Genetic Variability and Host Specialization in the Latin American Clade of *Ceratocystis fimbriata*

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ABSTRACT

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The *Ceratocystis fimbriata* complex includes many undescribed species that cause wilt and canker diseases of many economically important plants. Phylogenetic analyses of DNA sequences have delineated three geographic clades within *Ceratocystis fimbriata*. This study examined host specialization in the Latin American clade, in which a number of lineages were identified using sequences of the internal transcribed spacer (ITS) region of the rDNA. Three host-associated lineages were identified from cacao (*Theobroma cacao*), sweet potato (*Ipomoea batatas*), and sycamore (*Platanus* spp.), respectively. Isolates from these three lineages showed strong host specialization in reciprocal inoculation experiments on these three hosts. Six cacao isolates from Ecuador, Trinidad, and Columbia differed genetically from other cacao

isolates and were not pathogenic to cacao in inoculation tests. Further evidence of host specialization within the Latin American clade of *Ceratocystis fimbriata* was demonstrated in inoculation experiments in growth chambers using sweet potato, sycamore, *Colocasia esculenta*, coffee (*Coffea arabica*), and mango (*Mangifera indica*) plants; inoculation experiments in Brazil using Brazilian isolates from cacao, *Eucalyptus* spp., mango, and *Gmelina arborea*; and inoculation experiments in Costa Rica using Costa Rican isolates from cacao, coffee, and *Xanthosoma* sp. Hosts native to the Americas appeared to be colonized by only select pathogen genotypes, whereas nonnative hosts were colonized by several genotypes. We hypothesize that local populations of *Ceratocystis fimbriata* have specialized to different hosts; some of these populations are nascent species, and some host-specialized genotypes have been moved to new areas by humans.

Additional keywords: fungal phylogenetics, speciation.

Ceratocystis fimbriata Ellis & Halsted attacks an exceptionally wide range of economically important plants, causing wilt-type diseases, cankers, and rot of storage roots. The fungus usually enters woody plants through wounds. A total of 31 plant species representing 14 families have been confirmed as hosts, including trees such as sycamore (*Platanus* spp.), mango (*Mangifera indica* L.), *Gmelina arborea* Roxb., cacao (*Theobroma cacao* L.), *Citrus* spp., coffee (*Coffea arabica* L.), rubber tree (*Hevea brasiliensis* (Willd. ex A. Dr.) Juss.) Müll. Arg.), *Spathodea* sp., *Prunus* spp., fig (*Ficus carica* L.), poplar (*Populus* spp.), *Acacia* spp., and *Eucalyptus* spp. and root crops such as sweet potato (*Ipomoea batatas* (L.) Lam.) and edible aroids (*Colocasia esculenta* (L.) Schott and *Xanthosoma* spp.) (7). The fungus has a broad geographic range, and various host-associated forms appear to be native throughout North and Latin America as well as Asia (18). A close relative, *Ceratocystis albofundus* Wingfield, De Beer & Morris, is native to southern Africa (40,53).

Despite its wide host and geographic ranges, *Ceratocystis fimbriata* is generally known on each host in only a relatively restricted geographic area (7). For example, mango is reported as a host only in Brazil, even though mango trees are grown throughout northwestern South America and Central America, where the fungus is common on cacao and coffee. Several limited inoculation studies (4,9,21,23,29,30,38) have suggested the existence of host specialization within the *Ceratocystis fimbriata* complex. However, Webster and Butler (51) concluded that *Ceratocystis*

fimbriata was a single species because isolates from various hosts were sexually interfertile and morphologically indistinguishable.

Genetic analyses of rDNA sequences and partial sequences of the MAT-2 mating type gene delineate three geographic clades within *Ceratocystis fimbriata* centered in North America, Latin America, and Asia, respectively (18). Some lineages within these major clades appear to be associated with specific hosts. Barnes et al. (5) found that some of the variation in microsatellite markers among *Ceratocystis fimbriata* isolates correlated with the plant hosts from which the isolates were collected. Isolates from sycamore in Europe appeared to be distinct from sweet potato and *Populus* isolates based on random amplified polymorphic DNA markers (43). These observations suggest that genetically distinct groups within *Ceratocystis fimbriata* may have unique host ranges, but previous inoculation studies used only a few isolates on a few host plants, and no previous study has examined host specialization of *Ceratocystis fimbriata* within a phylogenetic context.

The aim of this study was to examine phylogenetic relationships and host specialization within the Latin American clade of *Ceratocystis fimbriata* (18), particularly focusing on isolates from cacao, sweet potato, and sycamore. Isolates from these and other hosts native or exotic to Latin America were used for rDNA-internal transcribed spacer (ITS) sequencing and in a series of inoculation studies.

MATERIALS AND METHODS

Fungal isolates. Isolates of *Ceratocystis fimbriata* were obtained primarily from trees showing wilt symptoms or cankers, rotted storage roots or rotted corms, or from culture collections. Representative isolates of the Latin American clade from particu-

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lar hosts were selected for genetic analysis and inoculation studies (Table 1).

ITS sequencing. Genomic DNA for use as template in polymerase chain reactions (PCR) was obtained from mycelium grown in 25 ml of broth (2% malt extract and 0.2% yeast extract) at room temperature (approximately 24°C) for 2 weeks. Extraction of DNA followed the method of DeScenzo and Harrington (10). Sequences of the ITS region and 5.8S gene of the rDNA were obtained by PCR amplification of genomic DNA using the primers ITS1F (5'-CTTGGTCATTAGAGGAAGTAA-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (16,52), following the protocol of Harrington et al. (19), with slightly different cycling conditions, which were an initial denaturation at 94°C for 95 s followed by 35 cycles of denaturation (94°C) for 35 s, annealing at 52°C for 60 s, and extension at 72°C for 60 s. Final extension was at 72°C for 15 min.

Phylogenetic analysis. Sequences were aligned manually by adding gaps and analyzed using parsimony with PAUP 4.0b10 (46). *Ceratocystis albofundus* isolate C1048 from *Acacia mearnsii* de Wild. in South Africa was used as the outgroup taxon, and the ingroup was considered monophyletic. Of 676 total characters including gaps, 89 were ambiguously aligned and excluded from the analysis, 150 remaining sites were variable, and of these, 52 were parsimony-informative. Gaps were treated as a fifth character. A maximum parsimony heuristic search was performed with all characters having equal weight. Stepwise addition was used to obtain starting trees, and tree-bisection-reconnection was used. Bootstrap analysis with 1,000 replications of heuristic searches was used to assess support for the internal branches (14).

Inoculation experiments. Because several lineages distinguished by the phylogenetic analysis correlated with host plants from which the isolates were collected, we performed a series of inoculation experiments with isolates from these lineages. Three series of inoculation experiments were performed using varying hosts and isolates in growth chambers at Iowa State University and in outdoor nurseries at Viçosa, Brazil and Turrialba, Costa Rica.

Growth chamber experiments. Three experiments were performed in growth chambers. In the three-host experiment, cacao, sweet potato, and sycamore plants were inoculated with nine isolates from these hosts. In the five-host experiment, sweet potato, sycamore, coffee, and *Colocasia* plants were inoculated with 13 isolates from various hosts and several different lineages, and mango plants were inoculated with four of those isolates. In a separate cacao experiment, cacao plants were inoculated with 14 cacao isolates of varying ITS sequences to examine variability in pathogenicity among cacao isolates (Table 1).

All plants for growth chamber experiments were grown in pasteurized potting mix containing 25% peat, 25% soil, and 50% perlite. Sycamore (*Platanus occidentalis* L.) seeds were half-sib progeny from an open-pollinated tree (Sheffield's Seed Company, Locke, NY). Cacao seeds for the three-host inoculation experiment were obtained from pods of a cacao tree grown in a greenhouse at the Iowa State University campus. The cultivar of this cacao plant was unknown, but fruits were smooth, slightly furrowed, yellow, approximately 15 to 20 cm by 10 cm, and 20 to 40 seeds per pod. Cacao seeds for the cacao inoculation experiment were obtained from pods of cv. Theobahia (Forastero type) from Bahia, Brazil. Sweet potato cuttings were propagated vegetatively from sprouts of a storage root (unknown cultivar, cream flesh and peach skin) obtained from a grocery store in Ames, Iowa. Mango plants were propagated from seeds of mango fruits (unknown cultivar), also obtained from a grocery store in Ames, Iowa. *Colocasia esculenta* plants were grown from small (5 to 8 cm long, white flesh) corms obtained from a grocery store in Des Moines, Iowa, and had green leaves and pseudopetioles. Coffee (*Coffea arabica*) plants were grown from seeds obtained from Sheffield's Seed Company. Cacao, sycamore, sweet potato, and coffee plants were

started in shallow trays in a high-humidity mist bay for approximately 4 weeks and then transplanted into 6-inch pots in a warm greenhouse. Mango seeds and *Colocasia* corms were directly planted in 6-inch pots in the same greenhouse. All plants were kept in full sun, except for cacao plants, which were grown under silver shade cloth that reduced sunlight to about 20% ambient. Plants were grown in the greenhouse with adequate water for 1 to 12 months, depending on the plant species and experiment, before being transferred to growth chambers. Plants used in the three-host experiment received weekly applications of fertilizer (25% Peter's Excel 21-5-20, 75% Peter's Cal-Mag Special 15-5-15) prior to and throughout each experiment; plants used in other experiments received weekly applications of Miracle Gro Excel 21-5-20 only prior to being moved to the growth chambers.

Cacao plants used in the three-host experiment were 6 months old; those used in the cacao inoculation were 4 to 5 months old. Sweet potato plants in the three-host experiment were 3 to 5 weeks old, and those used in the five-host experiment were 8 to 12 weeks old. Sycamore plants used in the three-host experiment were 4 months old, and those used in the five-host experiment were 6 months old. Mango plants were 12 months old, coffee plants were 7 months old, and *Colocasia* plants were 3 months old.

Seven days prior to inoculation, the potted plants were moved to a growth chamber set at 25°C with 16/8-h light/dark cycle and illumination of about 110 $\mu\text{mol cm}^{-2} \text{s}^{-1}$ of photosynthetically active radiation. High humidity was maintained with trays of water inside the chamber, except in the cacao inoculation experiment, which utilized a growth chamber with automatic humidity control (65 to 80% relative humidity). Each growth chamber experiment used a balanced, completely random design, in which individual plants were assigned to treatments and arranged in the growth chamber randomly, with five plants per treatment and five plants as control (except for one sycamore experiment, with six plants per treatment). All growth chamber experiments were repeated except for the inoculations of sweet potato, sycamore, and mango in the five-host inoculation experiment.

Inoculum was prepared from self-fertile, single-ascospore progeny of each isolate grown on malt yeast extract agar (MYEA; 2% malt extract, 2% agar, and 0.2% yeast extract) at room temperature for 7 days. Each culture was flooded with 10 ml of sterile deionized water and scraped with a sterile spatula, and the resulting spore suspension was filtered through four layers of sterile cheesecloth, which was then rinsed with an additional 5 ml of water. The concentration of the spores was estimated with a hemacytometer, diluted to 2.0×10^5 spores per ml, and loaded into sterile syringes (needle gauge 21). Spore suspensions consisted almost entirely of cylindrical endoconidia, although there were also some ascospores, aleurioconidia, and hyphal fragments. Controls were prepared by flooding and scraping a sterile MYEA plate.

Sweet potato plants were wounded by a sterile dissection needle used to punch a hole through each stem about 3 cm above the soil line. The pseudopetiole of the youngest, fully expanded leaf of each *Colocasia* plant was similarly wounded. For the woody plants, an approximately 3 mm deep, downward-slanting cut was made from the outer bark to the inner wood with a sterile scalpel, approximately 3 cm from the base of the plant. Each wound was injected with at least 0.2 ml of spore suspension with the syringe, and the inoculation site was wrapped in Parafilm. In the three-host inoculation experiment, plant height was recorded immediately before inoculation and at harvest. Plants were observed daily and harvested either when they died (no fleshy green leaf tissue present) or at 45 days (three-host experiment) or 28 days (all other experiments) after inoculation.

At harvest, each stem was sliced open vertically above and below the point of inoculation, and the length of xylem discoloration was recorded. To reisolate the fungus, discolored tissue was placed between slices of carrot and incubated under humid condi-

TABLE 1. Origin and rDNA-internal transcribed spacer accession numbers of *Ceratocystis fimbriata* isolates used in phylogenetic analysis and inoculation experiments

Isolate	Other number ^x	GenBank Accession No.	Host plant	Location collected	Inoculation experiment ^y
C940	CBS 152.62	AY157951	Cacao	Costa Rica	GC3
C1547			Cacao	Costa Rica	GC3, GCC, CRC
C1548		AY157952	Cacao	Costa Rica	GC3, GC5, GCC, CR3, CRC
C1549			Cacao	Costa Rica	CRC
C1550			Cacao	Costa Rica	CRC
C1634			Cacao	Costa Rica	CR3, CRC
C1635			Cacao	Costa Rica	CRC
C1636			Cacao	Costa Rica	CRC
C1637			Cacao	Costa Rica	CRC
C1638			Cacao	Costa Rica	CRC
C1639			Cacao	Costa Rica	CR3, CRC
C1640			Cacao	Costa Rica	CR3, CRC
C1642			<i>Herrania</i> sp.	Costa Rica	CRC
C1778			<i>Herrania</i> sp.	Costa Rica	
C1587		AY157953	Cacao	Brazil	GCC, Br
C1593			Cacao	Brazil	GCC, Br
C1596			Cacao	Brazil	
C1597			Cacao	Brazil	Br
C1600			Cacao	Brazil	
C1004	CBS 153.62	AY157950	Cacao	Ecuador	GCC
C1690			Cacao	Ecuador	GCC
C1691			Cacao	Ecuador	
C1695			Cacao	Ecuador	
C1696			Cacao	Ecuador	GCC
C1751			Cacao	Ecuador	GCC
C1756			Cacao	Ecuador	
C1833			Cacao	Ecuador	
C1834			Cacao	Ecuador	GCC
C1831			Cacao	Ecuador	GCC
C1832			Cacao	Ecuador	GCC
C1835			Cacao	Ecuador	
C1836			Cacao	Ecuador	GCC
C1584		AY157954	Cacao	Trinidad	GCC
C1750		AY157955	Cacao	Columbia	GCC
C1354			Sweet potato	Japan	GC3
C1390	IFO 30501		Sweet potato	Japan	
C1869			Sweet potato	Japan	
C854	CBS 141.37		Sweet potato	Louisiana, USA	
C926			Sweet potato	New Jersey, USA	
C1418	CBS 141.37	AY157956	Sweet potato	North Carolina, USA	GC3, GC5
C1484			Sweet potato	Maryland, USA	
C1475	ATCC 13323		Sweet potato	New Zealand	
C1476	ICMP 8579	AY157957	Sweet potato	Papua New Guinea	GC3
C1932	DAR 58857		Sweet potato	Papua New Guinea	
C858			Sycamore	California, USA	
C859			Sycamore	California, USA	
C1818			Sycamore	California, USA	
C1819			Sycamore	California, USA	
C1820			Sycamore	California, USA	
C1830			Sycamore	California, USA	
C809			Sycamore	Italy	
C812			Sycamore	Italy	
C1351			Sycamore	Kentucky, USA	GC3
C1317		AY157958	Sycamore	North Carolina, USA	GC3, GC5
C1339			Sycamore	Virginia, USA	GC3
C1543		AY157961	Coffee	Columbia	GC5
C1490			Coffee	Costa Rica	CR3
C1491			Coffee	Costa Rica	CR3
C1493			Coffee	Costa Rica	CR3
C1551		AY157962	Coffee	Costa Rica	GC5, CR3
C1005	CBS 103.40	AY157960	Coffee	Guatemala	GC5
C996	CBS 146.53	AY157959	Coffee	Suriname	GC5
C1641		AY157963	<i>Xanthosoma</i> sp.	Costa Rica	GC5, CR3
C994	CBS 600.70	AY157964	Mango	São Paulo state, Brazil	GC5, Br
C1558		AY157965	Mango	Rio de Janeiro state, Brazil	GC5, Br
C1591			Mango	Rio de Janeiro state, Brazil	Br
C1592			<i>Annona</i> sp.	Rio de Janeiro state, Brazil	Br
C1603			Cassava ^z	Rio de Janeiro state, Brazil	Br
C1345		AY157966	Eucalyptus	Bahia state, Brazil	GC5, Br
C1442			Eucalyptus	Bahia state, Brazil	GC5, Br
C1451			Eucalyptus	Bahia state, Brazil	Br
C925		AY157967	<i>Gmelina</i> sp.	Pará, Brazil	GC5, Br

^x ATCC = American Type Culture Collection; CBS = Centraalbureau voor Schimmelcultures; DAR = Orange Agricultural Institute, New South Wales, Australia; ICMP = Landcare Research New Zealand; and IFO = Institute for Fermentation, Osaka, Japan.

^y GC3 = three-host growth chamber experiment; GC5 = five-host growth chamber experiment; GCC = cacao growth chamber experiment; Br = Brazilian experiment; CR3 = three-host Costa Rican experiment; and CRC = Costa Rican cacao experiment.

^z Isolate C1603 was obtained from a dead cassava stem but was not pathogenic to cassava in inoculation experiments.

tions at room temperature (31). After 7 days, the carrot slices were observed for growth of *Ceratocystis fimbriata*. Representative isolates (at least two per host plant per source of inoculum) were subcultured from these samples and fingerprinted (using *Pst*I restriction enzyme and (CAT)₅ as a probe [10]), and the banding patterns were compared with those of the original strains used in the inoculations.

Brazilian experiments. Two separate inoculation experiments were conducted using plants grown in plastic bags or pots filled with soil in an outdoor nursery on the campus of the Universidade Federal de Viçosa during June to July 2000 and March to April 2001. The first experiment included cacao seedlings (cv. Theobahia, 4-month-old), mango seedlings (cv. Espada, 5-month-old), eucalyptus cuttings (*E. urophylla* × *E. grandis* hybrid, 3-month-old), *Crotalaria juncea* L. seedlings (8-week-old), rubber seedlings (5-month-old), cassava cuttings (*Manihot esculenta* Crantz., 3-month-old), and coffee seedlings (*Coffea arabica*, 4-month-old). Mango, coffee, rubber, and cacao were grown under shade, cassava and *Eucalyptus* plants were grown in full sun, and *Crotalaria* plants were grown in partial shade. There were one to three *Crotalaria* plants in each pot, but each plant was inoculated separately (with the same isolate) and treated as a replicate. This experiment used an unbalanced, completely random design with approximately equal numbers of plants inoculated by each isolate. Due to the different numbers of plants available, number of replicates per treatment per plant species varied from 2 or 3 (mango) to 27 (*Crotalaria* plants). Plants were inoculated with 12 isolates collected in Brazil from various hosts (Table 1) by the same method as the growth chamber inoculations. Length of xylem discoloration was recorded after 22 to 23 days. We did not attempt to reisolate the fungus from inoculated plants.

The second Brazilian experiment included cacao seedlings (cv. Theobahia, 6- to 8-week-old), eucalyptus cuttings (same clone, 5-month-old), mango seedlings (cv. Espada, 1-year-old), *Crotalaria juncea* seedlings (5-week-old), and *G. arborea* seedlings (3-month-old). Cacao plants were grown under shade before and during the experiment, whereas all other plants were grown in full sun. As in the first Brazilian experiment, there were one to three *Crotalaria* plants per pot. Each plant species was treated as a block, and plants were inoculated by the isolates in a randomly selected order. Plants were inoculated with 11 of the same isolates as the first Brazilian experiment (Table 1), using the same method as the growth chamber inoculations, except the spore concentration was 1.0×10^6 spores per ml. Length of xylem discoloration was recorded after 32 to 34 days. We did not attempt to isolate the fungus from the inoculated plants.

Costa Rican experiments. Cacao seedlings (Forastero type), coffee seedlings (*Coffea arabica* cv. Caturra), and *Xanthosoma sagittifolium* (L.) Schott. plants were grown in plastic bags of soil in an outdoor nursery at the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE) in Turrialba, Costa Rica. Cacao and *Xanthosoma* plants were 2 to 3 months old and coffee plants were 4 to 5 months old when inoculated in March 2001. Four isolates from each of cacao and coffee, and one isolate from *Xanthosoma* sp., all collected in Costa Rica, were inoculated into these three hosts by methods identical to those used in the growth chamber inoculations.

In a second experiment, 12 Costa Rican isolates from cacao were tested on 2- to 3-month-old cacao plants by methods identical to the growth chamber experiments. In both Costa Rican experiments, the plants were randomly assigned to treatments, 9 or 10 plants per isolate, in a completely random design. Xylem discoloration was recorded at 18 days (cacao plants) or 19 days (coffee and *Xanthosoma* plants) after inoculation.

Statistical analysis. For the three-host inoculation experiment, length of xylem discoloration was analyzed by inoculated host plant, source (host) of inoculum, experiment, and the host-source of inoculum interaction using a multifactorial analysis of variance

(ANOVA). Height growth of plants that survived to the end of the experiment was also analyzed by source of inoculum using an ANOVA, with each host species analyzed separately because of widely varying height growth among species. When ANOVA indicated significant variation among sources of inoculum ($P \leq 0.05$), Duncan's multiple range test was used to separate means. The proportions of plants killed and reisolation success for each source of inoculum were compared within each host species using chi-square tests adjusted for multiple comparisons by the Bonferroni method (39).

For all other experiments, length of xylem discoloration was analyzed for each inoculated host species separately. When ANOVA indicated that the variation among isolates was significant ($P \leq 0.05$), Duncan's multiple range test was used to separate means. For the Brazilian experiment, a multifactorial ANOVA also was used to analyze length of xylem discoloration by host species, isolate, experiment, and host-isolate and host-isolate-experiment interactions, using only data from host species that were inoculated in both experiments. All statistics were performed using SAS version 8.2 statistical software (SAS Institute, Cary, NC).

RESULTS

Phylogenetic analysis. The ITS sequences of the *Ceratocystis fimbriata* isolates were extremely variable, primarily due to numerous insertions and deletions (indels) in ITS1 and ITS2. There were 923 most parsimonious trees of 219 steps derived from 52 parsimony-informative positions and 98 variable but not parsimony-informative positions. Most of the variable sites that were not parsimony-informative were between the outgroup taxon (*Ceratocystis albofundus*) and the ingroup (Latin American clade of *Ceratocystis fimbriata*). The consistency index (CI), retention index (RI), and rescaled consistency (RC) index were 0.772, 0.919, and 0.709, respectively. Most isolates from cacao or the closely related *Herrania* spp. fell into a strongly supported lineage with two sublineages; one sublineage contained isolates from Ecuador and one isolate from Costa Rica, and the other sublineage contained Costa Rican and Brazilian isolates (Fig. 1). Six cacao isolates from Ecuador, Columbia, and Trinidad did not group into either of these cacao sublineages, nor did they group with each other. All sycamore isolates formed a strongly supported lineage of similar ITS sequences. Isolates from sweet potato all shared a unique ITS sequence, and the ITS sequence of isolate C1005 from a coffee plant in Guatemala was identical to that of the sweet potato strains, except for two one-base insertions. There was no bootstrap support for the clade containing isolate C1005 and the sweet potato isolates.

Because the ITS sequence of coffee isolate C1005 was very similar to that of the sweet potato isolates, we performed a separate analysis using sequences of all isolates except C1005. In this analysis, 26,623 most parsimonious trees with lengths of 217 were found. The CI, RI, and RC were 0.779, 0.921, and 0.717, respectively. Bootstrap analysis was restricted to a maximum of 100 trees for 1,000 replications due to the large number of most parsimonious trees. Bootstrap values for branches were nearly identical (no more than four percent points different) to those shown in Figure 1 (when C1005 was included), except that a branch containing all the sweet potato isolates had 79% bootstrap support, and a branch containing isolates C1442 and C1451 from Brazilian *Eucalyptus* spp. had 53% support.

We performed a third analysis in which indels were coded as present or absent and gaps were treated as missing data. This analysis yielded more than 433,000 most parsimonious trees with lengths of 171. The CI, RI, and RC were 0.6842, 0.8854, and 0.6058, respectively. Bootstrap analysis was again restricted to a maximum of 100 trees for 1,000 replications. Some of the branches shown in Figure 1 were not supported in this analysis, but the cacao lineage still had 75% bootstrap support, the

Ecuadorian and Costa Rican cacao sublineages had support of 100 and 93%, respectively, and the sycamore lineage had support of 94%. Although the sweet potato isolates all had the same unique ITS sequence, there was no bootstrap support for this branch.

Growth chamber experiments. Because the ANOVA indicated no significant difference between the two three-host growth chamber experiments (Table 2), these were combined for analysis. *Ceratocystis fimbriata* isolates from the cacao, sweet potato, and sycamore lineages caused dramatically more xylem discoloration on the hosts from which they were originally isolated than on the other two plant species (Fig. 2). For each of the three hosts, inoculation by isolates from the other two hosts did not result in xylem

discoloration significantly different from the control inoculations, which had a very small amount of discoloration, a host response to the wounding. Discoloration was not qualitatively different among the inoculated and control plants. An ANOVA of these discoloration data showed that the largest source of variation was the host-source of inoculum interaction (Table 2). With the exception of one sycamore plant inoculated with a cacao isolate, only cacao isolates killed cacao plants, only sweet potato isolates killed sweet potato plants, and only sycamore isolates killed sycamore plants (Table 3).

Height differences among surviving inoculated and control cacao and sycamore plants were not significant based on ANOVA,

Fig. 1. Strict consensus of the 923 most parsimonious trees from a heuristic search using rDNA-internal transcribed spacer sequences from *Ceratocystis fimbriata* isolates of the Latin American clade. The trees are based on 586 characters, including gaps, 52 of which were parsimony-informative. *Ceratocystis albofundus* was used to root the tree. Bootstrap values greater than 50 are shown above the branches.

probably because only a few plants inoculated with compatible isolates survived to the end of the experiment. However, the ANOVA of height growth of surviving sweet potato plants showed significant variation ($F = 4.39$; $P = 0.0062$). The 25 surviving sweet potato plants inoculated with sweet potato isolates had reduced growth compared with sweet potato plants inoculated with cacao isolates, sycamore isolates, or the control plants (Table 3).

Reisolation of *Ceratocystis fimbriata* was attempted from all inoculated and control plants, but the fungus was not recovered from control plants. Reisolation success varied significantly with source of inoculum from inoculated cacao plants ($F = 14.98$; $P =$

0.0003) and from inoculated sycamore plants ($F = 23.09$; $P < 0.0001$), but not from inoculated sweet potato plants ($F = 1.37$; $P = 0.2852$). Cacao isolates inoculated into cacao plants were reisolated from 73% of the seedlings, and sycamore isolates inoculated into sycamore plants were reisolated from 97% of the seedlings (Table 3). Cacao isolates were reisolated from 67% of the inoculated sycamore plants, even though these sycamore plants did not differ significantly from control plants in percent mortality, length of xylem discoloration, or height growth. The (CAT)₅ DNA fingerprints of cultures reisolated from the plants matched those of the original isolates (data not shown).

To see whether a wider range of *Ceratocystis fimbriata* isolates and host plants would show similar patterns of strong host specificity, we inoculated 13 isolates (Table 1) of diverse ITS sequences into coffee, *Colocasia*, sweet potato, and sycamore in a five-host growth chamber experiment. Four isolates were also inoculated into mango. Separate ANOVAs of the extent of xylem discoloration showed significant variation ($P \leq 0.05$) among isolates on each host, but no difference between the two experiments ($P = 0.6019$). Only the sweet potato isolate caused significant discoloration on sweet potato (Table 4). Isolates from cacao, coffee,

TABLE 2. Analysis of variance of the linear extent in xylem discoloration in cacao, sweet potato, and sycamore plants inoculated with *Ceratocystis fimbriata* isolates from these hosts

Source	df	Mean square	F value	P
Host	2	33.6532	13.55	<0.0001
Source of inoculum	2	17.6247	7.10	0.0010
Host × source of inoculum	4	775.7112	156.19	<0.0001
Experiment	1	0.8588	0.69	0.4063

Fig. 2. Average length (centimeters) of xylem discoloration caused by nine *Ceratocystis fimbriata* isolates 45 days after inoculation into cacao, sweet potato, and sycamore plants. Data are means of all replicates (10 for sweet potato and cacao plants and 11 for sycamore plants) from two experiments; error bars represent standard deviation.

TABLE 3. Percent mortality, average growth in height of surviving plants 45 days after inoculation, and percent successful reisolation of isolates from the three hosts

Inoculated host	Source of inoculum	No. of inoculated plants	Percent mortality ^z	Average height growth of survivors (cm) ^z	Percent reisolated ^z
Cacao	Cacao	30	93.3 a	1.0	73.3 a
	Sweet potato	30	0 b	1.3	0 b
	Sycamore	30	0 b	1.5	3.3 b
	Control	10	0 b	1.4	0 b
Sweet potato	Cacao	30	0 a	80.0 a	13.3 a
	Sweet potato	30	16.7 a	44.8 b	6.7 a
	Sycamore	30	0 a	89.4 a	0 a
	Control	10	0 a	83.0 a	0 a
Sycamore	Cacao	33	3.0 b	23.8	66.7 b
	Sweet potato	33	0 b	23.9	10.0 c
	Sycamore	33	69.7 a	21.0	97.0 a
	Control	11	0 b	25.0	0 c

^z Numbers within a column followed by the same letter within a host plant group are not significantly different according to Duncan's multiple range test (average growth; mean square error = 2,298; $P \leq 0.05$) or chi-square tests (percent mortality and percent reisolated, Bonferroni adjusted $\alpha = 0.0083$). All statistical tests were performed separately for each host plant, with three isolates for each source of inoculum and 10 or 11 replicates per isolate. An initial analysis of variance did not indicate significant differences in height growth among cacao or sycamore plants.

and mango caused more discoloration on sycamore plants than did the control, but the sycamore isolate caused a significantly greater amount of discoloration than did the other isolates. All isolates caused more discoloration on coffee than did the control, but a coffee isolate from Costa Rica caused the greatest amount of discoloration. Only an *Xanthosoma* isolate caused significant discoloration on *Colocasia* plants (both are members of the family Araceae), and only a mango isolate did so on mango plants.

To see if all isolates from cacao were pathogenic to cacao plants, a third growth chamber experiment utilized representative isolates from the two cacao sublineages and all other cacao isolates (Fig. 1; Table 1). Amount of xylem discoloration on inoculated cacao plants varied significantly by isolate ($F = 44.49$; $P < 0.0001$), experiment ($F = 39.86$; $P < 0.0001$), and isolate-experiment interaction ($F = 4.16$; $P < 0.0001$), so experiments were analyzed separately. In both experiments, all isolates of the two cacao sublineages, except C1004, caused significantly more discoloration than the other cacao isolates, which did not differ from the controls (Table 5). Isolate C1004 appeared to be debilitated because it produced an abnormal white, fluffy mycelium in culture and did not produce perithecia. When only isolates of the cacao sublineages were analyzed (but without the nonpathogenic C1004), the ANOVA indicated no significant variation in lesion size among isolates when experiments were combined ($F = 1.82$;

$P = 0.1113$) and no isolate-experiment interaction ($F = 0.57$; $P = 0.7546$).

Brazilian inoculations. Considering only plant species that were inoculated in both experiments, the amount of discoloration differed significantly between experiments according to ANOVA ($P < 0.0001$). There was also a significant host-isolate-experiment interaction ($P = 0.0001$). Thus, each experiment and host species was analyzed separately (Table 6). In the first experiment, there was no xylem discoloration in any of the inoculated or control cassava plants. Lesion length in coffee and rubber was small (0 to 1.4 cm and 0 to 1.1 cm, respectively) and did not vary among isolates and controls in the ANOVA ($P = 0.1636$ and $P = 0.4455$, respectively). Because of this, cassava, coffee, and rubber were not included in the second experiment.

In the first experiment, only the three cacao isolates caused more discoloration in cacao plants than the controls (Table 6). Two isolates from mango, two genetically similar isolates from cassava and *Annona* spp. (Fig. 1), and the *Gmelina* isolate all caused significant discoloration in inoculated mangos. Only isolates C1345 and C1442, both from *Eucalyptus* spp., caused significant discoloration on inoculated *Eucalyptus* plants. *Crotalaria* plants had the most discoloration of any of the inoculated plants and were significantly discolored by all isolates, except C1451 from *Eucalyptus* sp., although cacao isolates caused significantly less discoloration on inoculated *Crotalaria* plants than did the

TABLE 4. Mean xylem discoloration (centimeters) caused by *Ceratocystis fimbriata* isolates inoculated into five host plants in a growth chamber^z

Isolate	Source of isolate	Sweet potato	Sycamore	Coffee	<i>Colocasia</i>	Mango
C1548	Cacao, Costa Rica	0.4 b	1.4 bc	1.0 d-f	0.6 b	NT
C1418	Sweet potato, North Carolina	4.3 a	0.7 cd	1.2 c-f	0.4 b	1.0 b
C1317	Sycamore, North Carolina	0.4 b	5.5 a	1.1 d-f	0.6 b	NT
C1641	<i>Xanthosoma</i> sp., Costa Rica	0.4 b	0.7 cd	0.9 f	3.5 a	NT
C1543	Coffee, Columbia	0.9 b	0.8 cd	1.8 b	0.4 b	NT
C1551	Coffee, Costa Rica	0.5 b	1.9 b	3.8 a	0.4 b	0.7 b
C1005	Coffee, Guatemala	0.4 b	0.7 cd	0.9 ef	0.3 b	NT
C996	Coffee, Surinam	0.5 b	1.0 cd	1.3 c-f	0.5 b	NT
C994	Mango, Brazil	0.4 b	2.0 b	1.5 bc	0.3 b	NT
C1558	Mango, Brazil	0.4 b	0.9 cd	1.3 c-f	1.0 b	11.6 a
C1345	<i>Eucalyptus</i> , Brazil	0.3 b	1.0 cd	1.4 b-d	0.8 b	NT
C1442	<i>Eucalyptus</i> , Brazil	0.3 b	1.0 cd	1.4 b-d	0.6 b	NT
C925	<i>Gmelina arborea</i> , Brazil	0.6 b	1.0 cd	1.3 c-e	0.4 b	NT
Control		0.5 b	0.5 d	0.3 g	0.3 b	0.4 b

^z Means are of five plants per isolate for sweet potato, sycamore, and mango and 10 plants (two experiments of five replicates each) for coffee and *Colocasia esculenta*. Means within a column followed by the same letters are not significantly different according to Duncan's multiple range test (mean square error = 0.25 for sweet potato, 0.34 for sycamore, 0.19 for coffee, 1.11 for *Colocasia esculenta*, and 7.37 for mango; $P \leq 0.05$). Each plant host was analyzed separately. NT = not tested.

TABLE 5. Percent mortality and xylem discoloration (centimeters) in cacao plants inoculated with *Ceratocystis fimbriata* cacao isolates in two growth chamber experiments^z

Isolate	Country of origin	Internal transcribed spacer lineage	Experiment 1		Experiment 2	
			Percent mortality	Xylem discoloration	Percent mortality	Xylem discoloration
C1690	Ecuador	Cacao—Ecuador	100	6.4 a	100	9.9 ab
C1547	Costa Rica	Cacao—Costa Rica	80	6.3 a	60	8.6 a-c
C1587	Brazil	Cacao—Costa Rica	100	6.3 a	100	10.9 a
C1593	Brazil	Cacao—Costa Rica	100	5.6 a	100	10.7 a
C1548	Costa Rica	Cacao—Costa Rica	100	5.4 a	80	9.4 a-c
C1751	Ecuador	Cacao—Ecuador	100	5.1 a	100	7.4 c
C1834	Ecuador	Cacao—Ecuador	80	4.6 a	100	7.6 bc
C1004	Ecuador	Cacao—Ecuador	0	1.6 b	0	1.0 d
C1750	Columbia	Other	0	1.4 b	0	1.1 d
C1584	Trinidad	Other	0	1.2 b	0	1.3 d
C1832	Ecuador	Other	0	1.1 b	0	1.6 d
C1831	Ecuador	Other	0	1.1 b	0	1.0 d
C1696	Ecuador	Other	0	1.1 b	0	1.1 d
C1836	Ecuador	Other	0	1.0 b	0	1.1 d
Control			0	1.1 b	0	0.7 d

^z Means are of five plants for each isolate in each experiment, except for C1750 and C1696, which are of four plants each in experiment 1 and six plants each in experiment 2. Means followed by the same letter are not significantly different according to Duncan's multiple range test (experiment 1, mean square error = 2.03; $P \leq 0.05$; and experiment 2, mean square error = 2.88; $P \leq 0.05$).

other isolates. Isolate C1451 appeared to be debilitated in the first experiment and was not used in the second experiment.

In the second Brazilian experiment, the cacao plants were relatively young, and all cacao plants inoculated with cacao isolates died (Table 6). One cacao seedling inoculated with the *Gmelina* isolate also died, and among surviving cacao plants, only the *Gmelina* isolate caused more discoloration than did the controls. The isolates from cacao and *Gmelina arborea* did not cause significant discoloration on inoculated mango plants; but all three mango isolates and the related cassava and *Annona* isolates, and one *Eucalyptus* isolate caused more discoloration than the controls. As in the first Brazilian experiment, isolates C1345 and C1442 caused the most discoloration on *Eucalyptus* plants, although isolates from cassava, mango, and *Gmelina arborea* also caused more discoloration than did the controls. Substantial discoloration was found in *Crotalaria* plants inoculated by all isolates, and several *Crotalaria* plants inoculated with each isolate died. Only the *Gmelina* isolate caused significant xylem discoloration in *Gmelina* plants.

Costa Rican inoculations. The ANOVA of the extent of xylem discoloration in each of the three hosts indicated significant ($P < 0.0001$ in each case) variation among isolates. Cacao isolates caused extensive discoloration and death in inoculated cacao plants, whereas cacao plants inoculated with coffee or *Xantho-*

soma isolates did not differ from controls (Table 7). Only coffee plants inoculated with coffee isolates had xylem discoloration significantly greater than that of control plants, and only the *Xanthosoma* isolate caused significant xylem discoloration in *Xanthosoma* plants.

ANOVA indicated significant variation in the extent of xylem discoloration among the 12 Costa Rican cacao isolates inoculated into cacao plants and the control treatment ($F = 4.49$; $P < 0.0001$). All isolates caused more xylem discoloration than did the control inoculations (Table 8). Sixty-five percent of inoculated seedlings died, and there was an average of 4.3 cm of discoloration in the inoculated seedlings compared with no mortality and 0.7 cm in control plants. However, when control inoculations were removed, there was no variation among the 12 isolates in the amount of xylem discoloration produced ($F = 1.53$; $P = 0.1303$).

DISCUSSION

Analysis of rDNA-ITS sequences demonstrated substantial genetic variation and resolved some lineages within the Latin American clade of *Ceratocystis fimbriata*. Isolates from some of the lineages showed host specialization in a series of inoculation experiments, consistent with several previous, smaller inoculation studies (4,9,23,38). The association of specific ITS sequences

TABLE 6. Mean xylem discoloration (centimeters) caused by Brazilian isolates of *Ceratocystis fimbriata* in inoculated cacao, mango, *Eucalyptus*, *Crotalaria*, and *Gmelina* seedlings in Brazil^y

Isolate	Source of isolate	Cacao				Mango				<i>Eucalyptus</i>				<i>Crotalaria</i>				<i>Gmelina</i>	
		Exp. 1		Exp. 2		Exp. 1		Exp. 2		Exp. 1		Exp. 2		Exp. 1		Exp. 2		Exp. 2	
		N	XD	N	XD	N	XD	N	XD	N	XD	N	XD	N	XD	N	XD	N	XD
C1587	Cacao	8	11.0 a	0 (10)	—	3	0.9 c	10	2.0 cd	8	0.8 c	10	2.1 b-e	26	9.8 de	13 (4)	29.9 d-f	5	0.9 b
C1593	Cacao	8	10.4 a	0 (10)	—	3	0.7 c	10	2.8 cd	7	0.9 c	10	1.1 c-e	26	11.5 de	18 (1)	17.1 fg	5	0.4 b
C1597	Cacao	8	8.0 b	0 (10)	—	3	3.2 bc	10	2.0 cd	8	0.5 c	10	1.4 b-e	26	13.5 d	18 (1)	24.4 ef	5	0.4 b
C1345	<i>Eucalyptus</i>	8	0.4 c	10	0.5 bc	3	5.1 bc	10	5.4 bc	7	6.4 b	10	4.0 a	27	22.2 c	14 (3)	37.2 c-e	5	0.5 b
C1442	<i>Eucalyptus</i>	7	0.6 c	9	0.7 b	3	2.1 c	10	4.2 cd	8	9.1 a	10	4.3 a	27	20.8 c	12 (5)	56.6 ab	5	0.9 b
C1451 ^z	<i>Eucalyptus</i>	8	0.5 c	—	—	3	3.1 bc	—	—	7	0.9 c	—	—	25	5.3 ef	—	—	—	—
C1592	<i>Annona</i> sp.	6	0.5 c	10	0.8 b	3	8.7 ab	10	15.6 a	5	1.2 c	10	0.9 de	25	30.4 ab	6 (13)	58.1 ab	5	0.5 b
C994	Mango	9	0.5 c	10	0.6 bc	3	4.6 bc	10	8.8 b	8	0.6 c	10	1.3 b-e	24	23.9 bc	10 (5)	42.0 b-e	5	0.8 b
C1558	Mango	8	0.6 c	10	0.4 c	3	8.5 ab	10	16.3 a	7	0.8 c	10	1.0 de	25	22.7 c	9 (8)	50.6 bc	5	0.7 b
C1591	Mango	8	0.6 c	10	0.6 bc	2	13.6 a	10	13.4 a	9	1.3 c	10	2.8 a-c	26	30.3 ab	16 (8)	29.3 d-f	5	0.8 b
C1603	Cassava	7	0.4 c	10	0.5 bc	3	9.2 ab	10	15.2 a	8	0.8 c	10	2.6 a-d	23	35.9 a	7 (11)	44.9 b-d	5	0.6 b
C925	<i>Gmelina</i> sp.	8	1.3 c	9 (1)	1.4 a	3	8.3 ab	10	2.5 cd	8	0.7 c	10	2.9 ab	26	37.2 a	6 (12)	71.8 a	5	8.1 a
Control		7	0.6 c	10	0.6 bc	2	0.6 c	10	0.3 d	8	0.4 c	10	0.5 e	27	2.6 f	15	6.8 g	4	0.6 b
Mean square error			3.07		0.056		9.81		22.66		4.82		2.90		145.92		365.23		4.03

^y Experiment 1 lasted 22 to 23 days and experiment 2 lasted 32 to 34 days. N = number of surviving plants used in the analysis; numbers of plants that died during experiment 2 and from which discoloration data could not be obtained are given in parentheses. XD = xylem discoloration, in centimeters; means are from all surviving plants. Means followed by the same letters within a column are not significantly different according to Duncan's multiple range test ($P \leq 0.05$); each plant host in each experiment was analyzed separately.

^z Isolate C1451 was not used in experiment 2 because it appeared debilitated in experiment 1.

TABLE 7. Percent mortality and mean xylem discoloration (centimeters) caused by Costa Rican isolates of *Ceratocystis fimbriata* in inoculated cacao, coffee, and *Xanthosoma* plants in Costa Rica^z

Isolate	Source of isolate	Cacao		Coffee		<i>Xanthosoma sagittifolium</i>	
		Percent mortality	Xylem discoloration	Percent mortality	Xylem discoloration	Percent mortality	Xylem discoloration
C1640	Cacao	60	6.0 a	0	0.0 d	0	0.7 b
C1639	Cacao	80	4.7 b	0	0.0 d	11	0.7 b
C1548	Cacao	70	4.3 bc	0	0.0 d	0	0.7 b
C1634	Cacao	60	3.4 c	0	0.0 d	0	0.7 b
C1490	Coffee	0	0.8 d	0	1.4 a	0	0.7 b
C1491	Coffee	0	0.8 d	0	1.0 bc	0	0.6 b
C1493	Coffee	0	0.8 d	0	0.7 c	0	0.6 b
C1551	Coffee	0	0.7 d	0	1.3 ab	0	0.6 b
C1641	<i>Xanthosoma</i> sp.	0	0.8 d	0	0.0 d	100	10.8 a
Control		0	0.6 d	0	0.0 d	0	0.5 b

^z Means are of 10 plants per isolate for most isolates (nine per isolate for isolates C1639 and C1640 in both coffee and *Xanthosoma sagittifolium*). Means followed by the same letters within a column are not significantly different according to Duncan's multiple range test (mean square error = 1.47 for cacao, 0.12 for coffee, and 4.79 for *Xanthosoma sagittifolium*; $P \leq 0.05$); each plant host was analyzed separately. Percent mortality of cacao or coffee plants or inoculated pseudopetiole/leaf of *Xanthosoma sagittifolium*.

with host specialization suggests that some genotypes of *Ceratocystis fimbriata* have adapted to specific hosts.

Host susceptibility. The inoculation experiments provided generally consistent results despite differences in plant age, cultivars, and growth chamber and nursery conditions. Wounds on the trunk or branches are the most common infection court for natural infections of woody hosts, so the inoculation method used closely approximated natural conditions. Differences between the results of the two Brazilian experiments may be partly due to different ages of plants and experiments of different duration. Generally, there was more discoloration and mortality in the second experiment, which was harvested after 32 to 34 days rather than 22 to 23 days. However, the trends in host susceptibility were consistent for hosts such as cacao that were used in both growth chamber and nursery inoculations.

Many of the hosts not native to the Americas were susceptible to isolates from many hosts. For example, in the Brazilian inoculation study, all isolates tested caused more discoloration in *Crotalaria* plants than did the control inoculations, and all but the cacao isolates and one *Eucalyptus* isolate caused more discoloration in mango than did the controls. In growth chamber experiments, all isolates caused more discoloration in coffee than did the controls. In contrast, the American hosts, cacao (54), sweet potato (3,25), sycamore (42), and *Xanthosoma sagittifolium* (37), were highly resistant to all isolates except those from their respective hosts.

Several host resistance mechanisms may contribute to host specialization. A variety of phytoalexins are elicited in sycamore (2,8,11–13) and sweet potato plants (22,33,34,44,45,47–50) when challenged with *Ceratocystis fimbriata*. Several of these phytoalexins, especially spore agglutinating factors (26–28) and furanoterpenoid phytoalexins (29,55), have been suggested to play key roles in determining host specialization of sweet potato isolates and Asian isolates from *Colocasia esculenta*. It is possible that phytoalexins in the discolored tissue of inoculated sweet potato plants inhibited the fungus and reduced our recovery of *Ceratocystis fimbriata* from those plants. Sycamore isolates of *Ceratocystis fimbriata* produce phytotoxins (1,36), and these may also play a role in determining host specificity of the fungus. Mechanisms of resistance have not been well studied in cacao, but only cacao isolates could infect and survive in cacao seedlings, suggesting that the cacao sublineages of *Ceratocystis fimbriata* are uniquely adapted to cacao.

Host-specialized lineages. Two strongly supported lineages delineated by the phylogenetic analysis contained only isolates from cacao and sycamore. Sweet potato isolates all shared a unique, identical ITS sequence and also formed a moderately supported lineage when a related isolate from coffee was removed from the

analysis. The isolate from *Xanthosoma* sp. also had a unique ITS sequence. Isolates from these four host-associated lineages were uniquely pathogenic to their respective American hosts. The cacao lineage contained two geographic sublineages, one from Ecuador and the other from Costa Rica and Brazil, but isolates of these sublineages did not differ in aggressiveness to cacao in our growth chamber inoculations. Other cacao isolates with differing ITS sequences were not pathogenic to cacao, and at least some of these isolates were not from trees with cacao wilt.

Isolates from hosts not native to Latin America (i.e., from coffee, mango, *Eucalyptus* spp., and *Gmelina arborea*) generally showed some specialization to their respective hosts, but the specialization was not as strong as that shown by isolates from the four native hosts. Coffee isolates from Guatemala, Costa Rica, Columbia, and Suriname had very different ITS sequences, and these differed in their aggressiveness to coffee. Likewise, many ITS genotypes were found among isolates from mango and *Eucalyptus* spp. in various locales in Brazil. Isolates collected from mango, *Annona* sp., and cassava in a few small farms near São Fidelis, Rio de Janeiro, Brazil had identical ITS sequences and were highly aggressive to mango. These isolates were all collected from an area where the disease was severe on mango and apparently *Annona* sp., but the cassava isolate was from a cut stem to be used for propagation and was not pathogenic to cassava in our inoculations.

Local populations. Because *Ceratocystis fimbriata* is primarily disseminated by insects (15,21,24,32) and long-distance dispersal is not very efficient, we hypothesize that local populations of the fungus may become geographically isolated and can develop specialization to local, native hosts. Although these local populations may not be very aggressive to their native hosts (e.g., the cacao isolates with ITS sequences differing from those of the cacao sublineages), various exotic hosts may be seriously affected. This could explain much of the observed variation in hosts of *Ceratocystis fimbriata* in various regions (7). For instance, mango is known as a host only in the Mata Atlantica of Brazil, *Gmelina arborea* only near the mouth of the Amazon, and coffee only in Central America and northwestern South America. This study shows that these hosts are attacked by different, apparently local genotypes of the fungus.

Some local genotypes of *Ceratocystis fimbriata* may have been moved to other regions by humans. *Ceratocystis fimbriata* was originally described in 1890 on sweet potato in New Jersey (17). The sweet potato pathogen may be native to the eastern and southeastern United States, but it is also well known in Asia, New Zealand, and Papua New Guinea (7,18). The ITS sequences of isolates from these locations are identical to those from the eastern United States, and we suspect that the sweet potato fungus was moved to these locations on infected storage roots. *Platanus* spp. are known as hosts only in the United States and now southern Europe, where *Platanus* spp. are the only known hosts of *Ceratocystis fimbriata*. *Ceratocystis fimbriata* does not appear to be native to Europe (7), but the *Platanus* pathogen may have been introduced there on crating material made from diseased trees (35). Until recently, cacao was confirmed as a host of *Ceratocystis fimbriata* only in Central America, the Caribbean, and northwestern South America. Although *Ceratocystis fimbriata* has been known on the Atlantic Coast of Brazil for many decades on mango, the disease in cacao was recognized there in 1997 (6), presumably introduced in infected cacao cuttings (18). Cacao isolate C940 was collected in Costa Rica but was similar to Ecuadorian isolates in ITS sequence; it may represent an introduction of an Ecuadorian strain into Costa Rica. Genotypes of *Ceratocystis fimbriata* recently have been found in *Eucalyptus* plantations in Africa, perhaps introduced from South America (41), and the ITS sequences of the African isolates are similar to those of *Eucalyptus* isolates from Brazil (T. C. Harrington and C. J. Baker, unpublished data).

TABLE 8. Percent mortality and mean xylem discoloration (in centimeters) and percent mortality of cacao seedlings inoculated with Costa Rican cacao isolates

Isolate	Percent mortality	Length of discoloration ^z
C1638	100	5.9 a
C1634	30	4.7 ab
C1549	70	4.7 ab
C1548	90	4.5 ab
C1639	50	4.5 ab
C1550	50	4.4 ab
C1640	70	4.3 ab
C1635	90	4.2 ab
C1636	60	3.9 b
C1642	80	3.7 b
C1547	60	3.2 b
C1637	30	3.1 b
Control	0	0.7 c

^z Means (of 10 plants) followed by the same letters are not significantly different according to Duncan's multiple range test (mean square error = 3.30; $P \leq 0.05$).

Host specialization appears to be a major factor defining other groups of closely related, morphologically indistinguishable species of *Ceratocystis* (20). It is likely that several of the lineages of *Ceratocystis fimbriata* that have adapted to American hosts represent distinct species or populations in the process of speciation. Recognition of these unique populations as species would facilitate disease management and the development of more effective quarantine measures to minimize the risk of introducing specialized forms of the pathogen to new regions. Further work will focus on intersterility barriers and phylogenetic divergence among these putative species.

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