Molecular Taxonomy of *Colletotrichum* Species Causing Anthracnose on the Malvaceae

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ABSTRACT

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The taxonomic status of isolates of *Colletotrichum* (*C. gossypii*; *C. gossypii* var. *cephalosporioides*; *C. gloeosporioides* f. sp. *malvae*, including BioMal; and *C. malvarum*) from cotton, *Lavatera trimestris*, *Malva pusilla*, and *Sida spinosa* was studied. For a representative sample of these isolates, conidial morphology and differentiation, their affinity for the lectin *Bauhinia purpurea* agglutinin (BPA) and a monoclonal antibody (UB 20), and the nature of their infection hyphae were assessed in association with an analysis of rDNA sequence data. The results revealed that all the isolates from *L. trimestris*, *M. pusilla*, and *S. spinosa*, including BioMal and several other samples of *C. gloeosporioides* f. sp. *malvae*, were forms of the *C. orbiculare* aggregate species. All these isolates produced straight-cylindrical conidia that bound BPA and UB 20 and remained aseptate after germination. The similarity of these isolates to other forms of the *C. orbiculare* species aggregate was confirmed by examination of their initial infection process and by restriction analysis of their rDNA. Analysis of isolates from cotton showed that they were similar to each other and to several forms of *C. gloeosporioides*. Thus, it is probably not appropriate to regard *C. gossypii* as a species distinct from *C. gloeosporioides*. The suitability of the *C. orbiculare* species aggregate for studies on the molecular basis of host specificity is discussed.

Additional keywords: *Gossypium*, *lindemuthianum*, *trifolii*.

The family *Malvaceae* is large and consists of important agricultural crops (cotton, kenaf, and okra), ornamental species (abutilon, hollyhock, hibiscus, and lavatera), and some widespread weeds (*Malva* spp. and *Sida* spp.). The family, as a whole, is affected by several anthracnose diseases. Three main species of *Colletotrichum* have been recognized as the causal agents of anthracnose: *C. gossypii* Southworth (teleomorph *Glomerella gossypii*) from cotton; *C. malvarum* (A. Braun and Casp.) Southworth from hollyhock, lavatera, and *S. spinosa* L. (8,10,28); and *C. gloeosporioides* (Penz.) Penz. and Sacc. from many different malvaceous hosts including cotton and various weeds, e.g., *M. pusilla* Smith (8,14,28). The morphological differences between these species are not great and, often, the identification is based on the host from which they were obtained rather than on specific morphological characteristics. One other species, *C. coccodes*, was reported from abutilon (31).

C. malvarum is a synonym of *C. althea*, which was originally described from hollyhock (24). In a subsequent note, Southworth (25) observed that *C. althea* closely resembled *C. lindemuthianum*, that a similar pathogen affected *S. spinosa* in the United States, and that it had been described on *Malva* spp. in Europe several years earlier. It was on the basis of this earlier description that the pathogen was renamed *C. malvarum* (28). It produces darkly pigmented colonies with abundant setae. Conidia are straight-cylindrical to ellipsoid, 8 to 24 × 4 to 6 μm, with an obtuse apex and a truncate base (28). Sutton (28) regarded *C. malvarum* as distinct from *C. gossypii* and *C. gloeosporioides*, which he considered to be forms of the same species. In contrast, von Arx (29) regarded *C. malvarum* as a host-specific form of *C. gloeosporioides* that was distinct from *C. gossypii*. Recently, comparative morphological and molecular studies confirmed the close relationship between an isolate of *C. malvarum* from *S. spinosa* and isolates of *C. lindemuthianum* from bean, *C. orbiculare* from *Cucumis sativus*, and *C. trifolii* from alfalfa (21,23). As a result, it was proposed that *C. malvarum* should be regarded as a host-specific form of a species aggregate called *C. orbiculare* (23).

C. gloeosporioides is a large species aggregate that has been isolated from many different host plants. Often isolates from different hosts have been given names that reflect their origins and, thus, many hundreds of species and special forms have been described (28,29). Typically, cultures are very variable, setae may be present or absent, and conidia are cylindrical with an obtuse apex and a truncate base (12 to 17 × 3.5 to 6 μm). The teleomorph is regarded as *G. cingulata*. *C. gloeosporioides* has been reported on several species of the Malvaceae. On some hosts, e.g., *Hibiscus* spp., forms with the morphology of *C. gloeosporioides* have been given distinct names, e.g., *C. hibisci*, that reflect their host origin rather than any morphological differences (28). The anthracnose pathogens from *Malva* spp. and *Lavatera trimestris* L. have usually been regarded as *C. gloeosporioides* f. sp. *malvae* (11,14,16). One isolate of *Colletotrichum* from *M. pusilla* has been registered as a mycoherbicide for weed

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control in Canada. This fungus was identified as *C. gloeosporioides* f. sp. *malvae* and patented in Canada under the registered trademark BioMal (15).

C. gossypii is the name normally given to the cause of cotton anthracnose. It is generally considered to be the anamorph of *G. gossypii* (6,7,8). The pathogen affects bolls and seedlings, in which it may cause damping-off. *C. gossypii* causes little damage to mature plants. A virulent form causing ramulosis, i.e., abnormal branching of infected plants, occurs in Latin America (6,7). Ramulosis is considered to be caused by a distinct pathogen, *C. gossypii* var. *cephalosporioides*. No authoritative descriptions of the morphology of these pathogens have been reported, though their mycelia, conidia, and appressoria are generally similar to those of *C. gloeosporioides*. Several authors have already emphasized that there are no morphological differences between *C. gossypii* and *C. gloeosporioides* (6,7, 28).

To resolve the taxonomic status of the pathogens described above, the morphology and rDNA sequences (internal transcribed spacer 2 [ITS-2] and domain 2 [D2] of the 28S region) of isolates of *Colletotrichum* from a range of different malvaceous hosts were compared. Analysis of these regions has already been used to confirm and correct taxonomic uncertainties in the genus *Colletotrichum* (3,22,23,26,27). The selection of isolates did not embrace all the forms that have been isolated from this plant family, though it did include species from ornamental, agronomic, and weed species.

MATERIALS AND METHODS

Fungal and plant material. The isolates of *Colletotrichum* studied are described in Table 1. All isolates were obtained from plants grown in the regions of origin, except those from *L. trimestris* which were isolated from commercial seed samples of unknown geographic origin. Isolates were cultured on Mathur's agar medium (CM) (12,23). The affinity of their conidia for the lectin *Bauhinia purpurea* agglutinin (BPA) and the monoclonal antibody UB 20, which recognizes different sets of glycoproteins on the surface of *C. lindemuthianum* conidia, was determined by fluorescence microscopy (19,21). Isolates of *C. lindemuthianum* (LARS 137) and *C. gloeosporioides* (LARS 074, an isolate of the mycoherbicide Collego) were included in all experiments as positive and negative controls (19,21) to confirm the specificity of labeling by these probes. The production of a septum in conidia during germination was also investigated (19). Together, these characteristics have been used to define the *C. orbiculare* aggregate species, sensu Sherriff et al. (23), and to distinguish members of this group from all other species of *Colletotrichum* (19,21). The morphology of the initial infection structures produced within excised leaves of *S. spinosa* that had been grown and incubated at 25°C was studied by examination of cleared, infected tissues by differential and interference microscopy (20).

Preparation and amplification of rDNA. Conical flasks containing Czapek Dox-V8 liquid medium (23) were inoculated with conidia from 7-day-old cultures and incubated at 25°C for 3 to 4

TABLE 1. Origins of isolates from malvaceous hosts

LARS no.	Supplier's code	Supplied as	Original host	Geographical origin	EMBL accession no.[a]
Isolates from Malvaceae					
625[b]	Cm-9	*C. malvarum*	*Sida spinosa* (prickly sida)	Arkansas, United States	z74698[c], z74699[d]
626[b]	TN-1	*C. malvarum*	*Sida spinosa* (prickly sida)	Tennessee, United States	z74700[c], z74701[d]
627[b]	TN-3	*C. malvarum*	*Sida spinosa* (prickly sida)	Tennessee, United States	z74702[c], z74703[d]
629[b]	Cm-4	*C. malvarum*	*Sida spinosa* (prickly sida)	Arkansas, United States	z74704[c], z74705[d]
717[e]	Lav-1	*Colletotrichum* sp.	*Lavatera trimestris* (Mont Blanc)	United Kingdom[f]	z74706[c], z74707[d]
718[e]	Lav-2	*Colletotrichum* sp.	*Lavatera trimestris* (Mont Blanc)	United Kingdom[f]	z74708[c], z74709[d]
719[e]	Lav-3	*Colletotrichum* sp.	*Lavatera trimestris* (Mont Blanc)	United Kingdom[f]	z74710[c], z74711[d]
720[e]	Lav-4	*Colletotrichum* sp.	*Lavatera trimestris* (Mont Blanc)	United Kingdom[f]	z74712[c], z74713[d]
733[b]	83-43	*C. gloeosporioides* f. sp. *malvae*	*Malva pusilla* (round-leaved mallow)	Raymore, Saskatchewan, Canada	z74714[c], z74715[d]
734[b]	84-12	*C. gloeosporioides* f. sp. *malvae*	*Malva pusilla* (round-leaved mallow)	Lockwood, Saskatchewan, Canada	z74716[c], z74717[d]
735[b]	84-25	*C. gloeosporioides* f. sp. *malvae*	*Malva pusilla* (round-leaved mallow)	Estuary, Saskatchewan, Canada	z74718[c], z74719[d]
737[b]	84-15	*C. gloeosporioides* f. sp. *malvae*	*Malva pusilla* (round-leaved mallow)	Antler, Saskatchewan, Canada	z74720[c], z74721[d]
738[b]	83-43-1	*C. gloeosporioides* f. sp. *malvae*	*Malva pusilla* (round-leaved mallow)	Regina, Saskatchewan, Canada	z74722[c], z74723[d]
773[g]	94116	*C. gloeosporioides* f. sp. *malvae* (BioMal)	*Malva pusilla* (round-leaved mallow)	United States	z74724[c], z74725[d]
795[h]	IMI 82269	*C. gossypii*	*Gossypium* sp. (cotton)	Brazil	z74732[c], z74733[d]
794[h]	IMI 277115	*C. gossypii* f. sp. *cephalosporioides*	*Gossypium* sp. (cotton)	Bolivia	z74728[c], z74729[d]
796[h]	IMI 80023	*C. gossypii* f. sp. *cephalosporioides*	*Gossypium* sp. (cotton)	Brazil	z74730[c], z74731[d]
Reference isolates[i]					
058	IMI 165753	*C. acutatum*	*Musa nana*	West Indies	z18976
163	G1	*C. acutatum*	*Lupinus* sp.	France	z18989
079	IMI 62650	*C. capsici*	*Piper betle*	Pakistan	z18982
141	ODA 14 (1)	*C. capsici*	*Vigna unguiculata*	Nigeria	z18988
074	ATCC 20358	*C. gloeosporioides*	*Aeschynomene virginica*	United States	z18980
167	SR-24	*C. gloeosporioides*	*Stylosanthes scabra*	Australia	z18992
501	MC10	*C. gloeosporioides*	*Mangifera indica*	Malaysia	z18998
009	ATCC 56897	*C. lindemuthianum*	*Phaseolus vulgaris*	Europe	z18975
076	ATCC 58399	*C. malvarum*	*Sida spinosa*	United States	z18981
414	104-T	*C. orbiculare*	*Cucumis sativus*	Japan	z18997
507		*C. orbiculare*	*Cucumis sativus*	France	z19001
164	N85 ANW	*C. trifolii*	*Medicago sativa*	United States	z18990

[a] From the European Molecular Biology Laboratory database.
[b] Supplied by D. O. TeBeest, 217 Plant Science Building, Department of Plant Pathology, University of Arkansas, Fayetteville 72701.
[c] ITS-2.
[d] D2.
[e] Supplied by R. Maude, Horticultural Research International, Wellesbourne, United Kingdom.
[f] Commercial seeds of unknown geographical origin.
[g] Supplied by C. Kuiack, Philom Bios, 318-111 Research Drive, Saskatoon, Saskatchewan S7N 3RT, Canada.
[h] Purchased from International Mycological Institute, United Kingdom.
[i] According to Sherriff et al. (23).

days at 140 rpm. Mycelium from axenic cultures was harvested, ground to a powder in liquid nitrogen, and freeze-dried for 24 h. DNA was purified using a method adapted from Graham et al. (5) and treated with RNAase (23). ITS-2 of the rDNA and D2 of the 28S rDNA were amplified separately (23). The D2 region was amplified with primers Pn2 (5′-GTTCACCATCTTTCGCCTCC-3′) and Pn9 (5′-CTTAAGCATATCAATAAGCGGAGG-3′). The ITS-2 region was amplified with primers Pn3 (5′-CCGTTGGTG-AACCAGCGGAGGGATC-3′) and Pn8 (5′-GCTGCATTCCCA-AGCAACCCGACTC-3′). When the polymerase chain reaction (PCR) of the ITS-2 region was unsuccessful, Pn5 (5′-TTTCAAC-AACGGATCTCTTGG-3′) was used instead of Pn3. Sequencing was carried out using the Sequenase PCR Product Sequencing Kit (United States Biochemical, Amersham, United Kingdom) according to the manufacturer's recommendations. The sequencing primers used were Pn4 (5′-CCTTGGTCCGTGTTCAAGACGGG-3′) for the D2 region and Pn8/10 (5′-GCCCAAAGCATCCTCTGCA-AATTA-3′) for the ITS-2 region. Sequencing was performed according to the methods used previously (23).

Analysis of rDNA sequences. Sequences were aligned using GCG PileUp (Genetics Computer Group Inc., Madison, WI) (using all default settings). The D2 and ITS-2 regions of rDNA of *Colletotrichum* isolates from the Malvaceae were compared with the rDNA sequences of several reference species (22,23). A similarity matrix based on the proportion of different nucleotide sites was calculated from the data, with transitions and transversions given the same weight (deletions were ignored). A tree showing relatedness between isolates was constructed from the distance matrix by the neighbor-joining method using the MEGA software package (MEGA: Molecular Evolutionary Genetic Analysis, Version 1.01; Pennsylvania State University, University Park), and a bootstrap analysis based on 1,000 resamples of the data was carried out (23).

PCR amplification of rDNA spacers and restriction of products. Restriction digests of amplified rDNA have been used to characterize isolates of *C. lindemuthianum* (4). A region of the ribosomal genes containing ITS-1, ITS-2, and the 5.8S subunit was amplified using primers Pn3 and Pn10 (5′-TCCGCTTATTG-ATATGCTTAAG-3′). The amplification products were digested with *Hae*III and *Msp*I (4), and the products were separated on MetaPhor agarose (FMC BioProducts, Rockland, ME). The sizes of products were compared with those obtained from other members of the *C. orbiculare* aggregate, including isolates of *C. lindemuthianum* that represented examples of the two populations that had been described earlier (4), and with several other *Colletotrichum* species.

RESULTS

Morphology of the pathogen isolates. All isolates from *S. spinosa* produced cultures with dark mycelium and straight-cylindrical conidia, some of which had slightly pointed ends. Upon germination, conidia remained aseptate and the appressoria produced were sessile, or formed at the end of short germ tubes, and globose (Fig. 1A). Conidia fluoresced strongly when labeled with BPA or UB 20 (Table 2). All isolates from *L. trimestris* and *M. pusilla*, including the BioMal pathogen, had morphologies similar to the isolates from *S. spinosa*. Their conidia remained aseptate up-

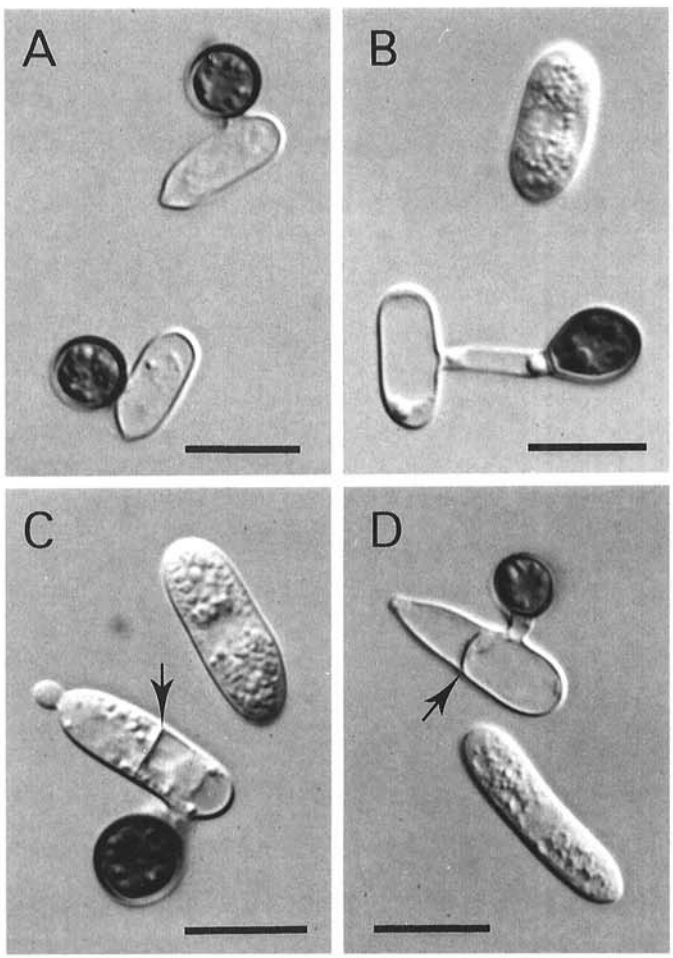

Fig. 1. Germinated and ungerminated conidia of **A,** LARS 629 from *Sida spinosa*; **B,** LARS 773 BioMal from *Malva pusilla*; **C,** LARS 794 from *Gossypium*; and **D,** LARS 795 from *Gossypium* viewed with a differential interference microscopy 18 h after placement of conidial suspensions on glass slides. All isolates have produced globose appressoria. Germinated conidia of LARS 794 and LARS 795 contained a single septum (arrows), while those of LARS 29 and LARS 773 remained aseptate. The absence of a septum in germinated conidia is a characteristic of the *Colletotrichum orbiculare* aggregate species. Bar = 10 μm.

TABLE 2. Morphological features of conidia of isolates of *Colletotrichum* from the Malvaceae

Isolate source LARS no.	Conidial length, μm	Conidial shape	Septation upon germination	BPA[a] labeling	UB 20[b] labeling
Isolates from *Lavatera*, *Malva*, and *Sida* (625-627, 629, 717-720, 733-738, 773)	9–15	Straight-cylindrical	No	Yes	Yes
Isolates from *Gossypium* (794-796)	12–20	Straight-cylindrical	Yes	No	No

[a] *Bauhinia purpurea* agglutinin (16).
[b] Monoclonal antibody raised to germlings of *C. lindemuthianum* (19).

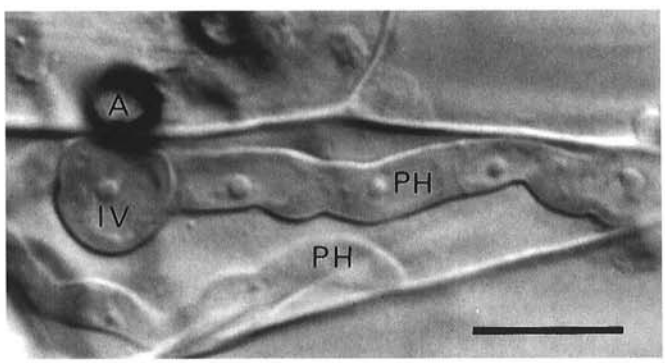

Fig. 2. Cleared leaf tissue of *Sida spinosa* 4 days after inoculation with LARS 629 (from *S. spinosa*) viewed with a differential interference microscopy. Beneath an appressorium (A) on the leaf surface, a large globular intracellular infection vesicle (IV) and primary hypha (PH) have developed inside the epidermal cell. The production of such vesicles is a characteristic of the *Colletotrichum orbiculare* aggregate species. Bar = 10 μm.

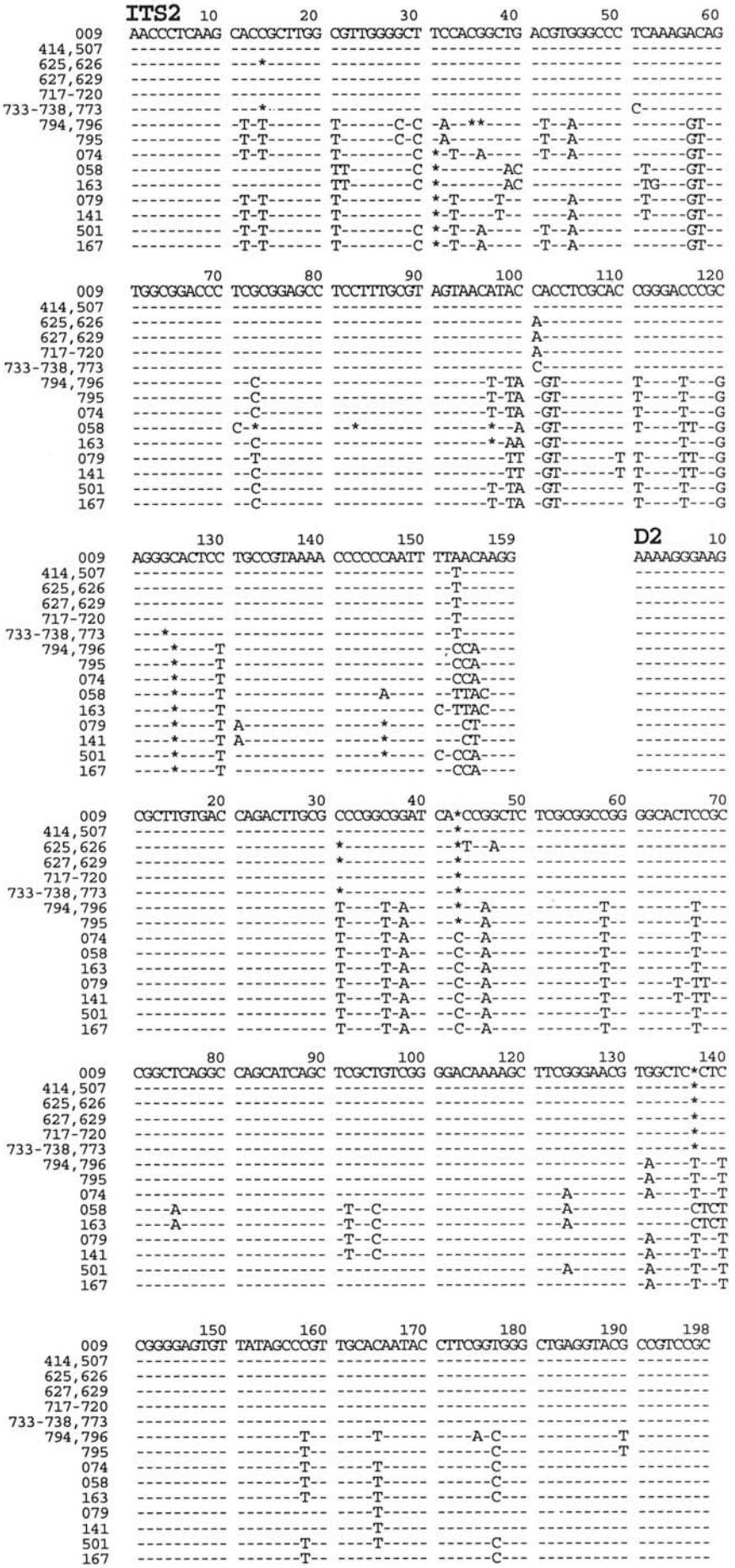

Fig. 3. Aligned sequences of the ITS-2 and D2 regions of rDNA of *Colletotrichum* isolates. Key to isolate numbers is in Table 1. – indicates base homologous with isolate 009. * indicates possible base deletion.

on germination (Fig. 1B) and showed identical affinities for BPA and UB 20 (Table 2). Isolates from *Gossypium* produced cultures that were similar to the other isolates when grown on CM, and the size and shape of their conidia and appressoria were also comparable. However, their conidia were not labeled by BPA or UB 20 and, upon germination in water, all conidia produced a septum (Fig. 1C and D; Table 2). Conidia of all the isolates were within the range of 9 to 20 μm in length, and all appressoria were 5 to 7 μm in diameter.

Examination of *S. spinosa* leaves that had been inoculated with isolates obtained from that host (LARS 625, 626, 627, and 629) revealed the presence of globose intracellular infection vesicles and primary hyphae in epidermal cells 4 days after inoculation (Fig. 2).

Comparison of rDNA sequences. The nucleotide sequences of the ITS-2 and D2 region of the 28S rDNA and the accession numbers of the European Molecular Biology Laboratory database are shown in Figure 3, along with data for isolates of *C. lindemuthianum*, *C. orbiculare* from *Cucumis sativus*, and *C. gloeosporioides* (23). From the difference matrix (Table 3) and corresponding tree and bootstrap analysis (Fig. 4), all the isolates from *L. trimestris, M. pusilla*, and *S. spinosa* appear to be very closely related (>99% homology) to each other and to members of the *C. orbiculare* aggregate, sensu Sherriff et al. (23).

The isolates from *Gossypium* had sequences that were different (10 to 12%) from those of the isolates from the other malvaceous hosts. The sequences of *C. gossypii* and *C. gossypii* var. *cephalosporioides* were almost identical (>99.5%) and, by comparison with other *Colletotrichum* species, were closely related to isolates of *C. gloeosporioides* (>97% homology). All the isolates from malvaceous hosts were distinct (8 to 13.5% differences) from the other species, i.e., *C. acutatum* and *C. capsici*, included in the comparison.

Restriction analysis of rDNA. The amplified fragments of all the isolates from *L. trimestris*, *M. pusilla*, and *S. spinosa* were of similar molecular weight, approximately 550 bp, which was identical to those obtained from *C. lindemuthianum*, *C. orbiculare* from *Cucumis sativus*, and *C. trifolii*. Digestion with *Hae*III and *Msp*I each produced distinct but identical patterns for all the isolates, except for one sample of *C. lindemuthianum* (Fig. 5). The patterns for isolates obtained for *C. gloeosporioides*, *C. capsici*, and *C. acutatum* were different from those illustrated in Figure 5 (C. Nash, *unpublished data*).

DISCUSSION

The concept of a species for anamorphic fungi is ill-defined. In this paper, "species" is used to group isolates that have highly conserved rDNA and have correlated morphological and biochemical characters. On this basis, the morphological and molecular data indicate that there are two distinct species in the sample of isolates of *Colletotrichum* obtained from four species of the Malvaceae.

The isolates from *L. trimestris*, *M. pusilla*, and *S. spinosa* are sufficiently similar to be regarded as the same species. They all have highly homologous rDNA sequences, show characteristic aseptate germinated conidia, and have identical affinities for the lectin BPA and the monoclonal antibody UB 20. In all these respects, the isolates from *L. trimestris*, *M. pusilla*, and *S. spinosa* are identical to *C. lindemuthianum* from *Phaseolus*, *C. orbiculare* from *Cucumis sativus*, and *C. trifolii* from *Medicago sativum* (21,23). In addition, a study of the infection processes of isolates from *S. spinosa* revealed the production of intracellular infection structures that were similar to those produced by *C. lindemuthianum* and *C. trifolii* (2,17,18) on their respective hosts. When the rDNA restriction patterns were compared, all the isolates from *L. trimestris*, *M. pusilla*, and *S. spinosa* produced the same patterns (type I, as described by Fabre et al. [4]), which were identical to those found for *C. lindemuthianum*, *C. orbiculare*, and *C. trifolii*.

In accordance with previous proposals (28), all the isolates from *L. trimestris*, *M. pusilla*, and *S. spinosa*, including BioMal, could be regarded as *C. malvarum*, and the existing names for *C. lindemuthianum*, *C. orbiculare* (synonym = *C. lagenarium*), and *C. trifolii* could be retained. This nomenclature, however, relies exclusively on the distinct host specificity shown by these pathogens. For example, isolates from *S. spinosa* and *M. pusilla* only affected the host from which they were obtained (14). Similarly, isolates from *Cucumis sativus* are specific for a few cucurbit species (30), while those from *Phaseolus vulgaris* only attack plants of the same genus (J. A. Bailey, *unpublished data*). However, such names totally ignore the close morphological and molecular similarities between these pathogens. For this reason, it is now proposed that all these pathogens be regarded as forma speciales of the *C. orbiculare* aggregate species (23), with their hosts indicated: *C. orbiculare* f. sp. from *L. trimestris* (previously *C. gloeosporioides* f. sp. *malvae*); *C. orbiculare* f. sp. from *S. spinosa* (previously *C. malvarum*); *C. orbiculare* f. sp. from *M. pu-*

TABLE 3. Difference matrix of a combined analysis of ITS-2 and D2 regions of rDNA of *Colletotrichum* isolates[a]

		058	163	079	141	074	167	501	794	795	009	076	625	627	717	733	414	507	164
058	*C. acutatum*																		
163	*C. acutatum*	0.018																	
079	*C. capsici*	0.078	0.090																
141	*C. capsici*	0.075	0.087	0.003															
074	*C. gloeosporioides*	0.057	0.063	0.054	0.051														
167	*C. gloeosporioides*	0.060	0.066	0.057	0.054	0.009													
501	*C. gloeosporioides*	0.060	0.060	0.057	0.054	0.003	0.012												
794	from *Gossypium*[b]	0.069	0.075	0.072	0.069	0.024	0.021	0.027											
795	from *Gossypium*	0.072	0.078	0.069	0.066	0.021	0.018	0.024	0.015										
009	*C. lindemuthianum*	0.111	0.105	0.096	0.099	0.090	0.081	0.093	0.090	0.087									
076	*C. malvarum*	0.126	0.120	0.117	0.120	0.108	0.099	0.111	0.108	0.105	0.024								
625	from *Sida*[c]	0.117	0.111	0.108	0.111	0.099	0.090	0.102	0.099	0.096	0.012	0.024							
627	from *Sida*[d]	0.111	0.105	0.102	0.105	0.093	0.084	0.096	0.093	0.090	0.006	0.018	0.006						
717	from *Lavatera*[e]	0.111	0.105	0.102	0.105	0.093	0.084	0.096	0.093	0.090	0.006	0.018	0.006	0.000					
733	from *Malva*[f]	0.111	0.105	0.102	0.105	0.093	0.084	0.096	0.093	0.090	0.006	0.024	0.012	0.006	0.006				
414	*C. orbiculare*	0.108	0.102	0.099	0.102	0.090	0.081	0.093	0.090	0.087	0.003	0.021	0.009	0.003	0.003	0.003			
507	*C. orbiculare*	0.108	0.102	0.099	0.102	0.090	0.081	0.093	0.090	0.087	0.003	0.021	0.009	0.003	0.003	0.003	0.000		
164	*C. trifolii*	0.117	0.111	0.108	0.111	0.099	0.090	0.102	0.099	0.096	0.012	0.012	0.018	0.012	0.012	0.012	0.009	0.009	

[a] Proportion of differences are calculated as the proportion of sites the same, with differences (transition and transversion only) counted as one.
[b] Also LARS 796.
[c] Also LARS 626.
[d] Also LARS 629.
[e] Also LARS 718–720.
[f] Also LARS 734–738 and 773.

silla (previously *C. gloeosporioides* f. sp. *malvae*); *C. orbiculare* f. sp. from *Medicago sativum* (previously *C. trifolii*); *C. orbiculare* f. sp. from *Phaseolus* spp. (previously *C. lindemuthianum*); *C. orbiculare* f. sp. from *Cucumis sativus* (previously *C. orbiculare*, synonym = *C. lagenarium*); and *C. orbiculare* f. sp. from *Xanthium* spp. (previously *C. orbiculare*).

BioMal, which originated from *M. pusilla*, was identified and registered as a form of *C. gloeosporioides* (11,14,15). However, the morphological and sequence data presented here indicate that the initial identification was incorrect and that the BioMal pathogen is similar to other pathogens isolated from *L. trimestris* and *S. spinosa*. Further support for this proposal comes from recent studies (13) on the initial infection process of BioMal on *M. pusilla*. That study revealed intracellular infection structures similar to those reported here (Fig. 2). BioMal has been extensively studied, and surveys of its pathogenicity, and that of other isolates of this species from *S. spinosa*, have failed to reveal any crops to be susceptible (14). However, since the pathogen is now shown to be a member of the *C. orbiculare* aggregate species, which contains pathogens that are important on a range of valuable crops, e.g., beans, cucurbits, and lucerne, the specificity and stability of BioMal may require further verification.

In contrast, conidia of the isolates from *Gossypium* became septate upon germination, showed no affinity for BPA or UB 20, and had rDNA sequences that were different from the other malvaceous isolates studied. Together, these data indicate that the pathogens from cotton are different from the other isolates from malvaceous plants. A comparison of their rDNA sequences shows that *C. gossypii* and *C. gossypii* var. *cephalosporioides* are almost identical. There is, thus, no justification from their sequence data to regard the cotton pathogens as species distinct from each other. When such sequences were compared with other *Colletotrichum* species, a close homology was found with several isolates of *C. gloeosporioides*. By analogy with the earlier discussion, the isolates from cotton should, therefore, be regarded as forma speciales of *C. gloeosporioides* from *Gossypium*.

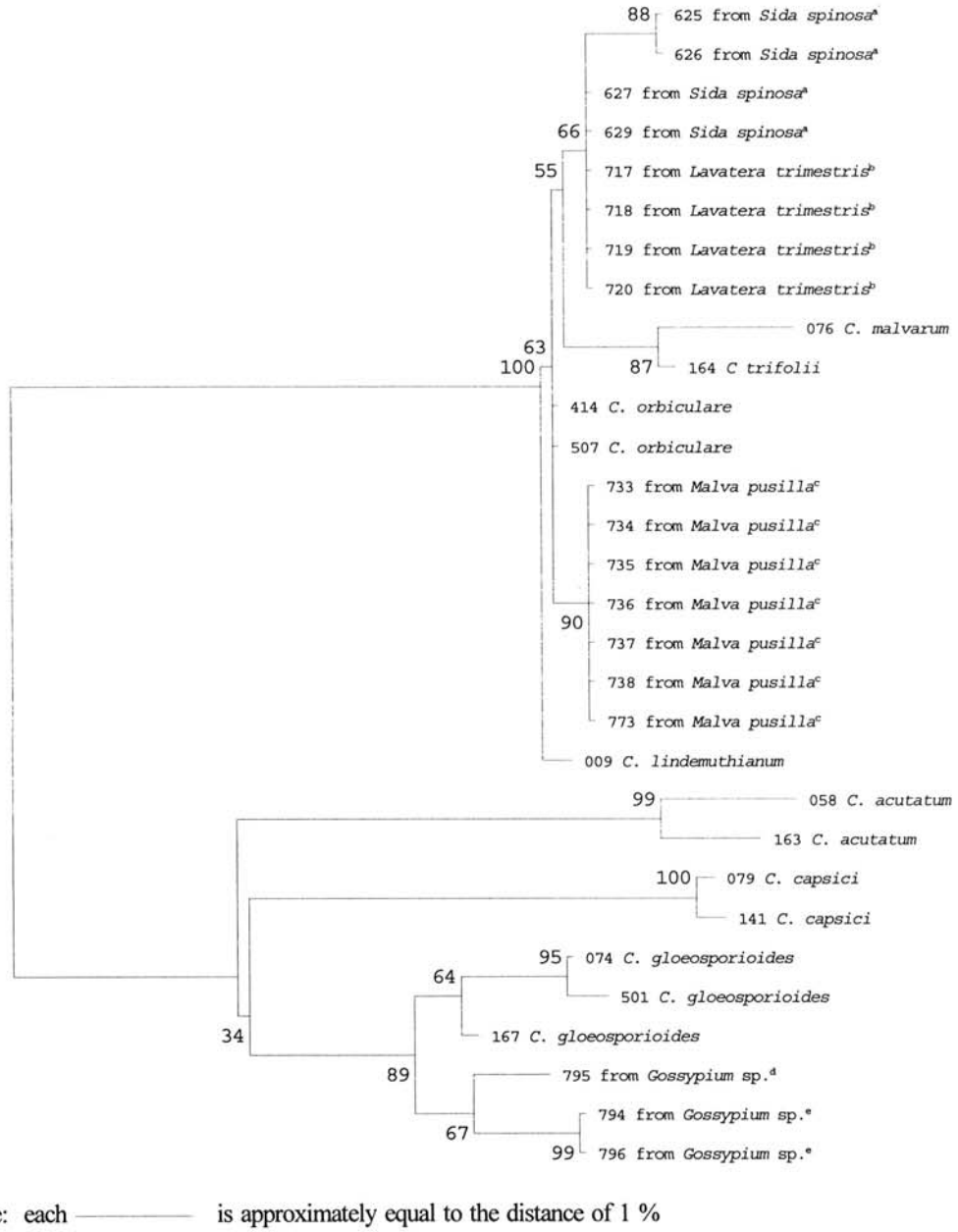

Fig. 4. Dendrogram, based on neighbor-joining analysis of ITS-2 and D2 rDNA sequences, illustrating the relationship of *Colletotrichum* isolates obtained from various malvaceous hosts with other species of *Colletotrichum*. Bootstrap confidence levels (MEGA, Version 1.01), based on 1,000 resamples, are given at the appropriate branches. [a] = supplied as *C. malvarum*. [b] = supplied as *Colletotrichum* sp. [c] = supplied as *C. gloeosporioides* f. sp. *malvae*. [d] = supplied as *C. gossypii*. [e] = supplied as *C. gossypii* var. *cephalosporioides*.

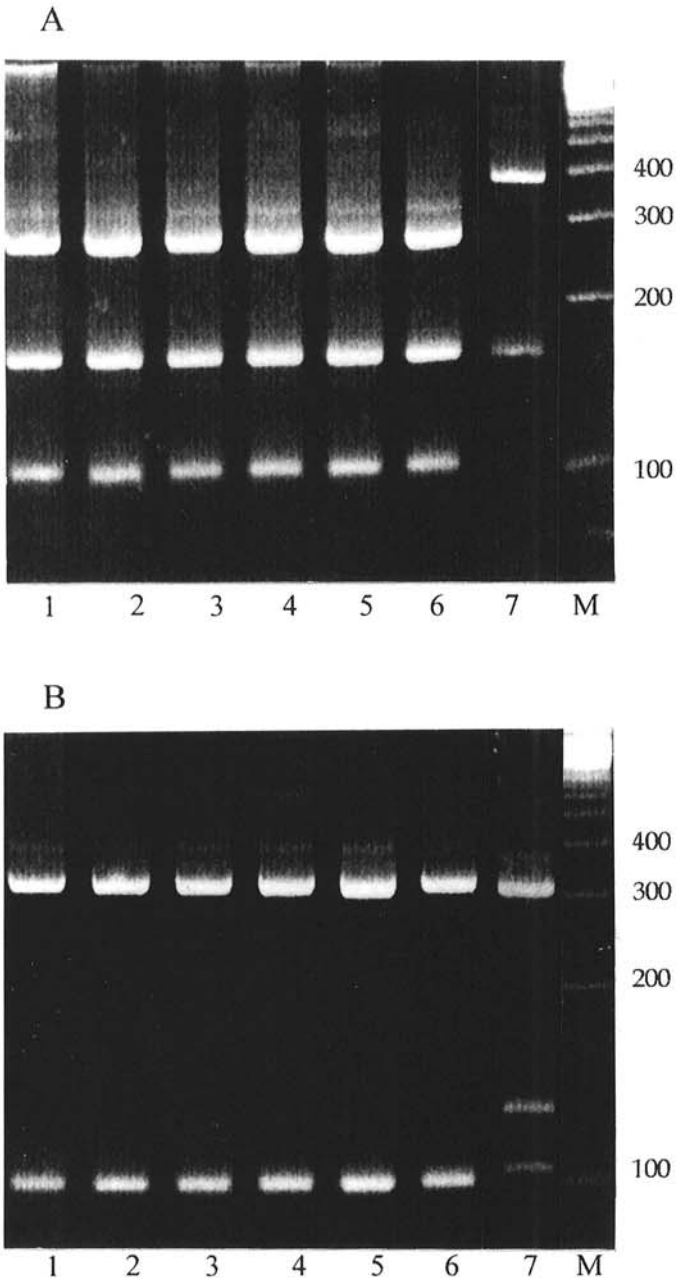

Fig. 5. Restriction analysis of polymerase chain reaction-amplified rDNA (ITS-1 and ITS-2). The restriction enzymes used were **A,** *Hae*III and **B,** *Msp*I. Lanes 1, 2, and 3: isolates 625, 720, and 773 from *Sida spinosa*, *Lavatera trimestris*, and *Malva pusilla*, respectively; lane 4: isolate 501 from *Cucumis sativus*; lane 5: isolate 164 from *Medicago sativa*; lanes 6 and 7: isolates from *Phaseolus vulgaris*, types I and II, respectively (5); and lane M: molecular weight markers (100-bp ladder, Pharmacia Biotechnology Inc., Uppsala, Sweden).

As indicated above, all forms of the *C. orbiculare* species aggregate show a high degree of host specificity. It has been suggested that this strict specificity arises because of the initial intimate biotrophic relationship that has to be established with the epidermal host cells (1,2,18). The existence of strict host specificity within a single species suggests that, as with *Magnaporthe grisea* (9), the *C. orbiculare* species aggregate provides excellent opportunities for dissecting the molecular basis of pathogen specificity.

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