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Phylogeny and taxonomy of the North American clade of the *Ceratocystis fimbriata* complex

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Abstract: *Ceratocystis fimbriata* is a widely distributed, plant pathogenic fungus that causes wilts and cankers on many woody hosts. Earlier phylogenetic analyses of DNA sequences revealed three geographic clades within the *C. fimbriata* complex that are centered respectively in North America, Latin America and Asia. This study looked for cryptic species within the North American clade. The internal transcribed spacer regions (ITS) of the rDNA were sequenced, and phylogenetic analysis indicated that most isolates from the North American clade group into four host-associated lineages, referred to as the aspen, hickory, oak and cherry lineages, which were isolated primarily from wounds or diseased trees of *Populus*, *Carya*, *Quercus* and *Prunus*, respectively. A single isolate collected from *P. serotina* in Wisconsin had a unique ITS sequence. Allozyme electromorphs also were highly polymorphic within the North American clade, and the inferred phylogenies from these data were congruent with the ITS-rDNA analyses. In pairing experiments isolates from the aspen, hickory, oak and cherry lineages were interfertile only with other isolates from their respective lineages. Inoculation experiments with isolates of the four host-associated groupings showed strong host specialization by isolates from the aspen and hickory lineages on *Populus tremuloides* and *Carya illinoensis*, respectively, but isolates from the oak and cherry lineages did not consistently reveal host specialization. Morphological features distinguish isolates in the North American clade from those of the Latin American clade (including *C. fimbriata sensu stricto*). Based on the phylogenetic evidence, interfertility, host specialization and morphology, the oak and cherry lineages are recognized as the earlier described *C. variospora*, the poplar lineage as *C. populicola* sp. nov., and the hickory lineage as *C. caryae* sp. nov. A new species associated with the bark beetle *Scolytus quadrispinosus* on *Carya* is closely related to *C. caryae* and is described as *C. smalleyi*.

Key words: Microascales, *Scolytus quadrispinosus*, speciation

INTRODUCTION

Species of *Ceratocystis* are largely insect-dispersed pathogens of woody plants, infecting their hosts through wounds. *Ceratocystis fimbriata* Ellis & Halsted is notable for its broad host range, with at least 31 plant species from 14 families confirmed as hosts (CABI 2001). Hosts of *C. fimbriata* include *Eucalyptus* spp., *Mangifera indica* (mango), *Theobroma cacao* (cacao), *Coffea arabica* (coffee), *Hevea brasiliensis* (rubber tree), *Platanus* spp. (sycamore or plane tree), *Prunus* spp. (almond and other stone fruits) and *Populus* spp. (aspen and other poplars). Nonwoody hosts include *Colocasia esculenta* (taro) and *Ipomoea batatas* (sweet potato), from which the species originally was described (Halsted 1890). The geographic range and genetic diversity of *C. fimbriata* is similarly impressive, although most of the diversity in the species is found in the Americas (Baker et al 2003, Barnes et al 2001, CABI 2001, Harrington 2000, Steimel et al 2004). *Ceratocystis albifundus*, a closely related species, is native to Africa (Roux et al 2000, Wingfield et al 1996).

Webster and Butler (1967) concluded that interfertility and lack of morphological differences precluded recognition of additional species within *C. fimbriata*, but sequences of the internal transcribed spacer region (ITS) of the nuclear ribosomal DNA and other genetic analyses show that there are several subgroups or clades within *C. fimbriata* (CABI 2001, Harrington 2000). One of these major clades seems to be centered in Latin America, where *C. fimbriata* infects numerous native and nonnative hosts. Two of the members of the Latin American clade, the sweet potato pathogen *C. fimbriata sensu stricto* and the sycamore pathogen *C. platani* (Walter) Engelbrecht & Harrington, are found in eastern North America and elsewhere (Baker et al 2003, Engelbrecht et al 2004, Engelbrecht and Harrington 2005). Other members of the Latin American clade include the cacao pathogen *C. cacaofunesta* Engelbrecht & Harrington, a *Xanthosoma* pathogen in the Caribbean, and various Central and South American populations (Baker et al 2003, Engelbrecht and Harrington

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TABLE I. Origin and GenBank accession numbers for ITS r-DNA sequences of *Ceratocystis fimbriata* isolates from the North American clade

Lineage[a]	Isolate or specimen number[b]	GenBank Accession No.	Additional isolate (specimen) numbers/collector[c]	Source	Collection Location
Aspen	C89	AY907027	CBS 114725/Hinds	*Populus tremuloides*	South Dakota
	C685	AY907028	CBS 11561, BPI 843723/ Smalley	*P. tremuloides*	Quebec
	C947		ATCC 36291/ Gremmen	*Populus* sp., from canker	Poland
	C995	AY907029	CBS 119.78/ Gremmen	*Populus* sp., from canker	Poland
	C1485		ATCC 24096/Hinds	*P. tremuloides*	Colorado
Hickory A	C682		Smalley	*Carya cordiformis*, *Scolytus*-infested	Wisconsin
	C683		Smalley	*Carya ovata*, from *Scolytus*	Wisconsin
	C684	AY907030	CBS 114724, BPI 843722/ Smalley	*C. cordiformis*, *Scolytus*-infested	Wisconsin
	C1410	AY907031	Harrington	*C. cordiformis*	Iowa
	C1411		Harrington	*C. cordiformis*	Iowa
	C1828	AY907032	Johnson	*C. cordiformis*, with *Scolytus*	Iowa
	C1839		Johnson	*C. cordiformis*, *Scolytus*-infested	Iowa
	C1840		Johnson	*C. cordiformis*, *Scolytus* beetle gallery	Iowa
	C1842		Johnson	*C. cordiformis*	Iowa
	C1844		Johnson	*C. cordiformis*, *Scolytus* beetle gallery	Iowa
	C1952[d]		Johnson	*C. cordiformis*, *Scolytus* beetle gallery	Iowa
Hickory B	C1412	AY907033	BPI 843728/Harrington	*C. cordiformis*, from wound	Iowa
	C1413		Harrington	*C. cordiformis*	Iowa
	C1827	AY907034	CBS 115168/Johnson	*Carya ovata*, wound	Iowa
	C1829	AY907035	CBS 114716, BPI 843735/ Johnson	*C. cordiformis*, wound	Iowa
	C1845		Johnson	*C. cordiformis*, wound	Iowa
	C1971[d]		Johnson	*Ostrya virginiana*, wound	Iowa
Oak	C1009		CBS 773.73, ATCC 12861/ Campbell	*Quercus* sp., from fresh stump	Minnesota
	C1483	AY907036	ATCC 12866/ Campbell	*Quercus ellipsoidalis*, from fresh stump	Minnesota
	C1843	AY907037	CBS 114715, BPI 843737/ Johnson	*Q. alba*, wound	Iowa
	C1846	AY907038	CBS 114714, BPI 843738/ Johnson	*Q. robur*, bleeding canker	Iowa
	BPI 595631[c]	AY907039	Davidson	*Q. prinus*, from inner bark	West Virginia
Oak, Japan	C1709	AY907040	MCC-NIES 323/ Matsuya	*Betula platyphylla*, from log	Japan
Cherry	C578	AY907041	Bostock	*Prunus dulcis*, canker	California
	C686		Smalley	*P. dulcis*	California
	C821		Rizzo	*P. dulcis*, canker	California
	C855		Harrington	*P. dulcis*, canker	California
	C856		Harrington	*P. dulcis*, canker	California
	C857		Harrington	*P. dulcis*, canker	California
	C1821		Harrington	*P. dulcis*, canker	California

TABLE I. Continued

Lineage[a]	Isolate or specimen number[b]	GenBank Accession No.	Additional isolate (specimen) numbers/collector[c]	Source	Collection Location
	C1822	AY907042	CBS 114717, BPI 843734/ Harrington	*P. dulcis*, canker	California
	C1837		Johnson	*Quercus rubra*, wound	Iowa
	C1841		Johnson	*Prunus serotina*, wound	Iowa
	C1953[d]		Johnson	*Populus grandidentata*, wound	Iowa
	C1954	AY907043	Johnson	*Tilia americana*, wound	Iowa
	C1955[d]		Johnson	*Quercus macrocarpa*, wound	Iowa
	C1956[d]		Johnson	*Q. macrocarpa*, wound	Iowa
	C1957[d]		Johnson	*Celtis occidentalis*, wound	Iowa
	C1958[d]		Johnson	*Carya ovata*, wound	Iowa
	C1959[d]		Johnson	*T. americana*, bark beetle (associated with wound)	Iowa
	C1963[d]		Johnson	*Prunus serotina*, wound	Iowa
	C1964		Johnson	*Q. macrocarpa*, bark beetle (associated with wound)	Iowa
	C2053		Harrington	*P. dulcis*, canker	California
Cherry, Japan	C1707	AY907044	MCC - NIES 321/ Matsuya	*Betula platyphylla*, from log	Japan
	C1762		MCC- NIES 335/ Matsuya	*Betula platyphylla*, from log	Japan
Cherry, WI	C1965[d]	AY907045	Johnson	*Prunus serotina*, wound	Wisconsin

[a] Based on ITS rDNA sequences and/or allozyme analysis.

[b] Isolate numbers preceded by C are from the collection of T. C. Harrington.

[c] ATCC = American Type Culture Collection; CBS = Centraalbureau voor Schimmelcultures; MCC-NIES = Microbial Culture Collection at National Institute for Environmental Studies, Heita, Kamaishi, Iwate, Japan; BPI = specimen from the U.S. National Fungal Collection.

[d] Extraction and analysis of allozyme electromorphs not replicated.

2005, Harrington 2000, Marin et al 2003, Thorpe et al 2005). A second clade occurs on fig (*Ficus carica*) and taro in Japan and the Pacific (Harrington 2000, Thorpe et al 2005), and the recently described *C. pirilliformis* I. Barnes & M. J. Wingf. from Australia (Barnes et al 2003) and Africa (Roux et al 2004) and *C. polychroma* M. van Wyk, M. J. Wingf. & E.C.Y. Liew from Indonesia (van Wyk et al 2004) are also in the Asian clade based on rDNA sequence analysis (Harrington unpublished, Thorpe et al 2005). Members of a third clade infect *Populus* spp., *Carya* spp., *Quercus* spp., and *Prunus* spp. in North America (Harrington 2000). A morphologically similar species, *C. variospora* (R.W. Davidson) C. Moreau, was described from *Quercus* in the eastern USA (Davidson 1944, Hunt 1956), but some have considered this species and *Rostrella coffaea* to be synonyms of *C. fimbriata* (Upadhyay 1981, Webster and Butler 1967, Zimmermann 1900).

This study applies analyses of allozymes, DNA sequences, interfertility tests, host specialization and morphology to identify cryptic species among isolates of the *C. fimbriata* complex from North America using the phylogenetic species concept (Harrington and Rizzo 1999). This concept recognizes species as populations or lineages with unique phenotypic characters, such as morphology and host specialization.

METHODS AND MATERIALS

Fungal isolates.—A limited number of isolates from *Populus tremuloides* (aspen), *Prunus dulcis* (almond), *Carya* spp. (hickory) and *Quercus* spp. (oak) were obtained from culture collections and plant pathologists. The second author collected isolates from *C. cordiformis* (bitternut hickory) in northeastern Iowa and *P. dulcis* in the Central Valley of California.

Further attempts were made during summer 2001 to collect isolates of *Ceratocystis* spp. from Iowa. We examined and obtained isolates from recently wounded *Quercus* spp. at the Yellow River State Forest in northeastern Iowa. An additional isolate was obtained from a bleeding canker on the European species *Q. robur* in an experimental planting at the Iowa State University (ISU) research farm at Rhodes.

TABLE II. Source of isolates from the Latin American and Asian clades of *Ceratocystis fimbriata* used in allozyme analysis

Isolate[a]	Additional numbers/ collector[b]	Source	Collection Location
C809	Capretti	*Platanus acerifolia*	Italy
C854	Clark	*Ipomoea batatas*	Louisiana
C858	Harrington	*Platanus* sp.	California
C859	Harrington	*P. acerifolia*	California
C868	CMW2220	*P. acerifolia*	France
C918	Alfenas	*Gmelina arborea*	Brazil
C925	CBS 115173, Alfenas	*G. arborea*	Brazil
C940	CBS 152.62/Hansen	*Theobroma cacao*	Costa Rica
C994	CBS 600.70/Figueiredo	*Mangifera indica*	Brazil
C1004	CBS 153.62/Hansen	*T. cacao*	Ecuador
C1022	Alvarez	*Citrus sinensis*	Colombia
C1024	Alvarez	*Coffea arabica*	Colombia
C1213	Somasekhara	*Punica granatum*	India
C1317	CBS 115162, Harrington	*Platanus occidentalis*	North Carolina
C1339	Britton	*P. occidentalis*	Virginia
C1345	Alfenas	*Eucalyptus* sp.	Brazil
C1351	Harrington	*P. occidentalis*	Kentucky
C1354	KFCF 9210/Kajitani	*I. batatas*	Japan
C1355	KFCF 9001/Kajitani	*Ficus carica*, from canker	Japan
C1391	IFO 30956/Kato	*F. carica*	Japan
C1392	IFO32968/ Mukobata	*F. carica*	Japan
C1393	IFO32969/ Mukobata	*F. carica*	Japan
C1418	Cubeta	*I. batatas*	North Carolina
C1421	CBS 114723, Cubeta	*I. batatas*	North Carolina
C1442	CBS 115174, Alfenas	*Eucalyptus* sp.	Brazil
C1451	Alfenas	*Eucalyptus* sp.	Brazil
C1473	ICMP 894	*I. batatas*	New Zealand
C1475	ICMP 1731	*I. batatas*	New Zealand
C1476	ICMP 8579	*I. batatas*	Papua New Guinea
C1547	Paulin	*T. cacao*	Costa Rica
C1548	CBS 114722, Paulin	*T. cacao*	Costa Rica
C1551	Paulin	*C. arabica*	Costa Rica
C1554	Alfenas	*M. indica*	Brazil
C1587	Harrington	*T. cacao*	Brazil
C1590	Harrington	*M. indica*	Brazil
C1592	Harrington	*Annona* sp.	Brazil
C1593	Harrington	*T. cacao*	Brazil
C1597	Harrington	*T. cacao*	Brazil
C1603	Harrington	*Manihot esculenta*	Brazil
C1637	Harrington	*T. cacao*	Costa Rica
C1641	Harrington	*Xanthosoma* sp.	Costa Rica
C1642	Harrington	*Herrania* sp.	Costa Rica
C1655	Baker	*M. indica*	Brazil
C1657	Baker	*M. indica*	Brazil
C1672	Baker	*Annona* sp.	Brazil
C1673	Baker	*Eucalyptus* sp.	Brazil
C1690	Harrington	*T. cacao*	Ecuador
C1713	Harrington	*Hevea brasiliensis*	Mexico
C1714	CBS 115164, Uchida	*Colocasia esculenta cv* bunlong	Hawaii
C1715	CBS 114720, Uchida	*C. esculenta*	Hawaii
C1717	CBS 114719, Uchida	*Syngonium* sp.	Hawaii
C1774	Norman	*Syngonium* sp.	Florida
C1780	CBS 115165, Baker	*Xanthosoma* sp.	Costa Rica
C1781	Harrington	*Syngonium* sp.	Florida
C1782	CBS 115166, Johnson	*Ficus carica*	Brazil

TABLE II. Continued

Isolate[a]	Additional numbers/ collector[b]	Source	Collection Location
C1809	CBS 115167, Harrington	*Syngonium* sp.	Florida
C1810	Grillo	*Spathodea campanulata*	Cuba
C1811	Harrington	*S. campanulata*	Cuba
C1812	Harrington	*S. campanulata*	Cuba
C1817	CBS 114718, Harrington	*Xanthosoma* sp.	Cuba
C1848	Harrington	*F. carica*	Brazil
C1849	Harrington	*F. carica*	Brazil
C1859	Harrington	*Colocasia esculenta*	Brazil
C1860	Harrington	*C. esculenta*	Brazil
C1863	Harrington	*C. esculenta*	Brazil
C1864	Harrington	*C. esculenta*	Brazil
C1865	CBS 114713, Harrington	*C. esculenta*	Brazil

[a] Isolate number, those preceded by C are from the collection of Dr. Thomas C. Harrington.

[b] ATCC: American Type Culture Collection; CBS = Centraalbureau voor Schimmelcultures; ICMP = Landcare Research New Zealand ; CMW = Forestry and Biotechnology Institute, University of Pretoria, South Africa; IFO = Institute for Fermentation, Osaka, Japan; KFCF = from collection of Y. Kajitani, Fukuoka Agricultural Research Center, Fukuoka, Japan.

At another site south of Boone, samples were taken from a *C. ovata* (shagbark hickory) tree with crown dieback and a *C. cordiformis* tree with recent attacks by a wood-boring beetle (*Agrilus* sp.). *Ceratocystis* species were not isolated from either hickory tree initially, but when the same trees were resampled 2 wk later, the wounded bark was found to be extensively colonized and isolates were recovered. Isolates also were obtained from *C. cordiformis* trees in a forest stand in Coggan, in northeastern Iowa, where an outbreak of the hickory bark beetle (*Scolytus quadrispinosus* Say) was ongoing; isolates were obtained from beetle galleries and from discolored wood associated with beetle attacks. Additional isolations were obtained from logging wounds on *C. cordiformis* and *P. serotina* (black cherry) trees in the same stand. Another outbreak of *S. quadrispinosus* was located near Cambria, in southern Iowa, and an isolate was obtained June 2002 from a *C. ovata* tree infested with the beetle.

In 2002 trees were wounded artificially at four locations in Iowa. Wounds were created at breast height (1.4 m) on the main stem by removing a 6 × 6 cm patch of bark with a flame-sterilized hatchet, then bruising the bark with the back of the hatchet along two sides of the wound to loosen the bark. Each site was revisited approximately 10 d after wounding, and samples were taken from the wound face and from bark surrounding the wound. Isolations were made with the carrot disk method of Moller and DeVay (1968). At the first site, north of Ogden, one tree from each of six species (*P. serotina*, *Q. macrocarpa* [bur oak], *C. ovata*, *Celtis occidentalis* [hackberry], *Populus grandidentata* [bigtooth aspen] and *Tilia americana* [basswood]) was wounded in mid-June. Isolates of a *Ceratocystis* sp. were recovered from all but the *Carya ovata* and *Celtis occidentalis* trees. At a second site near Lucas one tree each of five species was wounded at the end of June, and isolates of *Ceratocystis* were recovered from *Q. macrocarpa*, *Carya ovata* and *Celtis occidentalis*, but no *Ceratocystis* was recovered from *Prunus serotina* and *Ulmus rubra* (red elm). The third site, near Ames, contained both upland and bottomland species, and *Prunus serotina*, *Q. macrocarpa*, *Carya ovata*, *Ostrya virginiana* (ironwood), *Juglans nigra* (black walnut), *Gleditsia triacanthos* (honeylocust), *Fraxinus pennsylvanica* (green ash), and *Populus deltoides* (cottonwood) were wounded in mid-July, but *Ceratocystis* was isolated only from *O. virginiana*. A fourth site north of Boone was visited in August, wounds were made on various hardwood species, and isolations were attempted 10 d later, but *Ceratocystis* was not recovered.

Representative isolates from the above collections were selected for DNA sequence and allozyme analyses, inoculation studies and mating experiments (TABLE I). Additional isolates representing the Latin American and Asian clades of *C. fimbriata* were used for allozyme analysis (TABLE II). Isolates C904, C1062 (CMW 4081), C1083 (CMW 4110) and C1360 (JC 6885) of the African species *C. albifundus* were supplied by J. Roux and used as an outgroup taxon in DNA sequence and allozyme analyses.

ITS sequencing and analysis.—Template DNA for PCR was obtained from mycelium grown on 10 mL of broth (MYB, 2% malt extract and 0.2% yeast extract) at approximately 24 C for 7–10 d, or from mycelium scraped from 1–2 wk old cultures grown on plates of malt yeast-extract agar (MYEA, 2% malt extract, 0.2% yeast extract, 2% agar). DNA extraction was performed with micropestles and microcentrifuge tubes following the method of DeScenzo and Harrington (1994). The PCR primers, reagents and cycling conditions were as previously described (Harrington et al 2001). Sequencing was performed at the ISU DNA Sequencing and Synthesis Facility using the PCR primers. Sequences were aligned manually by adding gaps, and parsimony analysis was performed with PAUP 4.0b10 (Swofford 2002). *Ceratocystis albifundus* was used as outgroup taxon, and the ingroup was considered to be mono-

TABLE III. Abbreviations, buffer systems, and electromorphs found for 12 enzymes used in starch gel electrophoresis

Enzyme name	Enzyme abbreviations	EC numbers[a]	Buffer systems[b]	Electromorphs discerned
Acontinase	ACN	EC 4.2.1.3	MC 8.1	3
Fumarate hydratase	FUMH	EC 4.2.1.2	MC 8.1	7
Peptidase	PEP	EC 3.4.-.-	MC 8.1	5
Glucose-6-phosphate dehydrogenase	G6PDH	EC 1.1.1.49	MC 8.1	4
6-phosphogluconic dehydrogenase	PGD	EC 1.1.1.44	MC 8.1	5
Aspartase aminotransferase	AAT	EC 2.6.1.1	MC 8.1	10
NADH Diaphorase	DIA	EC 1.8.1.4	S-6	7
Phosphoglucomutase	PGM	EC 5.4.2.2	S-6	3
Mannose-6-phosphate isomerase	MPI	EC 5.3.1.8	S-6	5
Adenylate kinase	AK	EC 2.7.4.3	S-11	8
Malate dehydrogenase	MDH	EC 1.1.1.37	S-11	4
Fluorescent esterase	FE	EC 3.1.1.-	S-11	4

[a] Nomenclature Committee of the International Union of Biochemistry.

[b] Buffer MC 8.1 was a continuous morpholine citrate system, adjusted to pH 8.1, run at 40 amps constant amperage for 4.5 hours. Buffer S-6 was a discontinuous Tris, citric acid system, adjusted to pH 8.6, run at 20 amps constant amperage for 4.5 hours. Buffer S-11 was a discontinuous histine system, adjusted to pH 7.0, run at 40 amps constant amperage for 4.5 hours. Systems 6 and 11 modified from Soltis et al. (1983).

phyletic. Of 714 total aligned characters, including gaps, 217 were ambiguously aligned and excluded from the analysis, 110 remaining sites were variable, and of these, 32 were parsimony informative. Except for gaps between ingroup and outgroup, there were only single-base gaps, which were treated as a fifth character. A maximum parsimony heuristic search was performed with all characters having equal weight. Starting trees were obtained through stepwise addition, and tree-bisection-reconnection was used. Bootstrap analysis with 2000 replications of heuristic searches was used to determine support for internal branches.

Allozyme analysis.—One hundred ten isolates of *C. fimbriata*, including representatives from all three geographic clades, and three isolates of the outgroup taxon, *C. albifundus*, were tested for allozyme variation (TABLES I and II). Cultures were grown 14 d in 125 mL Erlenmeyer flasks containing 30 mL of MYB at room temperature. Enzymes were extracted from mycelial mats onto paper wicks and stored at −80 C until electrophoresis (Zambino and Harrington 1992), which was performed on 12% starch gels (Harrington et al 1996). Buffers and electrophoresis conditions are shown (TABLE III). With few exceptions (TABLE I), enzymes were extracted and tested for allozyme activity at least twice. Isolates C1418 and C1410 were included in each gel as reference isolates. For each allozyme, electromorphs were designated by numbers in order of decreasing anodal migration, and the electromorphs were considered to be alleles. These data were used to develop an uncorrected ''p'' distance matrix, and phenograms were generated with neighbor joining and UPGMA (unweighted pair group method with arithmetic mean) with PAUP 4.0b10. The neighbor joining tree was rooted to *C. albifundus*.

Interfertility tests.—*Ceratocystis fimbriata* is both a heterothallic and a homothallic fungus, with two mating types; MAT-1 strains are self-sterile, but MAT-2 strains are self-fertile. The MAT-2 strains have both *MAT-1* and *MAT-2* genes, but during unidirectional mating-type switching, the *MAT-2* gene is deleted, and progeny that have inherited nuclei with the deletion behave as MAT-1 and are self-sterile (Harrington and McNew 1997, Witthuhn et al 2000b). Thus MAT-2 (self-fertile) and MAT-1 (self-sterile) progeny are recovered from selfings of MAT-2 strains. Most field isolates are MAT-2, and pairing experiments are hampered in that MAT-2 strains are usually self-fertile. Some self-fertile MAT-2 strains produce mutant sectors that lack the ability to produce protoperithecia and perithecia, and these MAT-2 sectors are self-sterile and function poorly as females in crosses (Engelbrecht and Harrington 2005, Harrington and McNew 1997, Harrington et al 2002).

MAT-2 tester strains that were used in pairings were obtained by subculturing sterile sectors that arose spontaneously from fertile, selfing isolates. The presence of the *MAT*-2 gene in these tester strains was confirmed with PCR (Witthuhn et al 2000b). All MAT-2 testers were self-sterile, except isolates C1959 and C1483, which produced deformed perithecia that could be distinguished readily from normal perithecia produced in successful pairings with other strains. The MAT-1 testers were obtained by recovering single ascospore progeny from self-fertile isolates.

The MAT-1 testers were used as recipients (females), and MAT-2 testers served as donors (males). MAT-2 testers also were paired with each other, but no combination resulted in an interfertile cross. Both male and female cultures were grown on MYEA plates at room temperature. After 5 d male testers were flooded with 15 mL sterile, deionized water and scraped with a sterile spatula to suspend spores and mycelial fragments. Female testers were 5 d old colonies, which

received 1 mL of a conidial suspension from the male tester at the edge of the expanding female colony. Spermatized cultures were allowed to grow 7 d at room temperature (24 C) before initial evaluation with a dissecting microscope. Ambiguous reactions were re-inspected after an additional 4 d. When perithecia were found they were examined at 400× or 1000× with a compound microscope to see whether normal ascospores had formed.

Four host cross-inoculations.—We first tested whether isolates from the four main, host-associated lineages exhibited host specialization to four hosts: *Populus tremuloides*, *Q. rubra* (red oak), *Prunus serotina* and *Carya illinoensis* (pecan). Nine inoculation treatments, consisting of two isolates from each of the four major lineages and a control, were applied to each host.

Lateral roots of *P. tremuloides* were dug from a clone near Johnston, Iowa, and were maintained under mist until root sprouts appeared (Benson and Schwalbach 1970). Sprouts were harvested when they were 3–6 cm tall, dipped in 1000 ppm IBA (indolebutyric acid) and placed in peat pellets to root (Snow 1938). The plants were inoculated at 5–6 mo after rooting. *Carya illinoensis* seed (Sheffield's Seed Company, Locke, New York) were cold-stratified for 4 mo then germinated in Kimpak paper (Kimberly-Clark Corporation, Irving, Texas) in a growth chamber set to a 16 h day (30 C)/8 hour night (20 C) cycle. After 14 d the germinated seeds were transferred to 4-inch pots in the greenhouse and inoculated after 3–4 mo. Seed of *Q. rubra* (Sheffield's Seed Co.) were cold-stratified for 6 wk, planted directly to 4-inch pots in the greenhouse, and the seedlings were inoculated after 2.5–3 mo. Half-sib seedlings of *Prunus serotina* were dug from under a tree near Ames, Iowa, planted in 4-inch pots, and grown 3–4 mo before inoculation.

Before inoculation, plants were grown on greenhouse benches in a mixture of 50% perlite, 50% Peat-lite mix (Fafard, Aawam, Massachusetts). All plants received a slow release fertilizer (Osmocote 19-6-12) at the time of sowing and biweekly feedings with liquid fertilizer (Miracle-Gro EXCEL 21-5-20). Artificial light was used to maintain a 16 h day. Plants were transferred to growth chambers 7 days before inoculation, where they were maintained on a 16/8 h light/dark cycle at 25 C. Each experiment (a single host) was performed with a randomized complete block design with six blocks, and six replications per treatment. The *C. illinoensis* seedlings were inoculated on greenhouse benches using the same experimental design. All experiments were repeated.

Inoculum was prepared from 7 d old cultures on MYEA plates (Baker et al 2003). Sterile water was added to the plates, the colonies were scraped and the suspension was filtered through four layers of sterile cheesecloth. Inoculum primarily comprised endoconidia, which were adjusted to 1.0×10^6 spores per mL with a hemacytometer. Control inoculum was prepared by flooding sterile MYEA plates with water, scraping and filtering the resulting solution through sterile cheesecloth.

Plants were prepared for inoculation by making a downward-slanting horizontal cut through the bark and into the xylem of each stem with a sterile razor blade. Immediately after wounding 0.2 mL of inoculum was introduced into the wound with a syringe (21-gauge needle), and each wound was wrapped with parafilm. Plants were watered daily, and any mortality occurring before the end of the experiment was recorded and the plants harvested. *Populus tremuloides* and *C. illinoensis* plants were harvested at 40 d, while *Q. rubra* and *Prunus serotina* plants were harvested after 30 d. At harvest a shallow cut was made along the stem above and below the inoculation point, without cutting into the xylem, and the length of cankers (phloem necrosis) was recorded. A slightly deeper cut then was made, exposing the xylem tissue so that the total length of xylem discoloration could be measured. The fungus was re-isolated from inoculated plants by placing discolored tissue between carrot slices (Moller and DeVay 1968).

Length of xylem discoloration was analyzed by host plant, source of inoculum, experiment (within host), host × source interaction and source × experiment (within host) interaction using a multi-factorial analysis of variance (ANOVA) with controls excluded. For each inoculated host ANOVA indicated significant variation ($P = 0.05$) due to the two experiments (within host), so each experiment then was analyzed separately with one-way ANOVA. When the ANOVA indicated significant variation among isolates (without controls), then Duncan's multiple range test was used to separate means, including the controls. Statistics were performed with SAS statistical software (SAS Institute, Cary, North Carolina).

Prunus virginiana and Quercus macrocarpa cross-inoculations.—An additional experiment was performed in which two hosts, *P. virginiana* (common chokecherry) and *Q. macrocarpa*, were inoculated with isolates from the oak and cherry lineages: two isolates from the four-host inoculation experiment (C1009 from *Quercus*, and C821 from *Prunus*), two additional isolates from the oak lineage, six Iowa isolates from the cherry lineage, and isolate C1965 from *P. serotina* in Wisconsin.

One-year-old bareroot seedlings obtained from the Iowa State Forest Nursery were grown in the greenhouse 6 wk after bud break in 6-inch pots in greenhouse soil amended with Osmocote slow-release fertilizer. Inoculations were performed in the greenhouse as described above using a completely random design with nine replications per treatment. Plants were harvested 37 d after inoculation. Length of xylem discoloration for each inoculated host was analyzed separately as described above.

Host range of hickory isolates.—Four species of *Carya* and two species from the related genus *Juglans* were inoculated with isolates from the hickory lineage. Bareroot seedlings (2 y old) of *C. cordiformis*, *C. ovata*, *C. illinoensis*, *J. nigra* (black walnut), and *J. cineria* (butternut) were grown in 2-gallon pots as described above and inoculated in a growth chamber with four isolates of *C. fimbriata* collected from *C. cordiformis*. Inoculations were performed 67 d after planting (50 d after first flush for *Juglans* spp. and 40 d after first flush for *Carya* spp.). The experiment used a completely randomized design, with five replicates per treatment. Plants were harvested 6 wk after inoculation and evaluated for linear extent of xylem discoloration. The

experiment was repeated with the same hosts and procedures, except that *Carya* plants were planted 7 d before the *Juglans* species in an effort to ensure that plants would be in a similar growth stage at the time of inoculation. Two-way ANOVA and Duncan's multiple range tests were used as described above.

Host range of aspen isolates.—Two inoculation experiments were performed in a growth chamber to test the susceptibility of five *Populus* species to four isolates from the aspen lineage. In the first experiment, *P. tremuloides*, *P. nigra* (European black poplar), *P. balsamifera* (balsam poplar), and *P. trichocarpa* (black cottonwood) were inoculated. The *P. tremuloides* plants were generated from root sprouts and inoculated 3 mo later. All other plants were 3 mo old rooted cuttings from dormant twigs. The *P. tremuloides* sprouts were grown in 4-inch pots, while the other hosts were grown in 6-inch pots. Fertilization, care and inoculation procedures were as described above. A completely randomized design was used, with five replicates per treatment.

The experiment was repeated with an additional host, *P. deltoides* (eastern cottonwood). The *P. tremuloides* plants for the second experiment were 9 mo old; the other hosts were 4–5 mo old and were generated by rooting greenwood cuttings. The first experiment was harvested at 7 wk, and the second experiment was harvested after 5 wk. For each experiment, a two-way ANOVA was used to analyze the effects of isolate, host and isolate × host interaction. For each experiment there was no significant interaction between host and isolate, so the results from the four isolates were combined and one-way ANOVA was performed to compare the response of each host. Duncan's multiple range test was used to separate means in each experiment.

Morphology.—Isolates were grown on MYEA at room temperature (approximately 23 C) and lighting 5–12 d before measurements. Measurements of endoconidia and endoconidiophores were made after 4–7 d growth, while perithecia and ascospores were measured after 7–10 d. Aleurioconidia were measured from cultures that had grown 7–20 d. Material to be measured was mounted in lactophenol cotton blue and observed with Nomarsky interference microscopy (Olympus BH-2 microscope), photographed with a Kodak DC 120 digital camera and analyzed with Openlab digital imaging software (Improvision Inc., Lexington, Massachusetts). Perithecia were measured with an eyepiece reticule at 200× or 400× magnifications. For most structures 10 observations were recorded per isolate; when measuring endoconidia, however, 20 conidia were measured per isolate. Some structures were rare or hard to locate in a few isolates, and fewer observations were made.

RESULTS

Phylogenetic analysis of ITS data.—Parsimony analysis of aligned ITS rDNA sequences resulted in 138 most parsimonious (MP) trees of 136 steps. The other MP trees differed from that shown in FIG.1 only in the minor branches, those without bootstrap support. Four host-associated lineages were evident in all of the MP trees and in a neighbor joining analysis of the same dataset (not shown). All isolates from diseased *Populus* spp. grouped in a strongly supported branch. Similarly most isolates from *Carya* spp. grouped into a single lineage with 99% bootstrap support (FIG. 1). Four *Quercus* isolates formed a separate lineage (89% bootstrap support) with a *Betula* isolate from Japan and the holotype specimen of *C. variospora* (BPI 595631) (FIG. 1). Nearly all *Prunus* isolates grouped into a single lineage (96% bootstrap support) with a few isolates from *Quercus*, *Populus*, *Carya*, *Celtis occidentalis* and *T. americana*, all wound associated; as well as a *Betula platyphylla* isolate from Japan. An isolate (C1965) collected from a wounded *Prunus serotina* tree in Wisconsin did not group into any of the four host-associated lineages. These groups henceforth are referred to as the aspen, hickory, oak, cherry and cherry-Wisconsin lineages.

Allozymes.—Forty-four electrophoretic phenotypes (ETs) were identified among the 113 isolates of *C. fimbriata* and *C. albifundus* tested. The neighbor joining phenogram, which was rooted to *C. albifundus*, and the unrooted UPGMA phenogram had similar topologies (FIGS. 2 and 3). There was substantial variation in electromorphs among isolates from the North American clade. The Latin American clade was supported only weakly in both analyses, and there was little variation among the isolates tested. There was no bootstrap support for the Asian clade, but two distinct lineages were apparent, one comprising isolates from *Ficus carica* in Japan and the other from *Colocasia esculenta* in the Pacific.

The four host-associated lineages within the North American clade seen in phylogenetic analysis of ITS-rDNA data also were seen in the allozyme analyses. Isolate C1965 from *Prunus serotina* in Wisconsin was unique (FIGS. 2 and 3). Six isolates of the hickory ITS lineage formed a sublineage within the hickory lineage, and these two sublineages are designated here as hickory A and hickory B.

Pairings.—Self-sterile MAT-2 tester strains from the cherry, hickory B, aspen and oak lineages were used to spermatize MAT-1 testers of other representative isolates of the North American clade (TABLE IV). The MAT-2 testers formed successful, interfertile pairings (perithecia producing abundant, normal ascospores) only with MAT-1 testers from their respective lineages. When a MAT-2

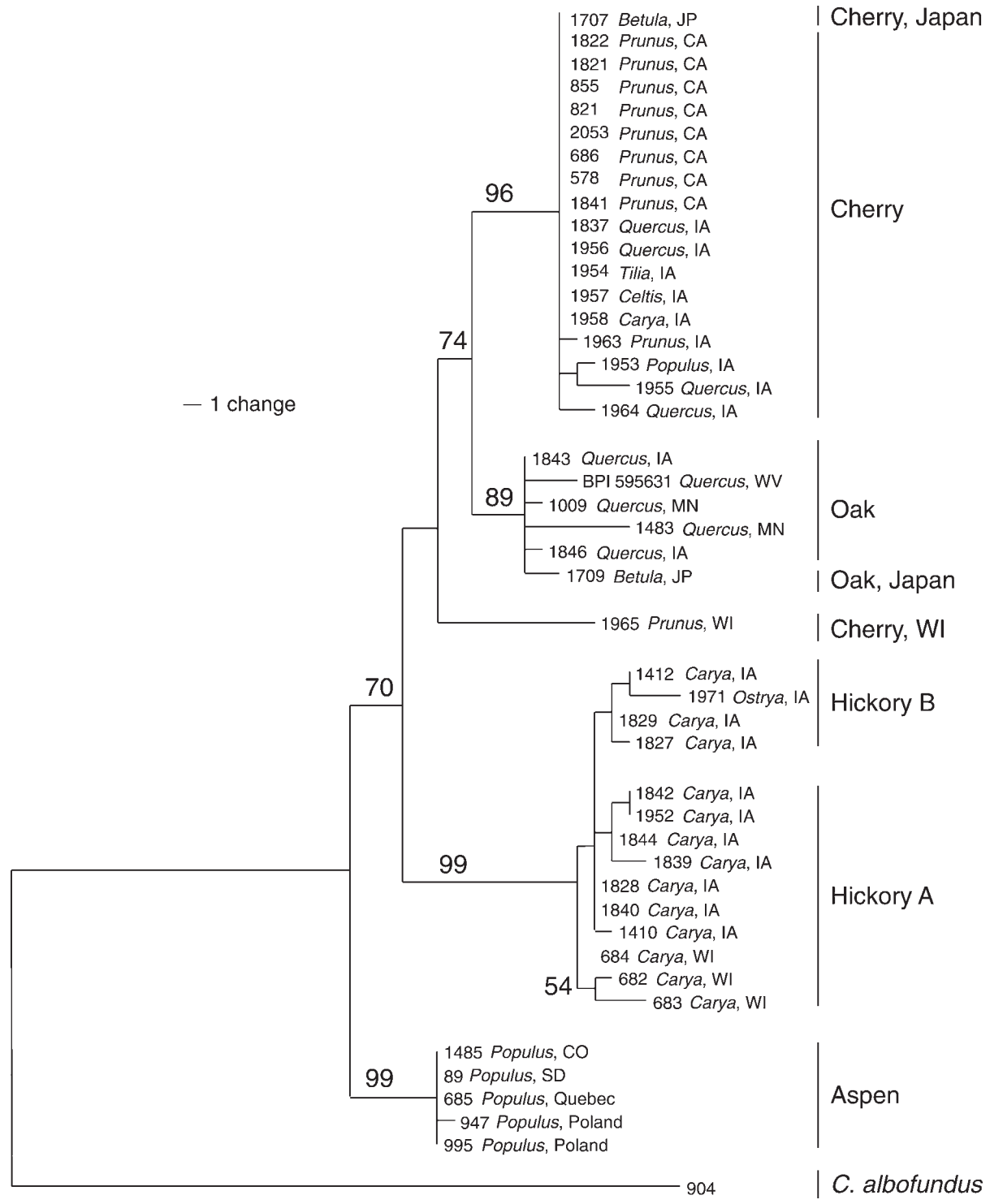


FIG. 1. One of 138 most parsimonious trees based on ITS-rDNA sequences of *Ceratocystis fimbriata* isolates from the North American clade. Consistency index (CI) = 0.897, rescaled consistency index (RC) = 0.863, retention index (RI) = 0.962. Bootstrap values greater than 50% are indicated above the branches. The tree is rooted to *C. albifundus*.

tester from the hickory B sublineage was used, perithecia and abundant ascospores were formed with MAT-1 testers from both the hickory A and hickory B sublineages. MAT-2 testers from C578 and C856, which are from the cherry lineage, paired with most other cherry testers but did not pair with MAT-1 testers from *T. americana*. Conversely the MAT-2 tester from *T. americana*

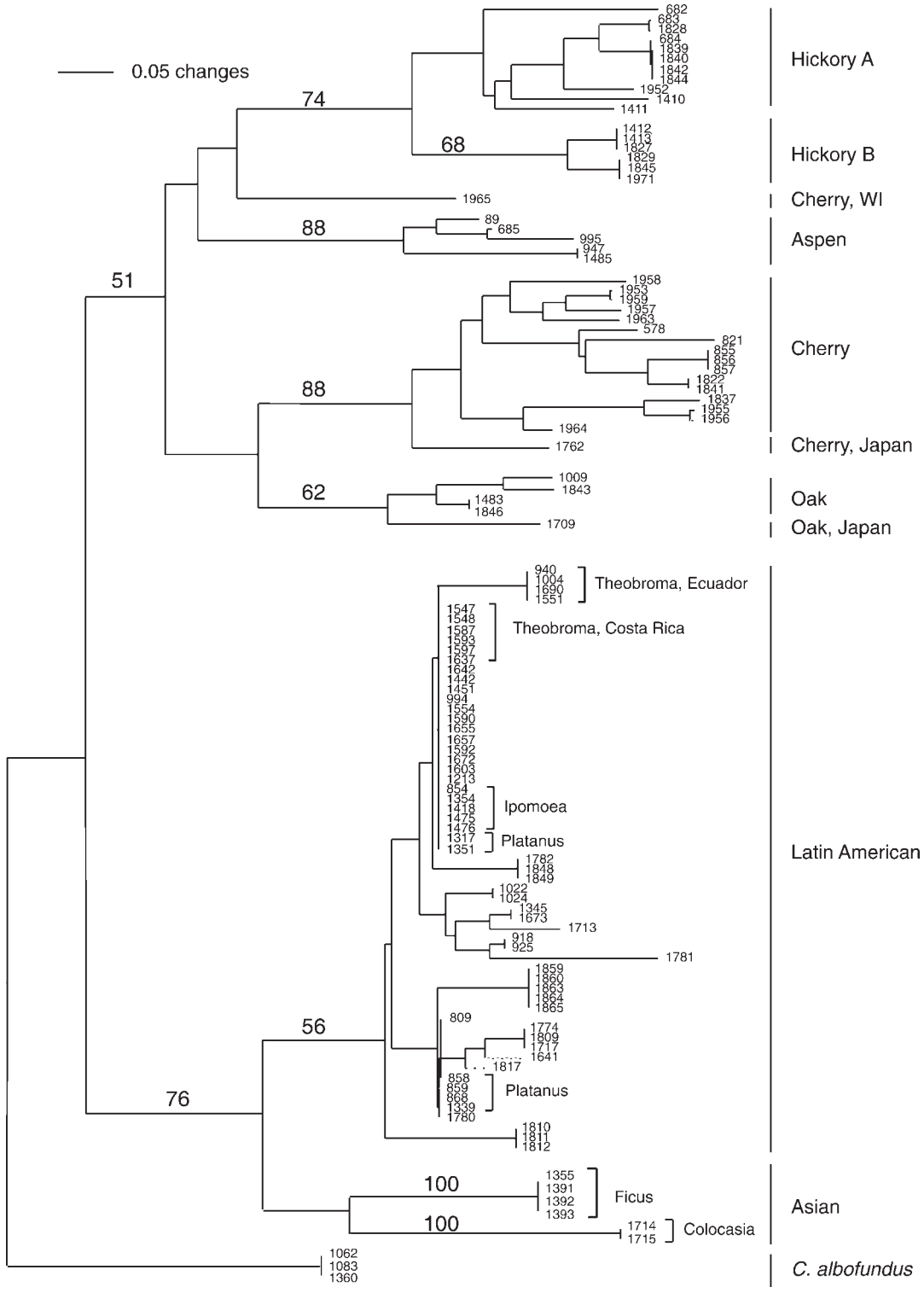


FIG. 2. Neighbor joining tree of 113 isolates of *C. fimbriata* based on allozyme electromorphs of 44 electrophoretic phenotypes. The tree was rooted to *C. albifundus*. Bootstrap values greater than 50 are shown above branches.

mated with MAT-1 testers from the same host (C1954 and C1959) but not with other testers of the cherry lineage. The MAT-1 testers from the Wisconsin *Prunus* isolate C1965 and the two Japanese isolates failed to mate with any MAT-2 tester.

Many pairings resulted in what appeared to be hybrid perithecia with watery ascospore masses

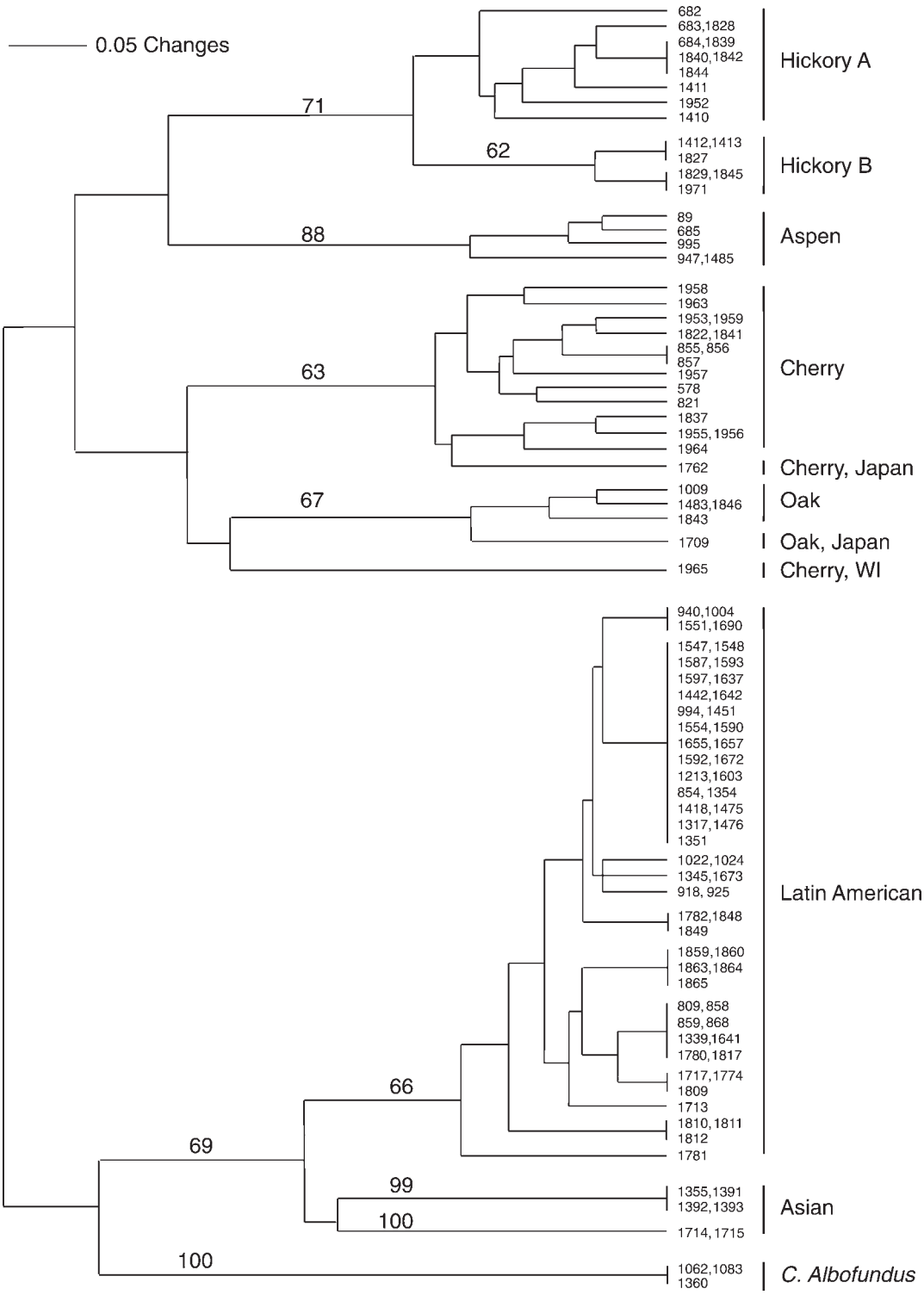


FIG. 3. Phenogram based on UPGMA analysis of 44 electrophoretic phenotypes from 113 isolates of *C. fimbriata* and three isolates of *C. albifundus*. Bootstrap values greater than 50% are shown above branches.

(TABLE IV). When observed microscopically these perithecia were filled mainly with cellular debris, apparently from aborted asci and ascospores. Ascospores, when present, were uncommon and often misshapen. These pairings were interpreted as partial interfertility due to interspecific crossing, consistent with the interpretation of interspecific pairings between other species of *Ceratocystis* (Engelbrecht

TABLE IV. Pairings for sexual compatibility among tester strains from isolates of *Ceratocystis fimbriata* representing lineages within the North American clade

		MAT-2, Males						
Genotype	Mat-1, Female	Cherry 578(A)	Cherry 856(B)	Cherry 1959(A)	Hickory B 1827(A)	Aspen 995(A)	Oak 1483(A)	Oak 1843(A)
Aspen	1485	—	H[a]	H	H	I[b]	—	S[c]
Hickory A	682	H	H	H	I	H	H	H
	684	H	—	H	I	—	H	H
	1410	H	H	H	I	H	H	H
	1411	H	H	H	I	H	H[e]	H[e]
	1828	H	H	H	I	H	H	H
	1844	H	H	H	I	H	H	H
Hickory B	1412	H	H	H	I	H	H	H
	1413	—	—	—	I	—	—	—
	1971	—	—	—	I	—	—	—
Oak	1009	—	—	—	—	—	—	I
	1483	—	—	—	—	—	I	I
	1843	—	—	—	—	—	I	I
	1846	—	—	—	—	—	I	I
Oak, Japan	1709	H	H	H	H	H	H	H
Cherry	578	I	I	H	—	—	H	—
	821	I	I	H	—	—	H	—
	855	I	I	H	—	—	H	—
	856	I	I	H	—	—	H	—
	857	I	I	H	—	—	H	H
	1822	I	I	H	—	—	H	—
	1841	I	I	—	—	—	H	S
	1953	I	I	H	—	—	H	H
	1955	I	I	H	—	—	H	—
	1957	—	—	—	—	—	—	—
	1964	I	I	H	—	—	S	S
	1954	H	H	I	H	H	—	—
	1959	H	H	I	H	H	—	—
Cherry, Japan	1707	H	H	H	—	—	—	—
	1762	H	H	H	H	H	H	H
Cherry, WI	1965	S	—	—	S	—	—	S

[a] H = hybrid: much cellular debris and few misshapen ascospores inside perithecium, exuded ascospore masses, when present, watery in appearance.

[b] I = interfertile: ascospores abundant, with normal form; exuded ascospore masses white to peach colored.

[c] S = sterile perithecia: perithecia produced, but no ascospores; perithecia often misshapen or poorly developed.

and Harrington 2005; Harrington and McNew 1997, 1998). Some pairings between testers also resulted in sterile perithecia that lacked ascospores.

Four host cross-inoculations.—The analysis of variance for the four-host inoculation experiment revealed a significant effect on linear extent of discoloration for each of the main factors, with the host plant showing the greatest effect ($F = 35.92$; $P < 0.0001$). Experiment (within host) was the second largest source of error ($F = 27.98$; $P < 0.0001$). There was also a significant host × source (of isolate) interaction ($F = 21.67$; $P < 0.0001$). Consequently xylem discoloration then was analyzed separately for each host and each experiment.

Isolates of the aspen and hickory lineage caused dramatically more discoloration (FIG. 4) on *P. tremuloides* and *C. illinoensis*, respectively, than did isolates from the other lineages. Hickory isolate C682 caused no more discoloration than the controls in both *Populus* experiments and was suspected to have deteriorated and lost pathogenicity. Thus hickory isolate C684 was substituted for C682 in the inoculation of other hosts.

Less evidence for host specialization was seen in the inoculations of *Q. rubra* and *Prunus serotina*. In the first *Quercus* inoculation, isolates from the oak and

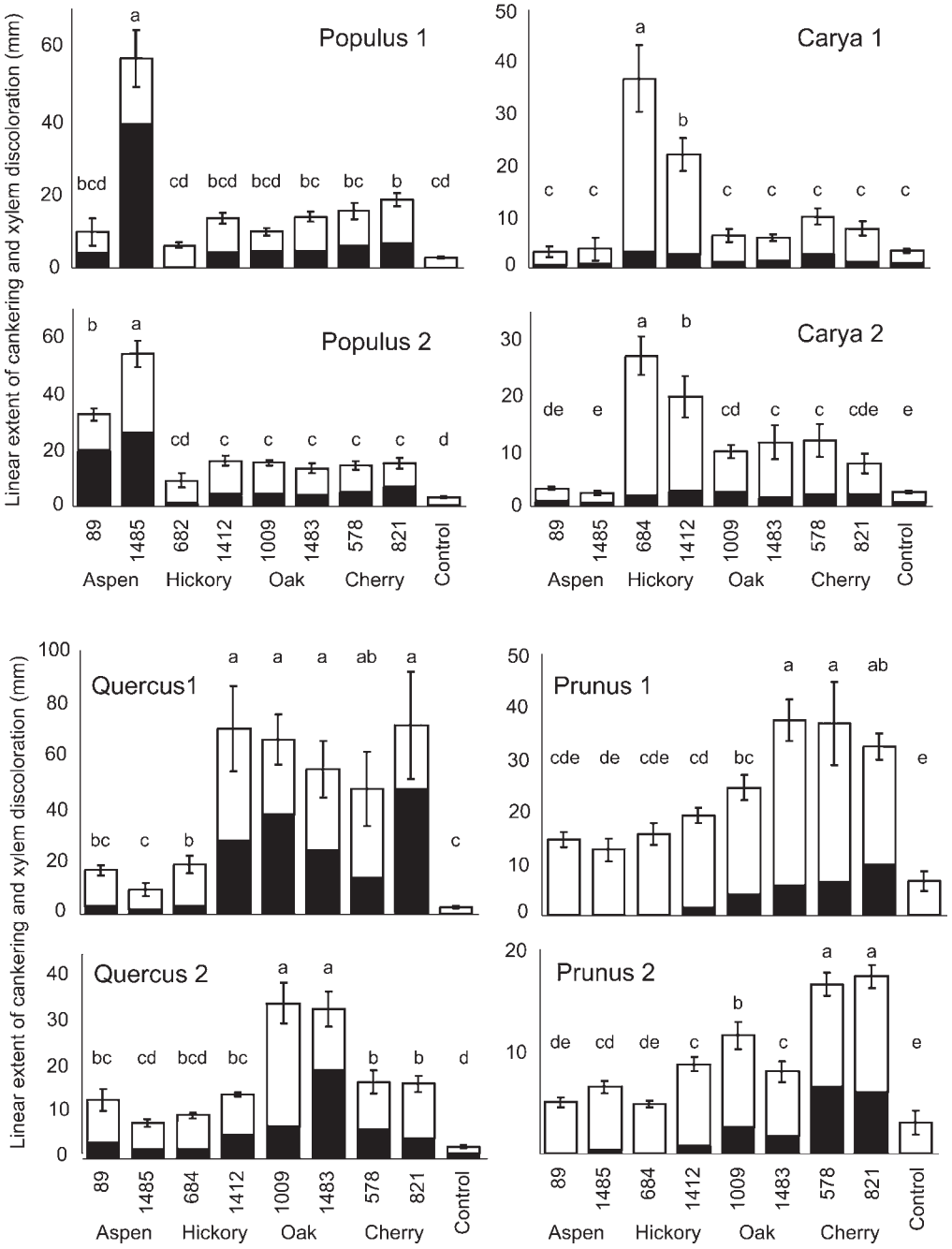


FIG. 4. Average length of cankers (black bars) and xylem discoloration (open bars) caused by *C. fimbriata* isolates at 30–35 d after inoculation into *Populus tremuloides*, *Carya illinoensis*, *Quercus rubra* and *Prunus serotina* plants. Bars are means for six replicates. Error bars represent standard error for xylem discoloration. Bars for xylem discoloration within a graph sharing the same letter are not significantly different based on Duncan's multiple-range test ($P = 0.05$).

cherry lineages and isolate C1412 from hickory caused significantly more discoloration than did the controls and the other isolates (FIG. 4). When the experiment was repeated, however, isolates of the oak lineage caused significantly more discoloration in *Quercus* than did the controls and the isolates from the other hosts, and the cherry isolates caused more discoloration than did isolates from aspen or hickory. Similarly, when *P. serotina* seedlings were inoculated, isolates of the oak lineage caused discoloration similar to that caused by isolates from the cherry lineage in one experiment, but only the cherry isolates caused substantial discoloration in *P. serotina* in a second experiment. In both experiments on *P.*

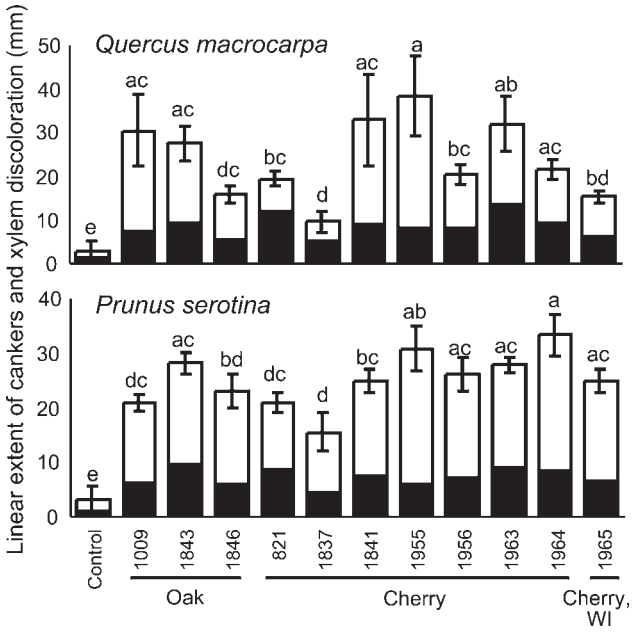


FIG. 5. Average length of cankers (black bars) and xylem discoloration (open bars) in *Quercus macrocarpa* and *Prunus serotina* plants inoculated with *C. fimbriata* isolates of the oak, cherry and cherry WI lineages. Errors bars represent standard error of xylem discoloration. Bars sharing the same letter on a given host are not significantly different based on Duncan's multiple-range test ($P = 0.05$) of xylem discoloration.

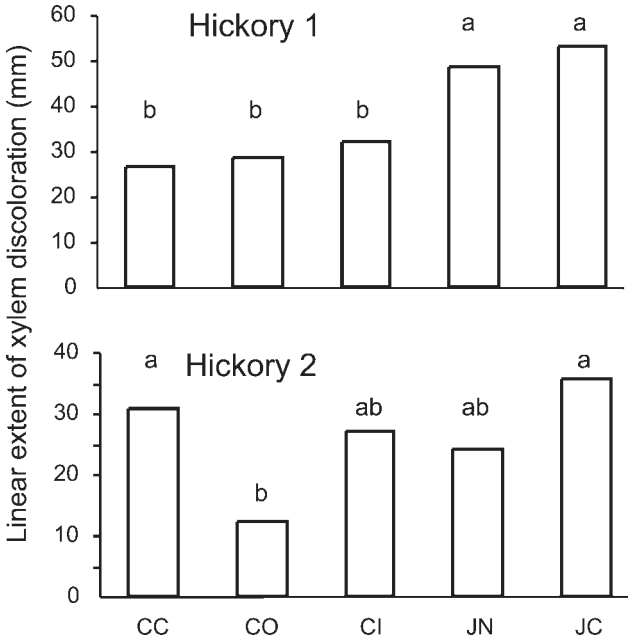


FIG. 6. Average length of xylem discoloration in three *Carya* species and two species of *Juglans* inoculated with four hickory-type isolates of *C. fimbriata*. Bars sharing the same letter in a given experiment are not significantly different based on Duncan's multiple-range test. CC = *Carya cordiformis*; CO = *Carya ovata*; CI = *Carya illinoensis*; JN = *Juglans nigra*; JC = *Juglans cineria*.

serotina isolates from the aspen and hickory lineages induced significantly less discoloration when compared to isolates from the cherry lineage.

Prunus virginiana and Quercus macrocarpa cross-inoculations.—Because of the ambiguous results seen when inoculating *Q. rubra* and *P. serotina*, another experiment was performed in which *Q. macrocarpa* and *P. virginiana* were inoculated with isolates from the oak and cherry lineages. For each host inoculations with all isolates resulted in significantly greater discoloration than was seen in plants that received control inoculations (FIG. 5). Significant differences among the isolates also were seen when the controls were excluded from the analysis (*Q. macrocarpa*: $F = 2.51$, $P = 0.0105$; *P. virginiana*: $F = 3.27$, $P = 0.0012$). However there was no evidence of host specialization, as isolates from each lineage produced similar amounts of discoloration in each host (FIG. 5).

Host range of hickory isolates.—When *Carya* spp. and *Juglans* spp. were inoculated with isolates from the hickory lineage, the ANOVA indicated that experiment, host and the host × experiment interaction all contributed significantly to the variation in response; most of the variation was due to the influence of experiment ($F = 13.12$, $P = 0.0004$), and host was the next largest factor ($F = 6.95$, $P < 0.0001$). The isolates, two of the hickory A sublineage and two of the hickory B sublineage, were not a significant source of variation in the two experiments, nor was there any isolate × host interaction. Because of the great differences in response between the two experiments, they were analyzed separately with one-way ANOVA. In the first experiment (FIG. 6) the two *Juglans* species had significantly more discoloration than the three *Carya* species. In the second experiment more discoloration was seen in *J. cineria* than in any of the other hosts, but the amount of discoloration was not significantly different than that seen in *J. nigra*, *C. cordiformis*, or *C. illinoensis* (FIG. 6).

Host range of aspen isolates.—Because the number of hosts in the two experiments differed, each experiment was analyzed separately. Most of the variation in the first experiment was explained by host species ($F = 56.83$, $P < 0.0001$), followed by isolate ($F = 15.42$, $P < 0.0001$). However there was no interaction between host and isolate ($F = 0.97$, $P = 0.4755$). The ANOVA for the second

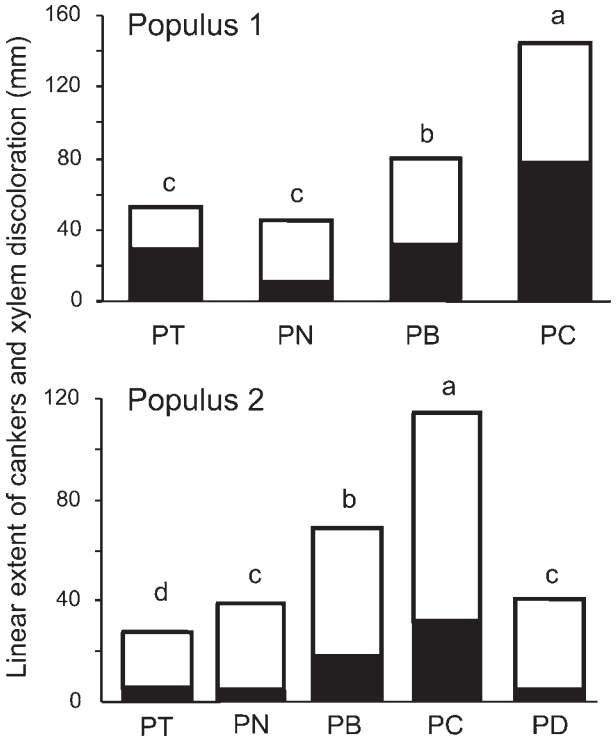


FIG. 7. Average length of cankers (black bars) and xylem discoloration (open bars) in five *Populus* species inoculated in two experiments with four aspen-type isolates *C. fimbriata* at 40 d after inoculation. Bars sharing the same letter within an experiment are not significantly different based on Duncan's multiple-range test of xylem discoloration. PT = *Populus tremuloides*; PB = *Populus balsamifera*; PC = *Populus trichocarpa*; PD = *Populus deltoides*.

experiment was similar to that of the first, with host species responsible for most of the variation ($F = 42.19$, $P < 0.0001$). In experiment 2, isolate was not a significant factor ($F = 0.25$, $P = 0.8644$) but there was a slight isolate × host interaction ($F = 2.05$, $P < 0.0299$). In the first experiment (FIG. 7) *Populus trichocarpa* was the most susceptible host, followed by *P. balsamifera*; *P. tremuloides* and *P. nigra* were less susceptible. The trend was similar in the second experiment, with significantly more discoloration in *P. trichocarpa* and *P. balsamifera* than in other hosts.

Morphology.—A wide range of perithecial sizes was seen in the isolates measured. In general isolates from sweet potato (*C. fimbriata sensu stricto*) produced smaller perithecial bases than isolates from the North American clade, but perithecia of isolates from *Platanus* spp. (*C. platani*) were comparable in size to isolates of the North American clade. Most perithecia of isolates from the North American clade produced a distinct collar at the point where the perithecial neck emerges from the base (FIG. 8), but this structure was absent in isolates of *C. fimbriata* ss and *C. platani*.

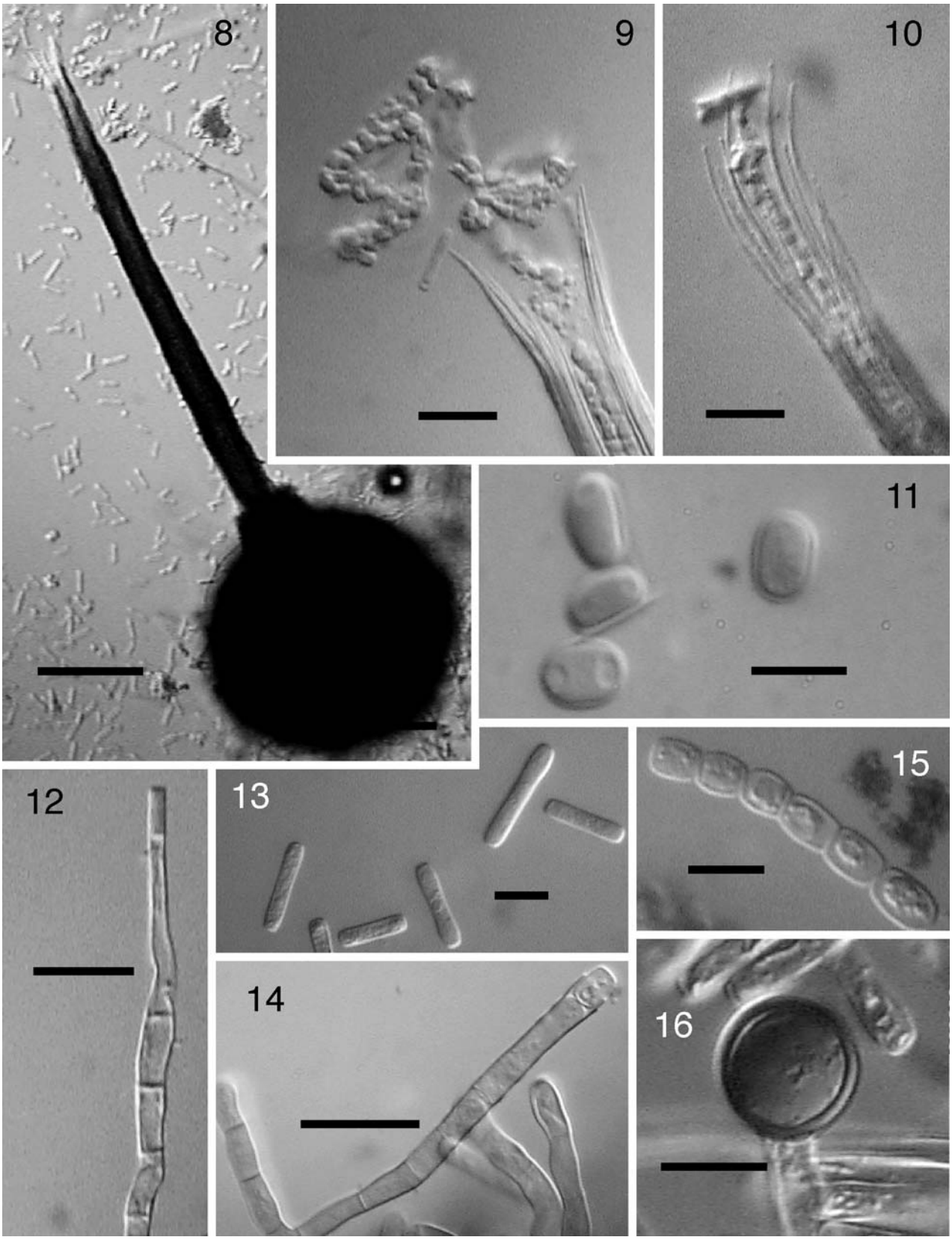
Davidson (1944) and Hunt (1956) reported that the ostiolar hyphae of *Ceratocystis variospora* are shorter than those of *C. fimbriata* isolates, and we found that the type specimen of *C. variospora* and isolates of the oak lineage had ostiolar hyphae considerably shorter than those of isolates from the Latin American clade (TABLE V). Isolates of the cherry lineage and the cherry-WI isolate also had relatively short ostiolar hyphae, although only the cherry-WI isolate had ostiolar hyphae that were consistently as short as those seen in isolates of the oak genotype.

Ascospores from North American isolates were 3.5–6.5 µm long and 3.0–5.0 µm wide (TABLE V), slightly smaller than the range found in *C. fimbriata* ss and *C. platani* (5.5–7.0 × 3.5–5.5 µm). Hunt (1956) reported ascospores 4–6 × 2.5–3.5 µm in *C. variospora* and 4.5–8 × 2.5–5.5 µm for *C. fimbriata*.

As is typical for *Ceratocystis* spp. the studied isolates produced two or three anamorphs, which are accommodated in the genus *Thielaviopsis* (Paulin-Mahady et al 2002). Flask-shape phialides (and the endoconidia produced from them) of isolates in the North American clade were similar in dimension to those reported by Hunt (1956) and Webster and Butler (1967) for *C. fimbriata* and *C. variospora*. Isolates from the hickory A sublineage conspicuously lacked flask-shape phialides (TABLE V).

All isolates in the North American clade produced a second endoconidial stage with doliiform conidia from wide-mouth phialides, but within the Latin American clade only isolates from *Platanus* produced these structures (TABLE V). This second type of endoconidiophore often was found clustered around the bases of perithecia produced in culture and in samples of naturally colonized plant tissue. Wide-mouth phialides were generally shorter (12–65 µm) than flask-shape phialides, similar to earlier reports (Webster and Butler 1967). Doliiform conidia were 4.5–19.5 µm long × 3.5 to 9.5 µm wide and often were found in long chains. Webster and Butler (1967) reported that doliiform conidia ''are at first hyaline, becoming subhyaline to light brown with age''; however we observed a change in the color of doliiform conidia only among isolates from the aspen lineage. Doliiform conidia from the aspen lineage frequently expanded in size after emerging from their phialides and developed into thick-walled, melanized chlamydospores.

Aleurioconidia were 8.5–26 µm long × 6.5–17.5 µm wide, were produced blastically and accumulated in chains. No obvious differences were seen in the size of aleurioconidia among isolates of the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various lineages, but isolates from the hickory A sublineage did not produce aleurioconidia.

These differences in morphology are incorporated into the emended descriptions of *C. variospora* and the newly recognized taxa in the North American clade.

TAXONOMY

Ceratocystis variospora (Davids.) C. Moreau, Reveu de Mycologie, Suppl. Colonial 17:22. 1952. FIGS. 8–16

≡ *Endoconidiophora variospora* Davids., Mycologia 36:303. 1944.

≡ *Ophiostoma variosporum* (Davids.) Arx, Antonie van Leeuwenhoek 18:212. 1952.

EMENDED DESCRIPTION: Cultures on malt yeast-extract agar hyaline at first with a fluffy appearance, becoming brown, gray or olive-green after 2–4 days, undersurface of agar turning dark; radial growth 18 mm at 5 d; odor sweet, often with banana scent. Hyphae hyaline to pale brown, often terminating as endoconidiophores. Perithecia (FIG. 8) with bases superficial to partially immersed, bases black or rarely brown, globose, 130–350(425) µm diam, unornamented or with undifferentiated hyphae attached; possessing a collar at the base of the neck 51–80 µm wide; necks black, slender, up to 830 µm long, 25–50 µm diam at base and 12- µm at the hyaline tip; ostiolar hyphae (FIGS. 9, 10) hyaline, 10–20 in number, 1–2 µm wide (Hunt), 22–50 µm long, tapering to a blunt tip; asci not seen; ascospores (FIG. 11) with outer cell wall forming a brim, hat-shaped, 3.5–6.0 × 3.0–5.0 µm. Endoconidiophores of two types; one flask-shape, hyaline to light brown, septate with conidiophores 52–198 µm long, conidiogenous cell 37–66 µm long, width 4.5–7.0 µm at base and 2.5–4.5 µm at the mouth; producing hyaline endoconidia (FIGS. 12, 13) 6.0–30.0 × 2.5–5.0 µm; the other endoconidiophores shorter, 32–90 µm long, not tapering, often flared at mouth, conidiogenous cell 16–38 µm long, width (3.0) 4.0–5.5 µm at base and 4.5–7.5 µm at mouth; producing doliiform endoconidia (FIGS. 14, 15), hyaline 5.5–10.0 × 5.0–8.0 µm. Aleurioconidia (FIG. 16) produced blastically, singly or in chains, orange-brown to brown, ovoid or obpyriform, smooth, 9.0–6.5 × 7.5–14.0 µm.

SPECIMEN EXAMINED: HOLOTYPE: USA. WEST VIRGINIA: Moorefield, from cambium side of *Quercus prinus* bark, May 1943, *M.E. Fowler*, BPI 595631.

CULTURES EXAMINED: USA. MINNESOTA: Ramsey County, from sapwood of *Q. ellipsoidalis* stumps cut 2–3 wk previously, 1955 or 1956, *R. Campbell*, isolate C1009 (= CBS 773.73, ATCC 12861). Ramsey County, from sapwood of *Q. ellipsoidalis* stumps cut 2–3 wk previously, 1955 or 1956, *R. Campbell*, from isolate C1483 (= ATCC 12866). IOWA: Harper's Ferry, from wound on *Q. alba* stem, Jul 2001, *J.A. Johnson*, isolate C1843 (= CBS 114715, BPI 843737). IOWA: Rhodes, from bleeding canker on *Q. robur*, Sep 2001, *J.A. Johnson*, isolate C1846 (= CBS 114714, BPI 843738).

Comments: This species is similar to *Ceratocystis fimbriata sensu stricto* (the sweet potato pathogen) but differs in the production of doliiform conidia from wide-mouthed phialides, and it differs from *C. fimbriata*, *C. cacaofunesta* and *C. platani* in its shorter ostiolar hyphae and slightly smaller ascospores. The presence of a distinct collar at base of perithecial necks distinguishes *C. variospora* from *C. fimbriata*, *C. cacaofunesta*, *C. platani*, *C. albifundus*, and *C. polychroma*. Cultures of the recently described *C. pirilliformis* from Australia were not available at the time of study, but the description by Barnes et al (2003) includes the presence of a collar at the base of the perithecial necks, as in *C. variospora*. Dimensions of ostiolar hyphae were not given for *C. pirilliformis*, but the ostiolar hyphae illustrated were up to 60 µm long (Barnes et al 2003), longer than those observed in isolates of *C. variospora*. Although *C. variospora* and *C. pirilliformis* are morphologically similar, the ITS sequences of isolates of *C. pirilliformis* are distinct from those of *C. variospora* (Thorpe et al 2005). *Ceratocystis variospora* differs from *C. albifundus* and *C. moniliformis* in the production of aleurioconidia and from *C. moniliformis* in the absence of ornamentation on the perithecial bases.

Ceratocystis variospora was described by Davidson based on fruiting structures found on the inner bark of chestnut oak (*Quercus prinus*) in West Virginia 1 wk after the bark was removed from a living tree (Davidson 1944). It also has been collected from *Q. ellipsodalis* stumps in Minnesota (Campbell 1957), from a wound on *Q. alba* in Iowa and from a bleeding canker on *Q. robur*, also in Iowa. Isolates from

←

FIGS. 8–16. *Ceratocystis variospora*. 8. Perithecium. 9, 10. Ostiolar hyphae and emerging ascospores. 11. Ascospores. 12. Flask-shape endoconidiophore producing cylindrical endoconidium. 13. Cylindrical endoconidia. 14. Wide-mouth endoconidiophore with emerging doliiform endoconidium. 15. Doliiform endoconidia in a chain. 16. Aleurioconidium. All features from isolate C1009 except FIG. 10, which was from isolate C1822. Bars: 8 = 100 µm; 9, 10,12, 14 = 20 µm; 11 = 5 µm; 13, 15, 16 = 10 µm.

TABLE V. Diagnostic features of *Ceratocystis fimbriata*, *C. platani*, and the taxa recognized within the North American clade of the *Ceratocystis fimbriata* complex

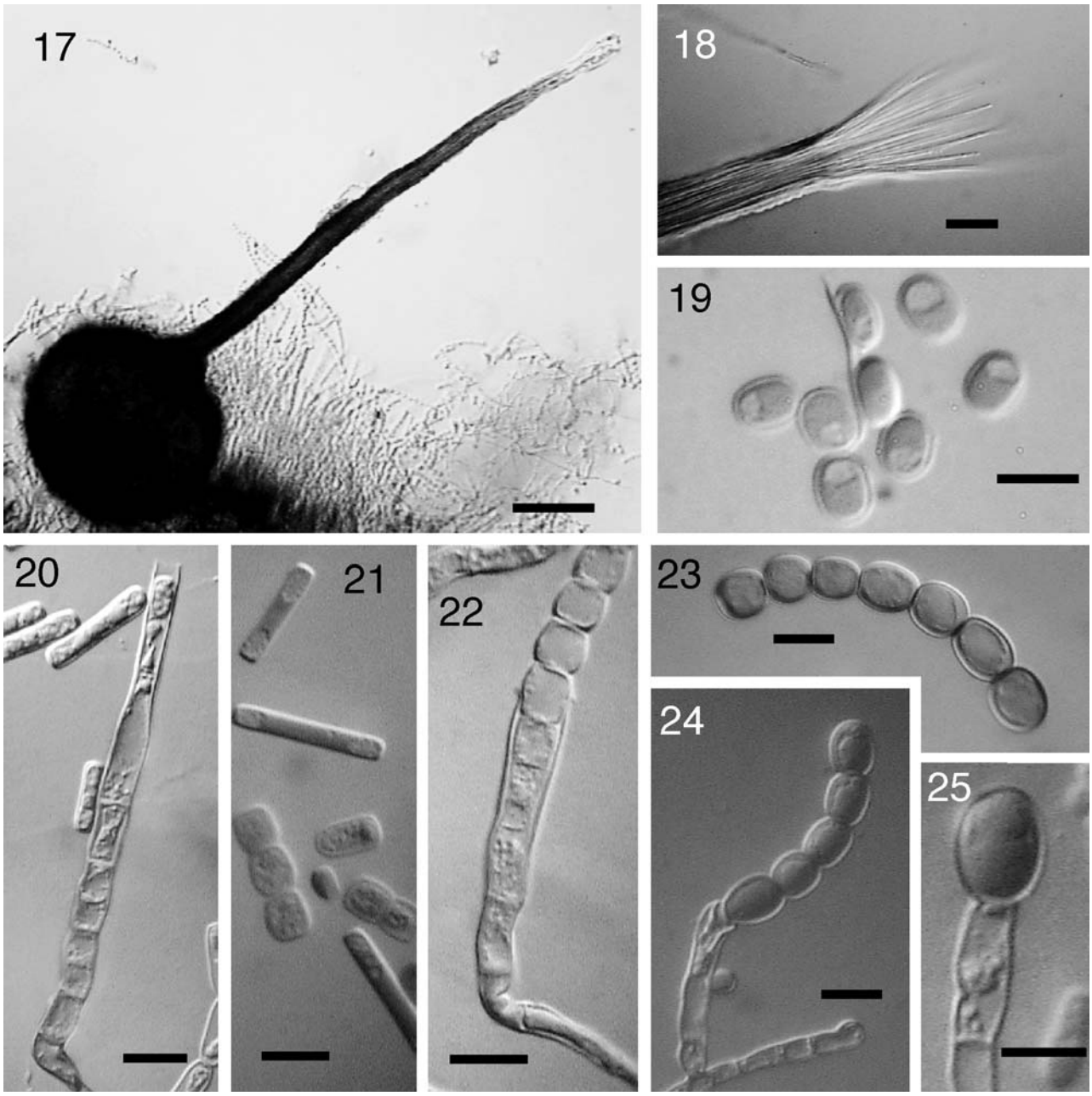
Taxon	Hosts/ Insects	Collar at base of perithecial neck	Ascospores (µm)	Length of ostiolar hyphae (µm)	Flask-shaped phialides and cylindrical conidia	Wide-mouthed phialides and doliform conidia	Melanized doliform conidia (chlamydospores)	Aleurioconidia
C. fimbriata sensu stricto	*Ipomoea batatas*	No	5.5–7.0 × 3.5–5.0	53–136	Present	Absent	Absent	Present
C. platani	*Platanus* spp.	No	5.5–6.5 × 4.0–5.0	57–77	Present	Present	Absent	Present
C. variospora oak lineage	*Quercus* spp.	Yes	3.5–6.0 × 3.0–5.0	22–50	Present	Present	Absent	Present
C. variospora cherry lineage	Various hardwoods	Yes	4.0–6.0 × 3.0–5.0	21–87	Present	Present	Absent	Present
C. populicola	*Populus* spp.	Yes	4.5–6.5 × 3.0–5.0	42–75	Present	Present	Present	Present
C. caryae	Primarily *Carya* spp.	Yes	4.0–6.0 × 3.5–4.5	32–79	Present	Present	Absent	Present
C. smalleyi	*Carya* spp./ *Scolytus quadrispinosus*	Yes	4.0–6.0 × 3.5–5.0	54–101	Absent	Present	Absent	Absent

Minnesota and Iowa had similar ITS sequences and were sexually interfertile, and the ITS sequence amplified from DNA extracted from the holotype specimen of *C. variospora* was also similar. A morphologically similar isolate (C1709) from a sporulating mat on a log of *Betula platyphylla* in Japan has a similar ITS sequence, but the Japanese isolate is not interfertile with the oak isolates from North America. Other isolates from wounds of various hardwoods in Iowa and a *Prunus* sp. in Wisconsin were morphologically indistinguishable from the *Quercus* isolates of *C. variospora*, but they differed in ITS sequence and allozyme electromorphs and the *Quercus* isolates were in a separate intersterility group. Most of these isolates from hosts other than *Quercus* formed ostiolar hyphae longer than 50 µm, longer than those found in the *Quercus* lineage of *C. variospora*, but no ostiolar hyphae longer than 50 µm were seen in the *Prunus* isolates C1822 and C 1841. Also host-specialization of isolates to *Quercus* spp. or *Prunus* spp. could not be demonstrated clearly. For the present, all these isolates are considered *C. variospora*, but the emended description of *C. variospora* is based solely on the *Quercus* isolates.

Ceratocystis populicola J. A. Johnson and Harrington, sp. nov. FIGS. 17–25

Culturae glycosmae, saepe bananae similes. Perithecia basibus atris, globosa, 110–275 µm diam, collari basim colli circumdante; collum usque ad 665 µm longum, diametro ad basim 24–45 µm et ad apicem 13–30 µm; hyphae ostioli hyalinae, 42–75 µm longae. Ascosporae 4.5–6.5 × 3.0–5.0 µm. Endoconidiophora hyalina ad fusca, formis duabus; forma prima cellulaconidiogena ampulliformi apicem versus angustata, endoconidiis cylindricis 10–33 × 2.0–5.0(5.5) µm; altera forma: cellula conidiogena breviore, saepeapicem versus dilatata. Endoconidiis doliiformibus, primo hyalinis, 6.5–12.0 × 3.5–8.5 µm, saepe tumidescentibus et fuscescentibus, crassitunicatis, 8.0–13.5 × 6.0–10.5 µm. Aleurioconidia singula vel catenata, cinnamomea vel brunnea, ovoidea vel pyriformia, levia, 9.0–18.5 × 8.0–17.5 µm.

Cultures on malt yeast agar hyaline to white initially, becoming darker, and turning brown or olive-green after 2–4 d, radial growth 17–21 mm at 5 d; cultures smell sweet or of banana oil. Perithecia on MYEA fully formed after 4–6 d, scattered on surface of agar or with bases partially submerged. Perithecia (FIG. 17) with black bases, globose, 110–275 µm diam; unornamented or with undifferentiated hyphae attached, possessing a collar at the base of neck, necks black, emerging from collars, hyaline at tip, slender, up to 665 µm long, 24–45 µm diam at



FIGS. 17–25. *Ceratocystis populicola*. 17. Perithecium. 18. Ostiolar hyphae. 19. Ascospores. 20. Flask-shaped endoconidiophore and cylindrical endoconidia. 21. Cylindrical and doliiform endoconidia. 22. Wide-mouth endoconidiophore with emerging doliiform endoconidia. 23. melanized doliiform endoconidia, most mature conidium at right. 24. Melanized doliiform endoconidia attached to wide-mouth endoconidiophore. 25. Aleurioconidium. All features from isolate C685. Bars: 17 = 100 µm; 18, 20, 22 = 20 µm; 19 = 5 µm; 21, 23, 24, 25 = 10 µm.

base and 13–30 µm at hyaline tip; ostiolar hyphae hyaline, slender, tapered to a blunt tip, 42–75 µm long (FIG. 18). Asci not seen; ascospores (FIG. 19) with outer cell wall forming a brim, hat-shape, 4.5–6.5 × 3.0–5.0 µm. Endoconidiophores of two types; one flask-shaped, hyaline to light brown, septate with conidiophores 45–200 µm long, conidiogenous cell 35–85 µm long, width 3.5–7.0 µm at base and 3.5–4.5 µm at mouth; producing hyaline endoconidia 10–33 × 2.0–5.0 (5.5) µm (FIGS. 20, 21); the other endoconidiophores shorter, not tapering, often flared at mouth; often produced in masses around perithecial bases (FIG. 17); conidiophores 17–95(125) µm long, conidiogenous cell 12–40 µm long; width 3.5–6.0 µm at base and 3.5–8.5 µm at tip of conidiogenous cell; producing doliiform endoconidia,

hyaline at first, 6.5–12.0 × 3.5–.5 μm (FIGS. 22), often becoming swollen and melanized with thick walls (FIGS. 23, 24), 8.0–13.5 × 6.0–10.5 μm. Aleurioconidia (FIG. 25) produced blastically, singly or in chains, orange-brown to brown, ovoid or pyriform, smooth, 9.0–18.5 × 8.0–17.5 μm.

HOLOTYPE: CANADA. QUEBEC: from *Populus tremuloides*, *E. Smalley*, BPI 843723, from isolate C685 (= CBS 115161).

CULTURES EXAMINED: CANADA. QUEBEC: from *Populus tremuloides*, *E. Smalley*, isolate C685 (= CBS 115161). USA. SOUTH DAKOTA: Black Hills, from *P. tremuloides*, 1980, *T.E. Hinds*, isolate C89 (= CO 301, = CBS 114725). COLORADO: from *P. tremuloides, T.E. Hinds*, isolate C1485 (= ATCC 24096). POLAND. KÓRNIK: from canker on *Populus* hybrid, Aug 1976, *J. Gremmen*, isolate C947 (= ATCC 36291). from canker on *Populus* hybrid, Aug 1976, *J. Gremmen*, isolate C995 (= CBS 119.78).

Etymology. *populicola*, Latin = on *Populus*.

Comments: This species is similar to *C. fimbriata ss* but differs in the production of doliiform conidia from wide-mouth phialides. The distinct collar at the base of perithecial neck distinguishes *C. populicola* from *C. fimbriata*, *C. cacaofunesta*, *C. platani, C. polychroma* and *C. albifundus*. *C. populicola* differs from *C. variospora* and *C. pirilliformis* in the production of chains of swollen, melanized chlamydospores from wide-mouth phialides. *Ceratocystis populicola* differs from *C. albifundus* and *C. moniliformis* in the production of aleurioconidia and from *C. moniliformis* in the absence of ornamentation on the perithecial bases.

In our inoculations only isolates of *C. populicola* were capable of causing disease in *Populus* spp. Distinctive, target-shape cankers caused by *C. fimbriata* have been noted on *P. tremuloides* in Minnesota (Manion and French 1967, Wood and French 1963), Pennsylvania, much of the western USA, including Alaska (Hinds 1972, Hinds and Laurent 1978) and Quebec, Manitoba and Saskatchewan in Canada (Zalasky 1965). All these reports are believed to be of *C. populicola*. It is likely that the pathogen is present wherever *Populus tremuloides* naturally occurs. Hybrid poplars were found infected at plantations in Poland (Gremmen and de Kam 1977, Przybyl 1984b), and isolates from these plantations are *C. populicola*. An additional report from Quebec (Vujanovic 1999) describes *C. fimbriata* infecting rooted cuttings of *P. balsamifera*, a host found susceptible to *C. populicola* in our inoculations.

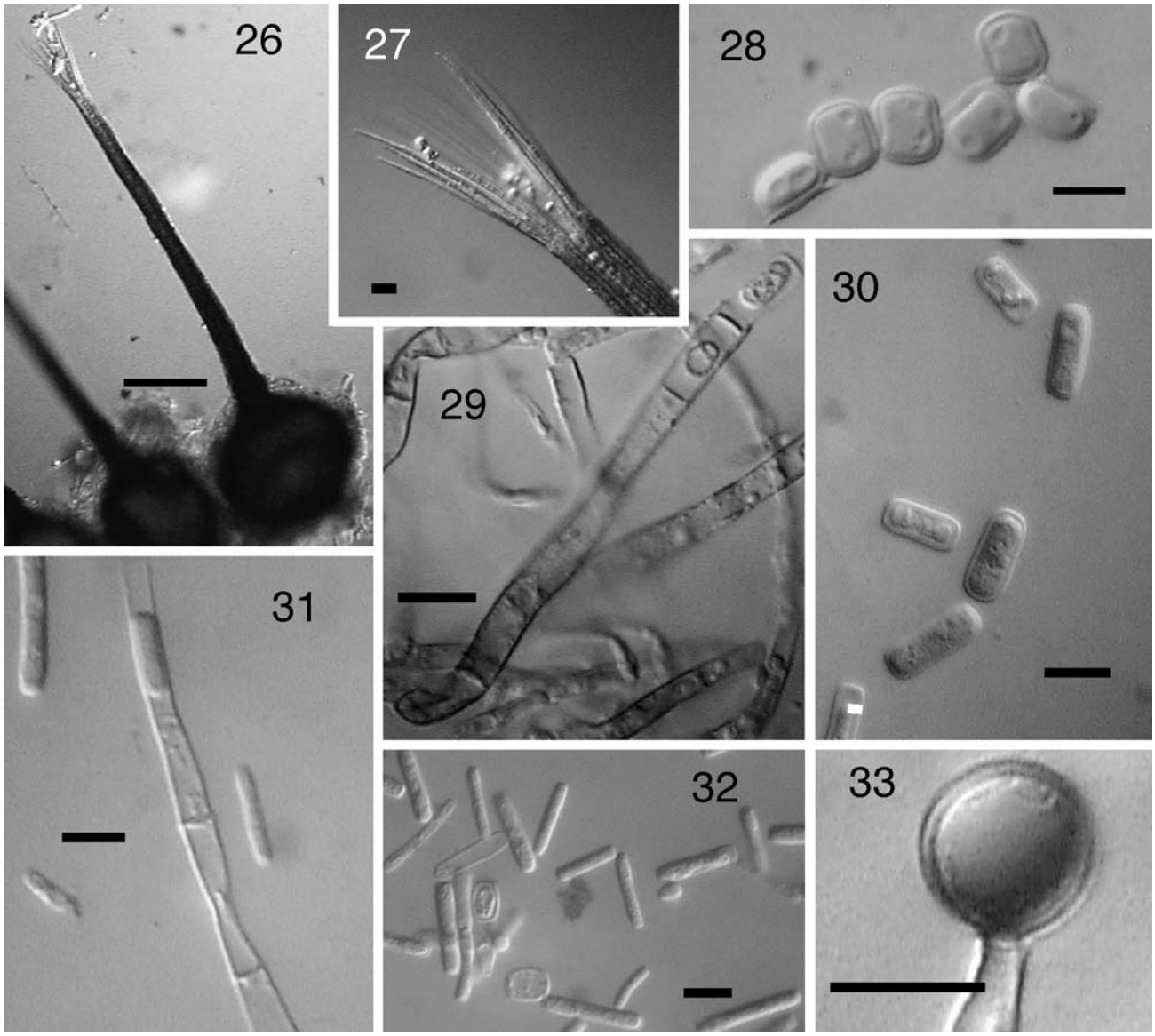
Ceratocystis caryae J.A. Johnson and Harrington, sp. nov. FIGS. 31–33

Culturae glycosmae, saepe bananae similes. Perithecia basibus atris, globosa, 135–340 μm diam, aliquando collari basim colli circumdante; collum atrum, gracile, usque ad 950 μm longum, diametro ad basim 25–52 μm et ad apicem 15–30 μm; hyphae ostioli hyalinae, gracile, 32–80 μm longae. Ascosporae 4.0–6.0 × 3.5–4.5 μm. Endocondidiophora hyalina ad fusca, formis duabus; forma prima cellulaconidiogena ampulliformi apicem versus angustata, endoconidiis cylindricis 8.5–27.0(43.0) × 2.5–6.0 μm; altera forma: cellula conidiogena breviore, saepeapicem versus dilatata. Endoconidiis doliiformibus, hyalinis, 6.0–13.5(16.0) × 5.5–9.5 μm. Aleurioconidia singula vel catenata, cinnamomea vel brunnea, ovoidea vel pyriformia, levia, 9.0–21.5 × 8.5–16.5 μm.

Cultures on malt yeast agar hyaline to white initially, becoming darker, and turning brown, gray or olive-green after 2–4 d, culture texture varying from fluffy to felty, undersurface of agar turning dark. Cultures with a sweet scent, often smelling like banana oil. Perithecia on MYEA fully formed after 4–6 d; perithecia scattered or clumped on surface of agar or with bases partially submerged. Perithecia with bases black, globose or broadly obpyriform, 135–340 μm diam; unornamented or with undifferentiated hyphae attached; occasionally with collar at apex 48–103 μm wide; necks black, tapering to a hyaline tip, up to 950 μm long, 25–52 μm diam at base and 15–30 μm at tip; ostiolar hyphae hyaline, slender, tapered to a blunt tip, 32–80 μm long. Asci not seen; ascospores with outer cell wall forming a brim, hat-shape, 4.0–6.0 × 3.5–4.5 μm. Endoconidiophores of two types; one flask-shaped, hyaline to light brown, septate with conidiophores 42–510 μm long, conidiogenous cell 33–80 μm long, width 3.8–7.5 μm at base and 3.2–4.8 μm at the mouth; producing hyaline endoconidia 8.5–27.0(43.0) × 2.5–6.0 μm (FIGS. 31, 32); the other endoconidiophores shorter, not tapering, often flared at mouth; often produced in masses around perithecial bases, conidiophores 40–100 μm long, conidiogenous cell 15–55 μm long; width 5.0–6.5(7.0) μm at base and 5.5–8.0 μm at tip of conidiogenous cell; producing hyaline doliiform endoconidia, 6.0–13.5(16.0) × 5.5–9.5 μm. Aleurioconidia produced blastically, singly or in chains, orange-brown to brown, ovoid or pyriform, smooth, 9.0–21.5 × 8.5–16.5 μm (FIG. 33).

HOLOTYPE. USA. IOWA: Coggan, from *Carya cordiformis* (Wangenh.) K. Koch, Aug 2001, *J.A. Johnson*, BPI 843735, from isolate C1829 (= CBS 114716).

CULTURES EXAMINED: USA. IOWA: Coggan, from *Carya cordiformis*, Aug 2001, *J.A. Johnson*, isolate C1829 (= CBS 114716). Clayton County, from *C. cordifomis*, Sep 1998, *T.C. Harrington*, isolate C1412



FIGS. 26–33. *Ceratocystis smalleyi and C. caryae*. 26. Perithecium. 27. Ostiolar hyphae. 28. Ascospores. 29. Wide-mouth endoconidiophore. 30. Doliiform endoconidia. 31. Flask-shape endoconidiophore. 32. Cylindrical endoconidia. 33. Aleurioconidia. 26–30 from isolate C684 from the holotype of *C. smalleyi*; 31–33 from C1829, the holotype of *C. caryae*. Bars: 26 = 100 µm; 27, 29, 30 = 20 µm; 28 = 5 µm; 31, 32, 33 = 10 µm.

(= BPI 843728). Clayton County, from *C. cordifomis*, Sep 1998, *T.C. Harrington*, isolate C1413. Boone County, from *C. ovata*, Jun 2001, *J.A. Johnson*, isolate C1827 (= CBS 115168). Boone County, from *C. cordiformis*, Jul 2001, *J. A. Johnson*, isolate C1845. Ames, from *Ostrya virginiana*, Aug 2002, *J.A. Johnson*, isolate C1971.

Etymology. *caryae*, Latin = on *Carya*.

Comments: This species is morphologically similar to *C. variospora* but differs in the length of the ostiolar hyphae. *C. caryae* differs from *C. fimbriata ss* in the production of doliiform conidia from wide-mouthed phialides, and from *C. fimbriata*, *C. cacaofunesta*, *C. platani*, *C. polychroma* and *C. albifundus* in the presence of a collar subtending the perithecial neck. The doliiform conidia and aleurioconidia of *C. caryae* are larger than those reported for *C. pirilliformis* (Barnes et al 2003). *C. caryae* differs from *C. moniliformis* in the absence of ornamentation on the perithecial bases. *C. caryae* lacks the melanized doliiform conidia seen in *C. populicola*. All isolates of *C. caryae sensu stricto* have been recovered from *Carya* spp., *Ulmus* spp. or *Ostrya virginiana*.

Ceratocystis smalleyi J.A. Johnson and Harrington, sp. nov. FIGS. 26–30

Culturae odore dulci bananae carentes. Perithecia basibus atris, globosa, 100–300 µm diam, aliquando collari basim colli circumdante; collum atrum, gracile, usque ad 570 µm longum, diametro ad basim 22–80 µm et ad apicem 15–40 µm; hyphae ostioli hyalinae, graciles, 55–100 µm longae. Ascosporae 4.0–6.0 × 3.5–5.0 µm. Endocondidiophora hyalina ad fusca, uniformia, brevia, cellulaconidiogena saepe dilatati versus apicem, endoconidiis doliiformibus, hyalinis, 7.5–13.5(16.0) × 5.5–9.5 µm. Aleurioconidia non visa.

Cultures on malt yeast agar hyaline to white initially, becoming darker and turning brown, gray or olive-green after 2–4 d, often with lighter colored gray to white patches, undersurface of agar turning dark, many isolates sectoring readily. Radial growth 21 mm at 5 d; cultures may have a sweet scent, but the banana odor typical of *C. caryae* is absent. Perithecia on MYEA fully formed after 4–6 d, often fruiting in concentric rings; perithecia on surface or with bases partially submerged. Perithecia (FIG. 26) with bases black, globose or broadly obpyriform, 100–300(350) µm diam; unornamented or with undifferentiated hyphae attached; occasionally with collar at apex 42–73(85) µm wide; necks black, tapering to a hyaline tip, up to 570 µm long, 22–80 µm diam at base and 15–37 µm at tip; ostiolar hyphae (FIG. 27) hyaline, slender, tapered to blunt tip, 55–100 µm long. Asci not seen; ascospores (FIG. 28) with outer cell wall forming a brim, hat-shape, 4.0–6.0 × 3.5–5.0 µm. Endoconidiophores (FIG. 29) of one type, not tapering, often flared at mouth; commonly produced in masses around perithecial bases, conidiophores multicellular, 35–105 µm long, conidiogenous cell 22–65 µm long; width 4.0–6.0 µm at base and 4.0–7.5 µm at tip of conidiogenous cell; producing doliiform to cylindrical hyaline endoconidia (FIG. 30), 7.5–31.5 × 4.0–7.5 µm.

HOLOTYPE. USA. WISCONSIN: Hickory Ridge, from *Carya cordiformis*, 1993, *E. Smalley*, BPI 843722, from isolate C684 (= CBS 114724).

CULTURES EXAMINED: USA. WISCONSIN: Hickory Ridge, from *Carya cordiformis*, 1993, *E. Smalley*, isolate C684 (= CBS 114724). La Crosse, from *C. cordifomis*, 1986, *E. Smalley*, isolate C682. Evansville, from *C. ovata*, 1993, *E. Smalley*, isolate C683. IOWA: Clayton County, from *C. cordiformis*, Sep 1998, *T.C. Harrington*, isolate C1410. Clayton County, from *C. cordiformis*, Sep 1998, *T.C. Harrington*, isolate C1411. Coggan, from *C. cordiformis*, Aug 2001, *J.A. Johnson*, isolate C1828. Coggan, from *C. cordiformis*, Aug 2001, *J.A. Johnson*, isolate C1839. Coggan, from *C. cordiformis*, Aug 2001, *J.A. Johnson*, isolate C1840. Coggan, from *C. cordiformis*, Aug 2001, *J.A. Johnson*, isolate C1842. Coggan, from *C. cordiformis*, Aug 2001, *J.A. Johnson*, from isolate C1844. Cambria, from *C. cordiformis*, Aug 2002, *J. A. Johnson*, isolate C1952.

Etymology. *smalleyi*, named after the late Eugene Smalley, who associated this fungus with *Scolytus quadrispinosus* and brought the new taxon to our attention.

Comments: This species differs from *C. caryae* in the absence of cylindrical conidia from flask-shape phialides and in the absence of aleurioconidia. All isolates of *C. caryae* from wounded *Carya* spp. or *Ostrya virginiana* are closely related to *C. smalleyi* based on ITS sequence analysis and allozyme banding patterns; they behave similarly in inoculation tests, and they appear to be sexually interfertile. The isolates from wounds produce pink ascospore masses, while ascospore masses of *C. smalleyi* are white to cream. Perithecia of *C. smalleyi* do not consistently produce a distinct collar at base of perithecial necks, but such swellings can be seen in at least some perithecia of all isolates, as they can in perithecia of *C. caryae*, *C. variospora* and *C. populicola*. Eugene Smalley first isolated the fungus from a tree that had been attacked by the hickory bark beetle (*Scolytus quadrispinosus*), and he later made collections in association with the beetle from other locations in Wisconsin (pers comm). We later collected isolates from northeastern and south-central Iowa. Isolates have been made from hickory bark beetle egg galleries, from stained wood surrounding galleries and from discolored sapwood associated with beetle attacks from previous years.

DISCUSSION

Analyses of ITS-rDNA sequences and allozyme electromorphs showed a great deal of variation in the North American clade of *C. fimbriata* and point to the existence of four host-associated lineages. Although relationships among lineages were not well resolved, there was general agreement between the ITS and allozyme analyses in delimiting the host-associated lineages, as has been found in other studies of *Ceratocystis* species (Witthuhn et al 2000a). Pairings between mutant MAT-2 tester strains that had lost the ability to self and MAT-1 testers provided evidence of many biological species, but not all of these biological species are formally recognized in this study.

To delimit species under the phylogenetic species concept supported by Harrington and Rizzo (1999), a lineage should have a unique combination of phenotypic characters. The taxa in the North American clade can be distinguished from the Latin American clade of *C. fimbriata* by their slightly smaller

ascospores and the collar present at the base of the perithecial necks. The taxa within the North American clade are distinguished from each other by a number of minor morphological characters, presence or absence of conidial states and by host range. Inoculation experiments distinguished some of the host-associated lineages, with strong evidence for host specialization shown by isolates from the aspen and hickory lineages. Isolates from the oak and cherry lineages showed little to no evidence of host specialization, so these are retained as a single species.

The name *C. variospora* is available for the oak lineage. *Ceratocystis variospora* originally was reported in West Virginia on the inner bark of *Q. palustris* collected for tanning and later was collected in Minnesota from a fresh stump of *Q. ellipsoidalis* (Davidson 1944, Campbell 1957). The ITS sequence generated from the holotype specimen was similar to that of the Minnesota isolates and Iowa isolates from *Q. alba*, a native tree, and *Q. robur*, a European species. The isolate collected from *Q. alba* was recovered from a wound, while the isolate from *Q. robur* was isolated from a bleeding canker in a small experimental planting where many of the *Q. robur* trees showed severe cankering.

We also are applying the name *C. variospora* to the lineage containing isolates from wounds on *Prunus* and other hardwood species. The cherry and oak lineages could be separated based on differences in ITS sequences, allozymes and interfertility, but they could not be consistently distinguished through morphology or host specialization. Isolates from a *Tilia* tree were typical of the cherry lineage in ITS sequence, allozymes and morphology but testers from these isolates were able to mate only with themselves. Isolates from *Betula platyphylla* logs in Japan also were morphologically similar to USA isolates from the oak and cherry lineages of *C. variospora*, but they were intersterile with the USA isolates and with each other. The ITS sequence and allozyme electromorphs of the Wisconsin isolate from *Prunus* were unique, but this isolate is morphologically indistinguishable from *C. variospora* and behaved similarly in inoculations of *Quercus* and *Prunus*.

Previous observations of *C. fimbriata* in North America have focused on mortality of infected trees, but *C. variospora* appears to be more common and occur on more hosts as a relatively innocuous wound colonizer. The cherry lineage of *C. variospora* appears to be particularly common as a wound colonizer on a wide range of tree species in Iowa, although it may act as a tree-killing pathogen on almond and other exotic *Prunus* species in California (DeVay et al 1968, Moller et al 1969, Teviotdale and Harper 1991). Although no member of the *C. fimbriata* complex had been reported previously in Iowa, isolates of *C. variospora* were readily collected from wounds, especially wounds made early in the summer. New host records for the *C. fimbriata* complex include *Carya cordiformis*, *C. ovata*, *Celtis occidentalis*, *Ostrya virginiana*, *Prunus serotina*, *Populus grandidentata*, *Quercus alba*, *Q. robur*, *Q. rubra* and *Q. macrocarpa*. New host genera and families include *Carya* (Juglandaceae), *Celtis* (Ulmaceae) and *Ostrya* (Betulaceae). The two Japanese isolates from logs of *Betula platyphylla* (Betulaceae) provided by H. Matsuya also represent a new host record for the *C. fimbriata* complex.

Isolates of *C. populicola* and *C. caryae* mated only with their respective testers, but the male tester of *C. caryae* mated successfully with MAT-1 females of both *C. caryae* and *C. smalleyi*. We have been unable to obtain a MAT-2 tester of *C. smalleyi* to perform the reciprocal crosses. The ITS analysis failed to distinguish *C. caryae* and *C. smalleyi*, although there was some support from the allozyme analyses for separation of these new taxa. We since have analyzed sequences from portions of the elongation factor-1α and β-tubulin-1 genes for the North American clade, and *C. caryae* and *C. smalleyi* appear as two well-resolved sister species in both of those gene trees (unpublished data).

Inoculations of *Carya* and *Juglans* spp. showed that *C. caryae* and *C. smalleyi* have a range of potential hosts within the Juglandaceae. *Juglans nigra*, *J. cineria*, and the *Carya* spp. are all susceptible to *C. caryae* and *C. smalleyi*, and only these two new species are pathogenic to *Carya*. It is interesting that these two species behave similarly in inoculation studies, both specialized to members of the Juglandaceae, and they appear to be fully interfertile, yet they differ substantially in morphology. *Ceratocystis caryae*, with a single exception, was isolated only from *Carya* spp. that had not been infested by the hickory bark beetle, although some of the trees were within 5 m of beetle-infested trees with *C. smalleyi*. Since completion of this study, we have isolated *C. caryae* from a wounded *Ulmus* sp.

Isolates of *C. smalleyi* were obtained from trees infested by the hickory bark beetle, which is common throughout the eastern United States and has been associated with substantial hickory mortality, especially in *C. cordiformis* (Felt 1914, St George 1929, Gange and Kearby 1979). *Ceratocystis smalleyi* might play a significant role in this mortality. The association of *C. smalleyi* with bark beetles is unique in the *C. fimbriata* complex, and this might be a newly diverged species with unique adaptations, perhaps evolving from the more typical wound-colonizing *C. caryae*. The absence of the endoconidial state with narrow

conidia and flask-shape phialides, the absence of aleurioconidia and the absence of fruity volatiles in culture are likely derived characters that somehow aid the unique association of *C. smalleyi* with *Scolytus quadrispinosus*. The three other species of *Ceratocystis* associated with bark beetles also show a loss of production of the narrow endoconidial state and a loss of aromatic volatiles when compared to their more typical relatives (Harrington and Wingfield 1998).

Although only a limited number of isolates were studied we conclude that Ceratocystis canker on aspen is caused by *C. populicola*. Published reports show that Ceratocystis canker on aspen occurs over a broad range, including neighboring Minnesota (Manion and French 1967, Hinds 1972, Hinds and Laurent 1978). However no cankers typical of those caused by *C. populicola* on aspen were observed in Iowa and no isolates of *C. populicola* were recovered during two summers of collecting. Our inoculations with isolates of *C. populicola* found that *Populus tremuloides*, the common host of *C. populicola*, had a less dramatic response to inoculation than did *P. balsamifera*, which has been reported as a host only once (Vujanovic 1999), or *P. trichocarpa*, which has not been reported as a host in North America. *Populus deltoides* and the European *P. nigra* also were shown to be susceptible to *C. populicola* in our inoculations. Given the susceptibility of many poplar species, the wide range of *P. tremuloides*, and the fact that *C. fimbriata* has been reported from much of this range (Manion and French 1967, Hinds 1972, Hinds and Laurent 1978), it is surprising that only *P. tremuloides* and *P. balsamifera* have been reported as North American hosts.

An outbreak of Ceratocystis canker on experimental plantings of hybrid poplars occurred in the late 1970s and early 1980s in Poland (Gremmen and de Kam 1977; Przybyl 1984a, b). Two isolates from *Populus* species in Poland were identified clearly as *C. populicola* based on ITS sequences, allozymes and morphology. It is likely that *C. populicola* is indigenous to North America and was introduced to Poland on infected poplar cuttings. Consistent with inoculation tests, Przybyl (1984a) found that clones from *P. nigra* were less susceptible than clones of *P. trichocarpa*.

Intersterility barriers have arisen within the North American clade of *C. fimbriata*, and some populations within the clade have begun to diverge genetically and phenotypically. Two host-associated lineages have been defined here as new species. *Ceratocystis smalleyi* associated with the bark beetle *Scolytus quadrispinosus* appears to have lost some spore stages and aroma production, and it may be a newly diverged species, still sexually compatible with *C. caryae*. Within *C. variospora*, the oak lineage, the cherry lineage, the *Tilia* genotype and the cherry-Wisconsin isolate may represent populations undergoing speciation and may prove to be true species.

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LITERATURE CITED

Baker CJ, Harrington TC, Krauss U, Alfenas AC. 2003. Genetic variability and host specialization in the Latin American clade of *Ceratocystis fimbriata*. Phytopathology 93:1274–1284.

Barnes I, Gaur A, Burgess T, Roux J, Wingfield BD, Wingfield MJ. 2001. Microsatellite markers reflect similar relationships between isolates of the vascular wilt and canker pathogen *Ceratocystis fimbriata*. Mol Plant Path 2:319–325.

———, Roux J, Wingfield BD, Dudzinski MJ, Old KM, Wingfield MJ. 2003. *Ceratocystis pirilliformis*, a new species from *Eucalyptus nitens* in Australia. Mycologia 95:865–871.

Benson MK, Schwalbach DE. 1970. Techniques for rooting aspen root sprouts. Tree Plant Note 21:12–14.

CAB International. 2001. *Ceratocystis fimbriata* [original text prepared by CJ Baker and TC Harrington]. Crop Protection Compendium. Wallingford, UK: CAB International.

Campbell RN. 1957. Studies on the biology of some wood-staining fungi. University of Minnesota. 69 p.

Davidson RW. 1944. Two American hardwood species of *Endoconidiophora* described as new. Mycologia 36: 300–306.

DeScenzo RA, Harrington TC. 1994. Use of $(CAT)_5$ as a DNA fingerprinting probe for fungi. Phytopathology 84:534–540.

DeVay JE, Lukezic FL, English H, Trujillo EE, Moller WJ. 1968. Ceratocystis canker of deciduous fruit trees. Phytopathology 58:949–954.

Engelbrecht CJB, Harrington TC. 2005. Intersterility, morphology and taxonomy of *Ceratocystis fimbriata* from sweet potato, cacao and sycamore. Mycologia 97:57–69.

———, ———, Steimel J, Capretti P. 2004. Genetic variation in eastern North American and putatively introduced populations of *Ceratocystis fimbriata* f. *platani*. Mol Ecol 13:2995–3005.

Felt EP. 1914. Notes on forest insects. J Econ Ent 7:373–375.

Gagne JA, Kearby WH. 1979. Patterns of host tree visitation by *Scolytus quadrispinosus* (Coleoptera: Scolytidae). J Kansas Ent Soc 52:112–118.

Gremmen J, de Kam M. 1977. *Ceratocystis fimbriata*, a fungus associated with poplar canker in Poland. Euro J Forest Path 7:44–47.

Halsted BD. 1890. Some fungous diseases of sweet potato. The black rot. N.J. Ag. Exp. Sta. Bull. 76:7–14.

Harrington TC. 2000. Host specialization and speciation in the American wilt pathogen *Ceratocystis fimbriata*. Fitopath Brasil 25S:262–263.

———, McNew DL. 1997. Self-fertility and uni-directional mating type switching in *Ceratocystis coerulescens*, a filamentous ascomycete. Cur Genet 32:52–59.

———, ———. 1998. Partial interfertility among the *Ceratocystis* species on conifers. Fung Genet Biol 25:44–53.

———, ———, Steimel J, Hofstra D, Farrell R. 2001. Phylogeny and taxonomy of the *Ophiostoma piceae* complex and the Dutch elm disease fungi. Mycologia 93:111–136.

———, Pashenova NV, McNew DL, Steimel J, Konstantinov MY. 2002. Species delimitation and host specialization of *Ceratocystis laricicola* and *C. polonica* to larch and spruce. Plant Dis 86:418–422.

———, Rizzo DM. 1999. Defining species in the Fungi. In: Worrall JJ, ed. Structure and Dynamics of Fungal Populations. Dordrecht, Netherlands: Kluwer Academic Press. p 43–71.

———, Steimel J, Wingfield MJ, Kile GA. 1996. Isozyme variation and species delimitation in the *Ceratocystis coerulescens* complex. Mycologia 88:104–113.

———, Wingfield MJ. 1998. The *Ceratocystis* species on conifers. Can J Bot 76:1446–1457.

Hinds TE. 1972. Ceratocystis canker of aspen. Phytopathology 62:213–220.

———, Laurent TH. 1978. Common aspen diseases found in Alaska. Plant Dis Report 62:972–975.

Hunt J. 1956. Taxonomy of the genus *Ceratocystis*. Lloydia 19:1–58.

Manion PD, French DW. 1967. *Nectria galligena* and *Ceratocystis fimbriata* cankers of aspen in Minnesota. For Sci 13:23–28.

Marin M, Castro B, Gaitan A, Presig O, Wingfield BD, Wingfield MJ. 2003. Relationships of *Ceratocystis fimbriata* isolates from Colombian coffee-growing regions based on molecular data and pathogenicity. J Phytopath 151:1–11.

Moller WJ, DeVay JE. 1968. Carrot as a species-selective isolation medium for *Ceratocystis fimbriata*. Phytopathology 58:123–124.

———, ———, Backman PA. 1969. Effect of some ecological factors on Ceratocystis canker in stone fruits. Phytopathology 59:938–942.

Moreau C. 1952. Coexistence des formes *Thielaviopsis* et *Graphium* chez une souche de *Ceratocystis major* (van Beyma) nov. comb. Remarques sur les variations des *Ceratocystis*. Rev Mycol Suppl Colonial 17:17–25.

Paulin-Mahady AE, Harrington TC, McNew D. 2002. Phylogenetic and taxonomic evaluation of *Chalara*, *Chalaropsis* and *Thielaviopsis* anamorphs associated with *Ceratocystis*. Mycologia 94:62–72.

Przybyl K. 1984a. Disease of poplar caused by *Ceratocystis fimbriata* Ell. et Halst. I. Isolation of *C. fimbriata*, symptoms of the disease and evaluation of resistance of poplar clones resulting from artificial infection. Arbor Korn 29:89–103.

———. 1984b. Disease of poplar caused by the fungus *Ceratocystis fimbriata* Ell. et Halst. II. Morphology of the pathogen. Arbor Korn 29:105–117.

Roux J, van Wyk M, Hatting H, Wingfield MJ. 2004. *Ceratocystis* species infecting stem wounds on *Eucalyptus grandis* in South Africa. Plant Path 53:414–421.

———, Wingfield MJ, Bouillet JP, Wingfield BD, Alfenas AC. 2000. A serious new wilt disease of *Eucalyptus* caused by *Ceratocystis fimbriata* in Central Africa. For Path 30:175–184.

Snow Jr AG. 1938. Use of indolebutyric acid to stimulate the rooting of dormant aspen cuttings. J For 36:582–587.

Soltis DE, Haufler CH, Gastony GJ, Darrow DC. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers and staining schedules. Am Fern J 73:9–27.

Steimel J, Engelbrecht CJB, Harrington TC. 2004. Development and characterization of microsatellite markers for the fungus *Ceratocystis fimbriata*. Mol Ecol Note 4:215–218.

St George RA. 1929. Weather, a factor in outbreaks of the hickory bark beetle. J Econ Ent 22:573–580.

Swofford DL. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods). Version 4.0b10a. Sunderland, Massachusetts, Sinaur Associates.

Teviotdale BL, Harper DH. 1991. Infection of pruning and small bark wounds in almond by *Ceratocystis fimbriata*. Plant Dis 75:1026–1030.

Thorpe DJ, Harrington TC, Uchida JY. 2005. Pathogenicity, internal transcribed spacer-rDNA variation and human dispersal of *Ceratocystis fimbriata* on the family Araceae. Phytopathology 95:316–323.

Upadhyay HP. 1981. A monograph of *Ceratocystis* and *Ceratocystiopsis*. Athens, Georgia: University of Georgia Press. 176 p.

Van Wyk M, Roux J, Barnes I, Wingfield BD, Liew ECY, Assa B, Summerell BA, Wingfield MJ. 2004. *Ceratocystis polychroma* sp. nov., a new species from *Syzgium aromaticum* in Sulawesi. Stud Mycol 50:273–282.

Vujanovic V. 1999. First report of *Ceratocystis fimbriata* infecting balsam poplar. Plant Dis 83:879.

Webster RK, Butler EE. 1967. A morphological and biological concept of the species *Ceratocystis fimbriata*. Can J Bot 45:1457–1467.

Wingfield MJ, DeBeer C, Visser C, Wingfield BD. 1996. A new *Ceratocystis* species defined using morphological and ribosomal DNA sequence comparisons. Sys Appl Microbio 19:191–202.

Witthuhn CR, Harrington TC, Steimel JP, Wingfield BD, Wingfield MJ. 2000a. Comparison of isozymes, rDNA spacer regions and MAT-2 DNA sequences as phylogenetic characters in the analysis of the *Ceratocystis coerulescens* complex. Mycologia 92:447–452.

———, ———, Wingfield BD, Steimel JP, Wingfield MJ. 2000b. Deletion of the *MAT*-2 mating-type gene during uni-directional mating-type switching in *Ceratocystis*. Cur Genet 38:48–52.

Wood FA, French DW. 1963. *Ceratocystis fimbriata*, the cause of a stem canker of quaking aspen. For Sci 9:232–235.

Zalasky H. 1965. Process of *Ceratocystis fimbriata* infection in aspen. Can J Bot 43:1157–1162.

Zambino PJ, Harrington TC. 1992. Correspondence of isozyme characterization with morphology in the asexual genus *Leptographium* and taxonomic implications. Mycologia 84:12–25.

Zimmermann A. 1900. Über den Krebs von *Coffea arabica*, verursacht durch *Rostrella coffeae* gen. et sp. nov. Bull. Inst. Bot. Gardens Buitenzorg 4:19–22.