensis* (= *Paracercospora fijiensis*) (ANON., 1983). In addition, in young lesions caused by *M. fijiensis*, a fungus called *Cercospora* "non-virulentum" was prevalent, especially in areas sprayed with benomyl (STOVER, 1977a, 1977b). Additional studies with *M. musae* and *Cercospora* "non-virulentum" are reported here and the results revealed that the latter is the samed as *M. musae.*

methods

Fungi were isolated by laying leaf pieces from the early streak stage of the Sigatoka disease on Martin's medium (MARTIN, 1950). Five pieces, about 5-11 x 1-2 mm, were taken from each young lesion, dipped in 5% chlorox (sodium hypochlorite 5.25%) for 5 min and then washed in distilled water. Hyphae growing from the leaf pieces were transferred to Mycophyl Agar (BBL Microbiology Systems). Fungi identified as one of the two Sigatoka pathogens or the faster growing *Cercospora* "non-virulentum" (STOVER, 1977b) were retained, all other fungi were discarded. Hyphae from the study fungi were streaked on 3% agar and the development of *Cercospora* conidia was observed over a period of about one week at room temperature (23°C-26°C).

Mycosphaerella spp. ascospores were discharged from leaf spot areas with mass infection according to the guidelines for monitoring ascospores for fungicide resistance (ANON., 1983). Ascospores characteristic of Sigatoka pathogens and *M. musae* were transferred to tubes containing Mycophyl Agar. Colony and sporulation characteristics of hyphae isolates from young lesions and *Mycosphaerella* ascospores were compared.

Young cv Valery plants about six months old derived from meristem cultures were inoculated by smearing a suspension of hyphae and conidia from agar cultures on the underside of the youngest leaves. The plants were then placed inside a closed clear plastic container where a saturated atmosphere was maintained for at least 12 h per day.

results

Most young streak lesions caused by *Mycosphaerella fijiensis* yielded *Cercospora* "non-virulentum" colonies, although usually fewer than the Sigatoka pathogen (Tables 1 and 2). The widespread use of benlate ended in 1977 and, in 1978, the proportion of *C.* "non-virulentum" colonies tended to be higher than in the 1980s. These colonies were previously shown to have resistance to up 7000 ppm of benomyl (STOVER, 1977b).

Table 1
Proportion of *Mycosphaerella fijiensis* and *Cercospora* "non-virulentum" isolated from young streak lesions.

Honduras Farm	*M. Fijiensis* n° colonies isolated	%	*C."non-virulentum"* n° colonies isolated	%
1978				
Mopala	90	100	0	0
Sta. Rosa	71	87	11	13
Ceibita	86	100	0	0
Indiana	49	58	36	24
Limones	44	54	37	46
Omonita	61	71	25	29
Echeverry	45	52	42	48
Coyoles	16	76	5	24
Isletas	40	80	10	20
1980-83				
Mopala	35	100	0	0
Sta.Rosa	68	100	0	0
Ceibita	42	71	17	29
Indiana	62	86	10	14
Limones	65	93	5	7
Omonita	75	89	9	11
Copen	56	62	13	14
Corozal	115	77	0	0
Cobb	67	75	0	0
Cobb	79	94	5	6
Las Flores	84	95	4	5
Naranjo Chino	67	91	7	9

C. "non-virulentum" was also present in most early streak lesions caused by *Mycosphaerella musicola* prior to the arrival of *M. fijiensis* in Central America, Mexico and Surinam (Table 2). In some instances, *C.* "non-virulentum" was more abundant than the Sigatoka pathogen.

When colonies from single ascospore isolates of *M. musae* were compared to colonies of *C.* "non-virulentum", they appeared to be identical (Photo 1). Conidia production on plain agar were also identical (Photo 2). Conidia were 55-200 µm long (average 127) x 2.6-3.2 µm (average 2.9). Conidiophores were 24-46 µm long (average 35) with one septum. Conidia were verruculose and usually had a scar. Colonies that produced smooth-walled conidia with a faint scar or no scar were occasionally isolated. Conidia were produced in 4-5 days. In addition to conidia, about one culture in twenty produced a few spermagonia with spermatia. Perithecia or ascospores were not found on Mycophyl Agar.

Inoculation of young cv Valery plants with a mixture of mycelium and conidia of *C.* "non-virulentum" resulted in the appearance of spots after 70 days incubation (Photo 3). Similar results were obtained with ascospore-derived cultures of *M. musae*. Symptoms of natural *M. musae* infection were not observed on Cavendish varities in the field.

Table 2
Proportion of *Mycospharella musicola* and *Cercospora* "non-virulentum" isolated from young streak lesions.

Location	Percentage *M. musicola*	Percentage *C.* "non-virulentum"
Puerto Cortes, Honduras	46	35
Coto, Costa Rica	23	77
Palmar, Costa Rica	39	61
Changuinola, Panama	56	21
Armuelles, Panama	39	61
Ecuador	91	9
Surinam	31	30
Tapachula, Mexico	95	5
Martinique	49	12
Guadeloupe	78	20

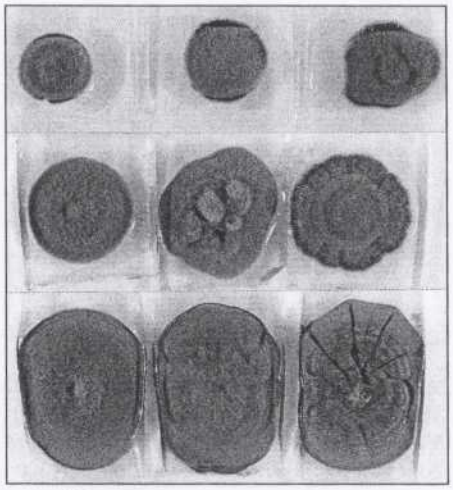

Photo 1
Cercospora *colonies on Mycophil Agar from single ascospores of* Mycosphaerella musae *from banana leaves infected with* M. musicola *in Guadeloupe. From top to bottom, colonies are 7 days (7-9 mm), 10 days (12-14 mm) and 14 days (21-22 mm).* Cercospora *"non-virulentum" colonies were identical to the colonies shown.*

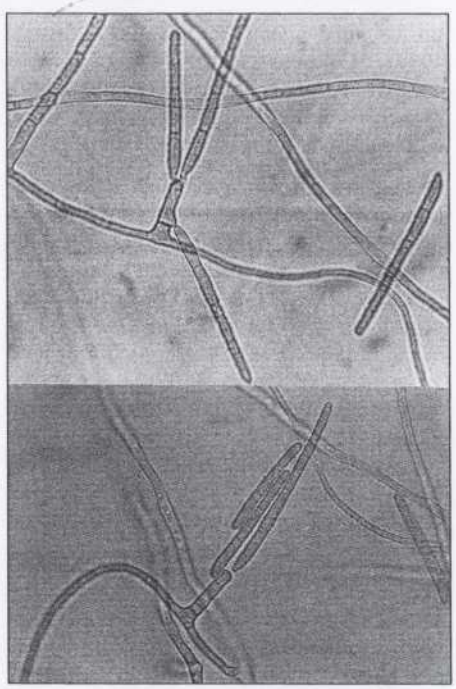

Photo 2
Verruculose conidia of Cercospora *"non-virulentum" (upper) and* Cercospora musae *(lower) on water agar after 5 days at room temperature in window light (1,000X). Several conidia were produced on each conidiophore and usually had a scar where attached.*

discussion

Cercospora "non-virulentum" was first detected as a prevalent co-inhabitant with the black Sigatoka pathogen of young lesions in plantations sprayed with benlate. It developed tolerance to benomyl before *Mycosphaerella fijiensis*, and this increased its prevalence in black Sigatoka lesions in benomyl-sprayed areas (STOVER, 1977a, 1977b). A similar *Cercospora* was also found to be common in young

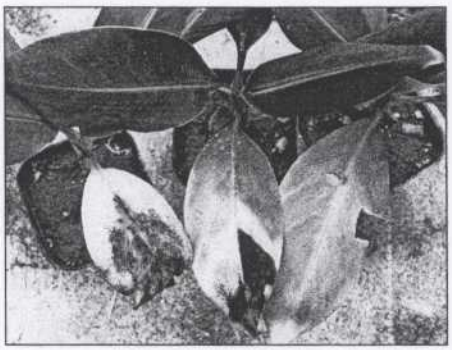

Photo 3
Lesions caused by inoculation of Mycosphaerella musae *on the underside of the two youngest leaves of cv Valery plants produced* in vitro. *The lesions appeared on the oldest leaves after 70 days.*

lesions of *Mycosphaerella musicola* in Martinique (CHEVALIER-CEDEÑO, 1983). Moreover, in Martinique, ascospores believed to be *M. musae* yielded verruculose *Cercospora* conidia and spermagonia on V8 juice agar (VAN DER BERG-LORIDAT, 1989). Cultures with a few smooth conidia were occasionally obtained and, in a few cultures, perithecia were formed with ascospores measuring 13-14 µm.

The source of *Cercospora* "non-virulentum" was believed to be related to the two Sigatoka pathogens. The results reported here showed that *C.* "non-virulentum" and *M. musae* were identical, producing the same colonies and conidia in culture. Both were pathogenic but with an incubation period of 70 days or more. In Australia, PONT (1960) demonstrated an incubation period of six weeks to the "brown blotch" stage when young leaves were inoculated with *M. musae*. Further development ceased until the inoculated leaf was well down the plant.

The unusual features of *M. musae* in the American tropics is its close association with *M. musicola* and *M. fijiensis*. It does not alone cause symptoms in the field, but appears to behave as an endophyte in lesions caused by the two Sigatoka pathogens. *M. musae* appears to be much more common and ascospore discharges occur more frequently from leaf spotting caused by *M. musicola* (STOVER, 1969).

There are two reports of isolates from *M. musicola* lesions producing perithecia and ascospores similar to *M. musae* in culture (CHEVALIER-CEDEÑO, 1983; VAN DER BERG-LORIDAT, 1989). In view of recently reported *in vitro* techniques for ascospore production by *M. fijiensis* (MOURICHON and ZAPATER, 1990), it should be possible to enhance ascospore production by *M. musae* in cultures producing spermagonia and spermatia. Although *M. musae* readily produces conidia *in vitro*, conidia have never been found *in vivo*.

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