The Proceedings of the 2nd National Oak Wilt Symposium

Edited by:

Ronald F. Billings
David N. Appel

Sponsored by

International Society of Arboriculture – Texas Chapter

Cooperators

Texas Forest Service
Texas AgriLife Extension Service
The Nature Conservancy of Texas
Lady Bird Johnson Wildflower Center
USDA Forest Service, Forest Health Protection

2009

THE GENUS *CERATOCYSTIS*: WHERE DOES THE OAK WILT FUNGUS FIT?

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ABSTRACT

Most species of *Ceratocystis* are plant pathogens, primarily colonizing sapwood near wounds on woody hosts, but only *C. fagacearum* causes a true vascular wilt. All species produce sexual spores in a sticky mass for insect dispersal, and most species, including *C. fagacearum*, produce fruity volatiles that are attractive to insects. Many *Ceratocystis* species produce sporulation mats on exposed wood, but only *C. fagacearum* forms pressure cushions or pads that push the bark away from the wood in order to crack the bark and expose the mats for fungal-feeding vectors (nitidulid beetles). Phylogenetic analyses of DNA sequences fail to identify a close relative of *C. fagacearum*. Limited genetic variation within *C. fagacearum* and the high susceptibility of some native oaks suggest that the fungus did not evolve in eastern U.S., but the evolutionary and geographic origins of *C. fagacearum* remain a mystery.

Key words: Evolution, fungal mats, genetic variation, taxonomy

Proper taxonomic placement of a species should say something about its biology. *Ceratocystis fagacearum* (Bretz) Hunt, the cause of oak wilt, is well placed in *Ceratocystis*, so we should not be surprised that it has insect vectors, produces fruity volatiles, and is a wound colonizer and vascular pathogen. Although its biology, morphology, and DNA sequences place *C. fagacearum* in the genus *Ceratocystis*, more precise placement within the genus has not proven possible, and there are several unique aspects to its biology. This paper reviews the genus *Ceratocystis* and discusses where *C. fagacearum* fits, or does not fit, based on phylogenetic analyses and biology. Genetic variation and possible origin of *C. fagacearum* also are discussed.

CERATOCYSTIS TAXONOMY AND EVOLUTION

The genus *Ceratocystis* once included the much larger genus *Ophiostoma* (Hunt 1956, Upadhyay 1981). The biology of *Ceratocystis* differs substantially from that of *Ophiostoma*, but they have converged on long-necked perithecia (sexual fruiting bodies) with sticky ascospore masses at their tip for insect dispersal (Harrington 1987). The two genera are not closely related and may have diverged more than 170 million years ago (Farrell et al. 2001). The genus *Ophiostoma* may be more than 85 million years old, near the time of radiation of coniferous bark beetles (Farrell et al. 2001, Harrington 2005), which are common vectors of *Ophiostoma* and related asexual genera, e.g., *Pesotum* and *Leptographium* (Harrington 1993). *Ceratocystis* may be younger than *Ophiostoma*, perhaps less than 40 million years (Farrell et al. 2001).

All *Ceratocystis* species have a Chalara-like endoconidial state, where the asexual spores are produced within deep-seated phialides. The genus name *Chalara* is restricted to the asexual state of another group of fungi, the discomycetes, but the genus name *Thielaviopsis* is available for the anamorphs of *Ceratocystis* (Paulin-Mahady, Harrington and McNew 2002). *Thielaviopsis* was

initially used to describe the two asexual states of *C. paradoxa*, the Chalara-like state and the aleurioconidial state. Aleurioconidia are thick-walled, chlamydospore-like, survival spores produced from specialized conidiophores. *C. fagacearum* does not form aleurioconidia but does form the endoconidial state (= *T. quercina*) (Paulin-Mahady, Harrington and McNew 2002).

Ceratocystis appears to be best placed with *Gondwanomyces*, *Petriella*, *Microascus*, and other members of the Microascales (Alexopoulos, Mims and Blackwell 1996), which also produce perithecia and sticky ascospore masses suitable for insect dispersal. Aside from *Ceratocystis*, Microascales are not plant pathogens; most are saprobes, and some are animal pathogens. The ancestor of *Ceratocystis* was probably a saprophytic species adapted to insect dispersal, and the ability to colonize wounds of living plants may have been crucial to its evolutionary success.

Phylogenetic analyses of ribosomal DNA sequences (Witthuhn et al. 1999, Paulin-Mahady, Harrington and McNew 2002) and DNA sequences of *MAT-2*, beta tubulin, and elongation factor-1 α (EF-1α) genes (Harrington, unpublished) show that there are at least four clades or complexes of species within *Ceratocystis* (Fig. 1). All but two species (*C. fagacearum* and *C. adiposa*) fall into four groups: 1) the *C. fimbriata* complex (Johnson, Harrington and Engelbrecht 2005), 2) the *C. paradoxa* complex (Paulin-Mahady, Harrington and McNew 2002), 3) the angiosperm and gymnosperm subclades of the *C. coerulescens* complex (Witthuhn et al. 2000a), and 4) the *C. moniliformis* complex (Van Wyk et al. 2006). Members of each of these clades share important ecological and morphological characters, such as ascospore morphology and presence or absence of aleurioconidia.

Four species of soil-borne pathogens with no known sexual state (*Thielaviopsis basicola*, *T. thielavioides*, *T. ovoidea*, and *T. populi*) are related to each other and appear related to *C. fimbriata* based on morphology and DNA sequences (Fig. 1) (Nag Raj and Kendrick 1975, Paulin-Mahady, Harrington and McNew 2002). These *Thielaviospsis* species, the *C. fimbriata* complex, and the *C. paradoxa* complex are joined by the common feature of aleurioconidia (Fig. 1). The unrelated *C. adiposa* and some Microascales also produce these survival spores, so it is hypothesized that the first *Ceratocystis* species produced aleurioconidia but that this character was lost in *C. fagacearum* and the *C. coerulescens* and *C. moniliformis* complexes.

Ceratocystis fagacearum and *C. adiposa* loosely group with the *C. monilformis* complex based on rDNA sequence analyses (Paulin-Mahady, Harrington and McNew 2002), but there is no statistical support for grouping these species using *MAT-2* sequences, beta-tubulin, or EF-1α (Fig. 1). Also, ascospores of *C. moniliformis*, *C. adiposa*, and *C. fagacearum* differ in shape (Hunt 1956).

Three *Ambrosiella* species that are symbionts with ambrosia beetles (Coleoptera: Curculionidae: Scolytinae) have no known sexual state but are placed within *Ceratocystis* based on DNA sequence analyses (Fig. 1) (Cassar and Blackwell 1996, Paulin-Mahady, Harrington and McNew 2002). *Ambrosiella xylebori* and *A. hartigii* are closely related to each other but not to *A. ferruginea*, which is the nearest neighbor to *C. fagacearum*. However, the relationship of *C. fagacearum* and *A. ferruginea* is not well resolved. The relatedness of *C. fagacearum* to *Ambrosiella* species is intriguing, but it is unlikely that the oak wilt fungus evolved directly from a highly-specialized, asexual ambrosia beetle symbiont (Harrington 2005). However, *C. fagacearum* may share a *Ceratocystis* ancestor with an ambrosia beetle symbiont.

INSECT ASSOCIATIONS IN *CERATOCYSTIS*

Ceratocystis species typically form a mat of mycelium on the diseased host. Black perithecia with long necks position masses of ascospores above the mat. The ascospores are held together in a sticky, hydrophobic matrix, so the spores are not readily separated by water but instead have an affinity for the hydrophobic exoskeleton of insects. Wind and rain dispersal of conidia from such mats may occur, but the mats are more important as the sight for fungal feeding and acquisition of spores by insects (Moller and DeVay 1968). Insects must leave the mats and then visit wounds on susceptible plants for successful pathogen transmission.

Most *Ceratocystis* species produce fruity odors similar to that of banana, while *C. fagacearum* has an aroma described as "cantaloupe." These aromas are due to small chain fatty acids and esters, or fusel oils, which are thought to be attractants for fungal-feeding insects (Lin and Phelan 1992, Kile 1993). These compounds are toxic and may reduce grazing by insects that are not regular fungal feeders, thus leaving the mats for vectors, such as Nitidulidae (Coleoptera), which typically tolerate high concentrations of mycotoxins (Dowd 1995). Fusel oils may also be phytotoxic, though their role in pathogenesis is not clear (Kile 1993).

Fungal-feeding insects such as drosophilid flies (Diptera) and nitidulids have been frequently associated with mycelial mats, but most *Ceratocystis* species do not have specific insect vectors (Kile 1993). For instance, Verrall (1941) isolated a common hardwood-staining species, *C. moniliformis*, from ambrosia beetles and three other families of beetles. *C. variospora* and *C. populicola*, cause of almond canker and aspen canker, respectively, have been associated with nitidulids, but also with other insects (Moller and DeVay 1968, Hinds 1972). Various species of nitidulids have been shown to be vectors of *C. paradoxa* (Chang and Jensen 1974).

The association of nitidulids with the oak wilt fungus is particularly strong and may be due to the latter's capability of producing mats under the bark of freshly-killed trees. *C. fagacearum* forms pressure cushions or pads that push the bark away from the wood in order to crack the bark for insect access and form cavities for mat formation (Fergus and Stambaugh 1957, True et al. 1960, Gibbs and French 1980). No other *Ceratocystis* species is known to produce such pressure pads. Many species of nitidulids inhabit *C. fagacearum* mats, but *Carpophilus sayi* and *Colopterus truncatus* appear to be particularly important vectors in the Upper Midwest (Cease and Juzwik 2001, Juzwik, Skalbeck and Neuman 2004, Ambourn, Juzwik and Moon 2005).

Bark beetles are not common vectors of *Ceratocystis* species. Only four species (*C. smalleyi*, *C. laricicola*, *C. polonica*, and *C. rufipenni*) are known to be adapted to bark beetle vectors (Harrington and Wingfield 1998, Johnson, Harrington and Engelbrecht 2005). These species lack fruity odors, which would not be needed to attract vectors because the fungus sporulates in the bark beetle galleries. Fusel oils are toxic, and bark beetles may not survive well in galleries heavily colonized by *Ceratocystis* species producing these volatiles. Each of the bark beetle associates is homothallic (self-fertile), which would be important for sexual reproduction as there is not likely to be suitable movement of insects between beetle galleries to assure cross-fertilization. Also, conidium production is absent or rare in *Ceratocystis* species associated with bark beetles, presumably because there is no need for cross-fertilization.

Although there has been considerable debate about bark beetles as vectors of *C. fagacearum* (Gibbs and French 1980, Merrill and French 1995), comparisons with other *Ceratocystis* species suggest that the oak wilt pathogen is not well adapted to such a vector. Bark beetles lay eggs in trees weakened by oak wilt, but the next generation of beetles would not likely carry *C. fagacearum* propagules in high numbers or frequently introduce propagules into living branches. Trees killed by oak wilt tend to be dominated by one or the other of the two mating types of *C.*

fagacearum (Apple et al. 1985), and without an insect to cross-fertilize the mycelia, the fungus would not be able to produce fruiting bodies and ascospores in beetle galleries. Also, *C. fagacearum* produces toxic aromatic compounds, and mycelial mats would tend to plug the beetle galleries and suffocate larvae and teneral adults.

Bark beetles may be significant vectors in regions where nitidulids are less effective in carrying *C. fagacearum* (Rexrode and Jones 1970). In Europe, the bark beetle *Scolytus intricatus* has been suggested as a potential vector because it has a life history and behavior more suited to overland transmission than that of the bark beetles implicated as North American vectors, i.e., *Pseudopityophthorus* spp. (Webber and Gibbs 1989). However, it is questionable if *C. fagacearum* could become established in an ecosystem without suitable nitidulid vectors.

Only three species of *Ambrosiella* related to *Ceratocystis* have been described (Fig. 1), but ambrosia beetle symbionts are not well studied and there are probably many more relatives of *Ceratocystis* that serve as food for these highly specialized, xylem-inhabiting Scolytinae (Harrington 2005). Most ambrosia beetles have specific symbiotic fungi that colonize the wood and produce special spores or modified hyphal endings for insect grazing (Batra 1967, Beaver 1989). Many of the ambrosia beetles have special spore-carrying sacs, called mycangia, and the fungal symbionts are transported in these sacs (Batra 1963, Francke-Grosmann 1967, Beaver 1989). Glandular secretions into the mycangium facilitate yeast-like growth (Norris 1979).

Ceratocystis fagacearum has been isolated from mycangia of ambrosia beetles (Batra 1963), but it is unlikely that ambrosia beetles introduce *C. fagacearum* into living oaks. Still, an evolutionary link between *C. fagacearum* and ambrosia beetle symbionts is intriguing, and there should be further work on the associations of oak wilt and ambrosia beetles.

Another form of dispersal for *Ceratocystis* species is in ambrosia beetle frass. Members of the Latin American subclade of *C. fimbriata*, including *C. cacaofunesta* and *C. platani* (Harrington 2000, Ocasio, Tsopelas and Harrington 2007), and *T. australis* (Kile 1963) have been shown to be dispersed in frass when ambrosia beetles attack trees previously colonized by the pathogens. The sawdust and fungal propagules expelled from the trees as the adult beetles clean their tunnels may be dispersed by wind or rain splash for relatively short distances. The *C. fimbriata* species produce long-lived aleurioconidia in wood. The myrtle wilt pathogen, *T. australis*, does not produce aleurioconidia, but viable conidia and conidiophores are expelled by the insect tunneling. *C. fagacearum* was not isolated from frass expelled by ambrosia beetles attacking trees with oak wilt (Peplinski and Merrill 1974), but this dispersal mechanism needs further study.

DISEASES CAUSED BY *CERATOCYSTIS* SPECIES

Ceratocystis species grow mostly on woody angiosperms, and the *Ceratocystis* ancestor may have grown on a range of dicots. The *C. paradoxa* complex attacks monocots, especially palms, and a subclade of the *C. coerulescens* complex is found exclusively on gymnosperms (Fig. 1). Adaptations to these host groups may be derived characters. The non-aligned *C. adiposa* colonizes a wide range of hosts, sometimes as a saprophyte on conifer wood, but it also causes a root rot of sugarcane (Kile 1993). Colonizers of oaks and other Fagaceae are found in the *C. fimbriata*, *C. coerulescens* (angiosperm subclade), and *C. moniliformis* complexes. With the possible exception of soilborne pathogens, *Ceratocystis* and *Thielaviopsis* species are wound colonizers (Kile 1993), and in their native ecosystems most species appear to colonize only a limited area around the wound and are relatively benign pathogens.

Most economically-important diseases caused by *Ceratocystis* species are associated with a high incidence of wounding (Kile 1993). Ceratocystis wilt of cacao caused by *C. cacaofunesta* has been called "mal de machete" because of infection through machete wounds (Engelbrecht et al. 2007), and canker stain of plane tree caused by *C. platani* is also strongly associated with human-caused wounds (Engelbrecht et al. 2004, Ocasio, Tsopelas and Harrington 2007). In addition, these pathogens and the cause of Ceratocystis wilt of eucalyptus (*C. fimbriata sensu stricto*) can be transmitted in infected cuttings (Harrington 2000, Engelbrecht et al. 2007). *Quercus* species are not often propagated in this manner, and transmission of *C. fagacearum* in rooted cuttings is not likely.

Some *Ceratocystis* species colonize the host xylem far from the wound, but only *C. fagacearum* causes a true vascular wilt (Kile 1993). As *Ceratocystis* species colonize the sapwood of trees, they attack living parenchyma cells, inducing a dark discoloration of the xylem. In addition to colonizing sapwood, some *Ceratocystis* species cause cankers by killing the cambium and inner bark tissue (Kile 1993). These "sapstreak" or "canker stain" diseases differ from true vascular wilts, in which the pathogen moves systemically through the host in the non-living vessels and tracheids, at least in the early stages of colonization (Dimond 1970).

From an evolutionary perspective, the switch from a sapstreak to a true vascular wilt pathogen like *C. fagacearum* may have been simple. In the case of the saprophytic *Ophiostoma querci*, the experimental transfer of a single gene (the gene coding for cerato-ulmin, a hydrophobin) from *O. novo-ulmi* allowed *O. querci* to systemically colonize elm and cause vascular streaking and leaf symptoms typical of Dutch elm disease, a true vascular wilt disease (Del Sorbo et al. 2000). A related hydrophobin, cerato-platanin, has also been implicated as a pathogenicity factor for *C. platani* (Carresi et al. 2006), and phytotoxins have also been speculated as pathogenicity factors in oak wilt (Dimond 1970). Thus, one or a few introgressed or mutated genes may have made *C. fagacearum* a true vascular wilt pathogen.

Few tree diseases caused by *Ceratocystis* species result in significant mortality in native ecosystems. One possible exception is myrtle wilt, a sapstreak disease of *Nothofagus cunninghamii*, in which *T. australis* moves readily from tree-to-tree through functional root grafts (Kile 1993). In spite of the rapid spread of the pathogen through sapwood and rootwood of *N. cunninghamii*, myrtle wilt is believed to be an important player in the natural stand dynamics of these Australian forests. However, genetic evidence suggests that *T. australis* may not have evolved in this forest type (Harrington, Steimel and Kile 1998). *Ceratocystis platani* also spreads through functional root grafts and causes substantial mortality of planetree (*Platanus acerifolia*) in urban plantings. However, neither root-graft transmission nor substantial mortality of sycamore (*P. occidentalis*) has been noted in natural forest stands in eastern U.S., where the pathogen is indigenous (Engelbrecht et al. 2004).

Initiation of new disease centers is relatively rare in myrtle wilt and canker stain of planetree; many more trees are killed through root graft transmission than through wound colonization. This also is true with oak wilt (Appel 1995b). With time, one would expect that an oak ecosystem with such highly root-grafted and susceptible species like Texas live oaks (*Quercus virginiana* and *Q. fusiformis*) would shift to a forest type with more resistance to oak wilt and/or less root grafting.

It is noteworthy that few vascular wilt diseases of forest trees have been recognized and few (or none) of these are thought to be endemic (Sinclair, Lyon and Johnson 1987). Verticillium wilt of trees in the U.S. is exclusively a disease of urban and agricultural landscapes (Harrington and Cobb 1984). Dutch elm disease causes a vascular wilt on continents where *Ophiostoma*

novo-ulmi or *O. ulmi* has been introduced because there has not been sufficient selection pressure on American or European elms for the development of the level of resistance found in Asian elms (Brasier 2001). Species of persimmon (*Diospyros* spp.) in the southeastern U.S., likewise, lack the resistance of Asian species to persimmon wilt, caused by *Acremonium diospyri*, a likely exotic vascular wilt pathogen (Sinclair, Lyon and Johnson 1987). The extreme susceptibility of many eastern North America oak species to oak wilt argues that these oaks did not evolve with *C. fagacearum.*

GENETIC VARIATION IN *CERATOCYSTIS*

Most species of ascomycetes are heterothallic, meaning that they can reproduce sexually only if two strains of opposite mating type come in contact. The MAT-1 and MAT-2 mating types are determined by different genes at the mating type locus. Homothallic species usually have both *MAT-1* and *MAT-2* genes at the mating type locus and thus have all the genes necessary for sexual reproduction without mating. There is a surprising amount of homothallism in the genus *Ceratocystis*, perhaps because of the unreliability of insect dispersal of conidia for cross-fertilization of mycelia. All known species in the *C. fimbriata* complex, all species in the gymnosperm subclade of the *C. coerulescens* complex, and *C. virescens* are homothallic through unidirectional mating type switching (Harrington and McNew 1997, Witthuhn et al. 2000b). Homothallism is also found in some species in the *C. paradoxa* and *C. moniliformis* complexes, and in *C. adiposa*, but the genetic basis of homothallism in these species is unknown.

In the heterothallic *C. fagacearum*, developing ascogonia on mycelial mats of one mating type are fertilized by conidia of the opposite type via insects from other mats (Hepting, Toole and Boyce 1952, True et al. 1960). The importance of sexual reproduction and ascospores in the epidemiology of oak wilt is supported by the fact that the two mating types occur in nature in roughly equal proportions (Yount 1954, Appel, Drees and Johnson 1985).

Natural populations of *Ceratocystis* spp. have considerable genetic variation, and introduced populations have very limited variation due to a genetic bottleneck associated with the founding of the population by a single strain or a few strains. The question of whether *C. fagacearum* is native to a portion of its known range within the USA or if it was introduced from some other region can be addressed by studying genetic variation in the pathogen, as has been done with *C. albofundus* (Roux et al. 2001), *C. platani* (Engelbrecht et al. 2004, Ocasio, Tsopelas and Harrington 2007), and *C. cacaofunesta* (Engelbrecht et al. 2007). In these three homothallic species, there is substantial genetic variation where the pathogens are native, and the populations are essentially clonal where they have been introduced. A natural population of a heterothallic species like *C. fagacearum* should have substantial genetic variation, as was found for the heterothallic *C. eucalypti* in Australia (Harrington, Steimel and Kile 1998).

Kurdyla et al. (1995) found surprisingly little variation among isolates of *C. fagacearum* using restriction fragment length polymorphisms (RFLPs) of mitochondrial and nuclear DNA. The 27 isolates obtained from throughout the known range of the species (mostly from Texas, but also from West Virginia and Wisconsin) showed no variation in the mitochondrial DNA markers. There was some limited RFLP variation among nine isolates using anonymous nuclear DNA probes, but the variation was substantially less than that found with similar markers in introduced populations of other out-crossing pathogens (Milgroom and Lipari 1993).

Mitochondrial DNA markers that were used with the *Ceratocystis* species mentioned above were applied to 37 isolates of *C. fagacearum* from Iowa, six from Minnesota, and one from Illinois (Harrington, unpublished). The mitochondrial RFLP polymorphisms were identified

using *Hae*III digestion of genomic DNA (Wingfield, Harrington and Steimel 1996). There were 24 scorable bands, and surprisingly, the 44 isolates had the identical banding pattern, except that one isolate from Iowa had an extra band of 2.4 kb (Fig. 2A).

Nuclear DNA fingerprinting was applied to the same 44 isolates (Harrington unpublished) by probing *Pst*I-digested genomic DNA with the oligonucleotide $(CAT)_5$ (DeScenzo and Harrington 1994). Out of 35 $(CAT)_5$ bands, only two were polymorphic; one band of 2.7 kb was present or absent, and another band was polymorphic, with one of four different bands (alleles) ranging in size from 2.8-2.9 kb present in each of the isolates (Fig. 2B). The level of variation found in *C. fagacearum* was dramatically less than that found in the heterothallic *C. eucalypti* (Harrington, Steimel and Kile 1998) and substantially less than in natural populations of the homothallic species. The low level of variation found in *C. fagacearum* was comparable to that of the introduced populations of homothallic *Ceratocystis* species and the putatively indigenous population of the asexual *T. australis* (Harrington, Steimel and Kile 1998).

Mitochondrial DNA in *Ceratocystis* species is inherited maternally (Harrington, Steimel and Kile 1998), so the mitochondrial DNA of the progeny in an ascospore mass is identical to the parental strain that produced the fruiting body. In contrast, the ascospore progeny from a fruiting body of a heterothallic species should have variation in nuclear DNA markers because the alleles of each parent would be recombined through meiosis. The essentially clonal nature of the mitochondrial genome of *C. fagacearum* in eastern North America and the limited variation found in the nuclear genome suggest that U.S. populations were derived from a single sporulating mat.

THE ORIGIN OF *CERATOCYSTIS FAGACEARUM*

There is little agreement on whether or not *C. fagacearum* is native to eastern North America. The pathogen was first reported in the Upper Mississippi River Valley in 1944, and it may have spread from there to the Appalachian Mountains (True et al. 1960, McDonald 1995). However, the fungus was likely killing oak trees in the Upper Midwest in the late 1800s (Gibbs and French 1980) and may have been present in Texas since the 1930s (Appel 1995b). The fact that the pathogen readily colonizes and kills many oak species in eastern North America supports the argument that *C. fagacearum* evolved elsewhere. Unfortunately, we have no close relative that could be used as a point of reference to surmise a continent of origin for *C. fagacearum*. Nonetheless, portions of the world with oak forests would be a good place to start.

Quercus and other potential host genera in the Fagaceae are distributed widely throughout the Northern Hemisphere, and the related *Nothofagus* is found in the Southern Hemisphere. The susceptibility of European and other exotic oaks has been demonstrated (MacDonald et al. 2001), and Chinese chestnut (*Castanea mollisima*) is also highly susceptible, suggesting that *C. fagacearum* is not a natural component of Eurasian forest ecosystems. Furthermore, no near relatives of *C. fagacearum* have been identified in either Europe or eastern Asia, where the mycoflora of oaks has been extensively studied.

Western U.S. and Canada also have a number of *Quercus* species, but the *Ceratocystis* species on these hosts are reasonably well-characterized, and none are morphologically or genetically close to *C. fagacearum*. However, *C. fagacearum* may be native to Mexico, Central America, or northern South America because the *Ceratocystis* species there, other than the agriculturally-important species, are not well known. Many species of oak occur in these regions, especially in cool, high elevation cloud forests (Ingens-Moller 1955), and it is possible that *C. fagacearum* is a wound colonizer of relatively resistant oaks there.

Even if a Latin American origin of *C. fagacearum* was accepted, the pathway of its arrival is difficult to envision. Introduction from another continent by human activity would be unlikely because mycelial mats form under bark and only when the sapwood and inner bark tissue are very moist (Gibbs and French 1980). Movement of oak logs from another region to the Upper Midwest in the late 1800s, if such shipments occurred, would probably have taken too long for the logs to arrive with fresh mycelial mats for nitidulid dispersal.

Another pathway could have been a spore-laden insect blown into the U.S. by a hurricane or other storm event. Bark beetles contaminated with *C. fagacearum* would likely be contaminated with only conidia, and the genetic data suggest that the introduction of *C. fagacearum* was via an ascospore mass. A storm-dispersed nitidulid beetle could have been contaminated with a single ascospore mass, but it would have had to visit a fresh wound on a susceptible oak tree in order to establish the pathogen. The possibility that *C. fagacearum* was established in eastern North America via an insect or group of insects from a single mycelial mat cannot be discounted, but an animal vector capable of wounding oaks should also be considered.

Birds have been discussed as potential vectors of *C. fagacearum*, but they have not been thought to efficiently or frequently carry the fungus and establish new infections (True et al. 1960, Gibbs and French 1980). However, a bird may have been responsible for the hypothesized single event that brought *C. fagacearum* to the eastern U.S. Sapsuckers (*Sphyrapicus* spp.), for instance, will remove bark to forage for insects (Walters, Miller and Lowther 2002), so they may rarely feed on insects on oak wilt mats and acquire spores on their beak. They also drill through the bark of healthy trees to produce sap, which attracts insects, and sapsuckers will consume inner bark and cambium tissues, thus potentially introducing the fungus into a suitable wound. The yellow-bellied sapsucker (*S. varius*) is migratory, overwintering in Central America and Mexico and migrating north to the Upper Midwest in the spring, the right season for infection.

CONCLUSIONS

The oak wilt pathogen is a typical member of the genus *Ceratocystis*, but there are several unique and noteworthy aspects to its biology. Phylogenetic analyses have failed to identify a close relative, though there is some relation to an ambrosia beetle symbiont. Like *C. fagacearum*, many *Ceratocystis* species form sporulation mats that emanate fruity odors, presumably to attract their fungal-feeding vectors, and nitidulids have been shown to be vectors of other *Ceratocystis* species. However, only *C. fagacearum* is known to form pressure pads on mats. Though other insect vectors may have importance in some regions, *C. fagacearum* does not have the adaptations found in other species of *Ceratocystis* that have bark beetle vectors.

Ceratocystis contains mostly plant pathogens that are wound colonizers, but only *C. fagacearum* causes a true vascular wilt disease. The high susceptibility of *Quercus* species in eastern North America to oak wilt suggests that *C. fagacearum* did not evolve here. Genetic data also indicate that the fungus has been introduced, perhaps as a single ascospore mass. Humans may not have been the agent of introduction, however. Instead, a storm-blown insect or migrating bird may have brought the pathogen from Mexico or Central America.

Ceratocystis fagacearum is now well established and causes substantial mortality of oak in some regions, especially in the Upper Midwest and parts of Texas. Suitable nitidulid populations, abundant mycelial mats and wounds, and root-grafted and highly susceptible oaks appear to be major contributors to the relative importance of oak wilt in these regions. If the pathogen is a relatively recent arrival, we might see it expand its range to similar oak forests.

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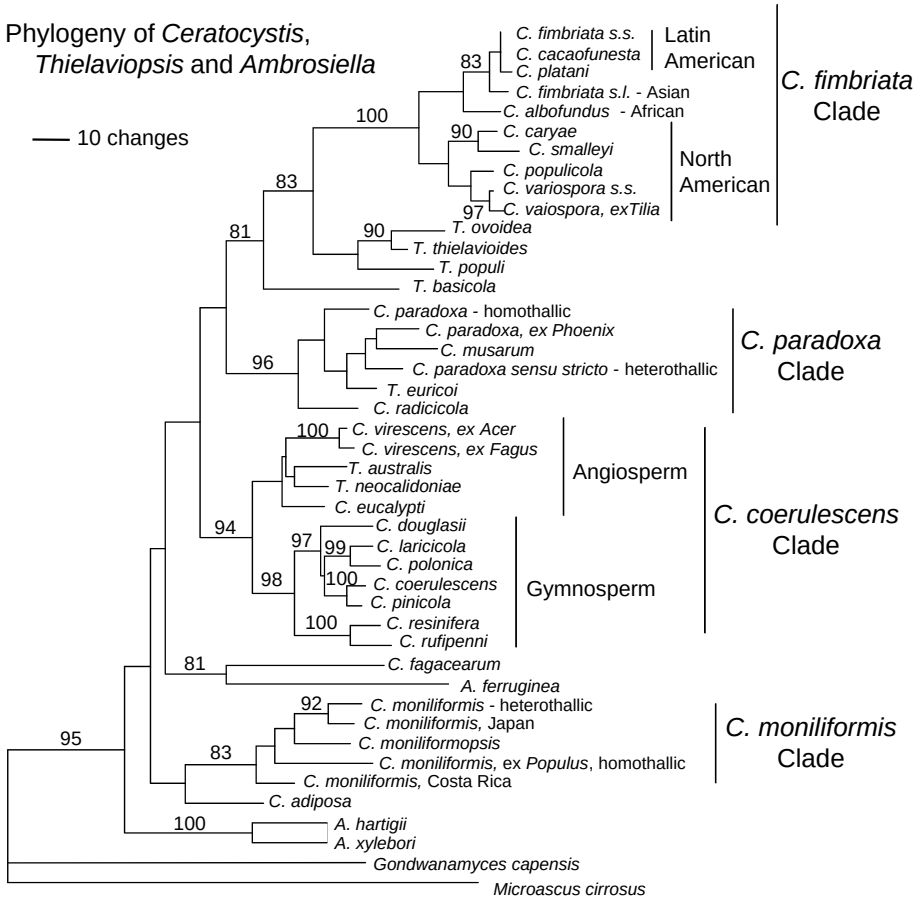

Figure 1. One of four most parsimonious trees of 1139 steps based on the DNA sequence of a portion of the elongation factor-1 α gene for *Ceratocystis*, *Thielaviopsis*, and *Ambrosiella* species. Of 1844 total aligned characters, 632 had to be eliminated from intron regions because of ambiguous alignment, 818characters were constant, 138 characters were parsimony un-informative, and 256 characters were parsimony informative. Gaps were treated as a fifth character. The consistency index was 0.3618 and the retention index was 0.6935. The tree was rooted to *Microascus cirrosus*. Bootstrap values (from 1000 replications) greater than 80% are shown above branches.

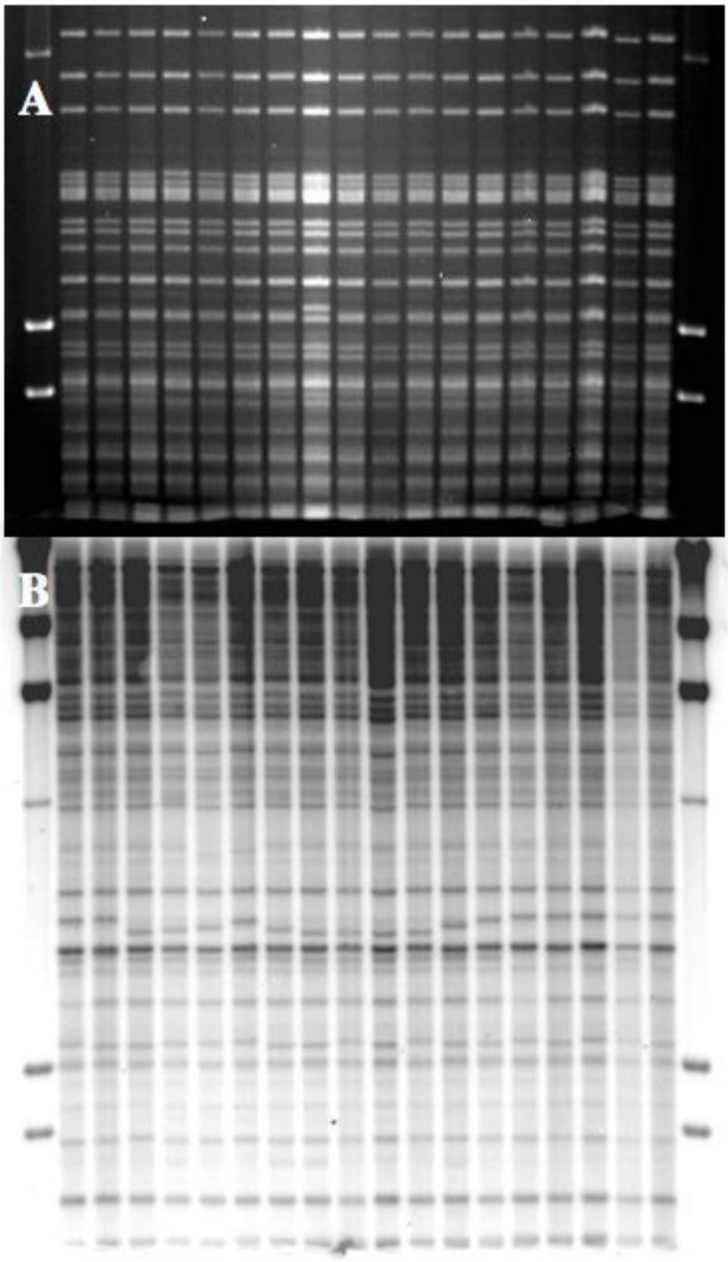

Figure 2. Mitochondrial (A) and nuclear (B) DNA fingerprints of *Ceratocystis fagacearum* isolates from Iowa. The markers in the outer lanes of A are 2.0, 2.3 and 4.3 kb from bottom to top. The markers in the outer lanes of B are 2.0, 2.3, 4.3 (faint), 6.5, 9.4, and 23 kb from bottom to top.