Bot Canker of Oak in Florida Caused by *Diplodia corticola* and *D. quercivora* [1]

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Emergent Pathogens on Oak and Grapevine in North America

In 2010, the fungal pathogen *Diplodia corticola* was discovered in Florida, associated with tip dieback and branch cankers in landscape live oaks (*Quercus virginiana*) (Dreaden et al., 2011) (Figures 1 and 2). In the same year, *D. corticola* was discovered in coast live oaks in San Diego County, California. *D. corticola* is now known to be an important factor in the deaths of thousands of acres of coast and canyon live oaks seen in California since 2002, as well as to have killed grapevine in Texas, California, and Mexico (Lynch et al., 2010, 2013; Urbez-Torres et al., 2010b; Urbez-Torres, 2011; Candolfi-Arballo et al., 2010). Descriptions of this fungus appeared in the literature beginning in the 1980s, when it was identified as a chief cause of a serious decline of cork oaks (*Quercus suber*) in western European countries, although originally misidentified as *Botryosphaeria stevensii* (Alves et al., 2004; Luque and Girbal, 1989). *D. corticola* has also been implicated in a malady of other European oaks generally known as "oak decline" (Alves et al., 2004; Linaldeddu et al., 2009; Vajna, 1986).

In May 2013, a second fungal species, *Diplodia quercivora*, closely related to *D. corticola* and causing similar symptoms, was identified on three oak species in Tunisia (Linaldeddu et al., 2013). Several Florida samples regarded initially as variants of *D. corticola* have even greater (99%) homology with two regions of the DNA of *Diplodia quercivora*. Both *D. quercivora* and *D. corticola* are, therefore, found in Florida, where they are causing damage to oaks (Dreaden et al., 2013; Mullerin et al., in press; Dreaden et al., 2011).

The disease *D. corticola* and *D. quercivora* cause is commonly called "bot canker." The same name is used for diseases caused by other members of the same family, Botryosphaeriaceae, which are generalist pathogens on hundreds of plant genera (Bush, 2009; Sinclair, 2005).

Signs and Symptoms of *D. corticola* Infection

Both *D. corticola* and *D. quercivora* infections in Florida oaks are characterized by branch cankers (elongated cracks in the bark) that often bear pycnidia, tiny, black, flask-shaped fruiting bodies of the fungus that erupt through the dead bark (Figure 3). Clumps of large dead branches randomly distributed in the crown are diagnostic, as is twig dieback (Figure 1), often throughout the tree (Mullerin et al., in press). Cutting into the branch at the cankers reveals black necroses in the phloem, and some trees exude amber-colored sap. When inoculations are made on the trunk, light brown streaking in the sapwood, extending upwards from the inoculation site for a much greater distance than the phloem necrosis, is usually evident (Mullerin et al., in press) (Figure 4).

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Inoculations of coast live oaks with *D. corticola* in California produced additional symptoms not seen in Florida, including epicormic shoots, leaf desiccation, and lesions on roots (Lynch et al., 2013).

In grapevine, *Vitis vinifera, D. corticola* is much less aggressive than in oak. On its own, it produces a wedge-shaped canker (see Figure 3 in Urbez-Torres, 2011, for photographs). When occurring with the related grapevine pathogens *Neofusicoccum vitifusiforme* and *Neofusicoccum australe*, the symptoms of *D. corticola* in *V. vinifera* are "black streaks and brown-red wood" (Urbez-Torres, 2011). There are no reports of *Diplodia quercivora* infecting *Vitis vinifera*.

Mode of Infection

It is unknown how *Diplodia corticola* and *Diplodia quercivora* colonize oaks. However, members of the Botryosphaeriaceae, the family to which the genus *Diplodia* belongs, are generally known to enter plants through wounds, including leaf scars, or stomata open for gas exchange (Bush, 2009; Sinclair et al., 2005; Venkatasubbaiah et al., 1991). They often live harmlessly as endophytes within the plant, becoming pathogenic when the plant is stressed by environmental factors such as drought, flooding, heat, freezing, herbicide use, or soil compaction (Bush, 2009; Vajna, 1986). The fungus may colonize dead tissue, then move into healthy tissue in the branch. It can be spread by air, water splash, or contaminated pruning tools (Bush, 2009; Sinclair et al., 2005; Urbez-Torres, 2011). In the case of cork oak, *Quercus suber*, the traditional practice of removing cork from the trunks of oak trees causes mild injury, thus likely providing entry for *Diplodia corticola*, as evidenced by cankers on the trunk (Luque and Girbal, 1989). In Florida, a trunk canker has been observed only once: the most common diagnostic features are branch cankers and twig dieback. Thus, *D. corticola*/*D. quercivora* may enter through lenticels. Their close relative *Botryosphaeria obtusa* is known to enter plants through lenticels and stomata (Venkatasubbaiah et al., 1991).

In Florida, almost all symptomatic trees have been planted live oaks (*Q. virginiana* Mill.) growing in cultivated settings (Mullerin et al., in press). Oaks in cultivated landscapes, unlike those in forests, are exposed to stresses such as transplanting, pruning, herbicides, and water stress brought on by extensive periods of both drought and flooding. Pruning might create the wounds that provide entry to these pathogens. Also, most of Florida experienced severe drought in 2010, the year reports of dieback began.

Attempts to tie entry of the fungus in oaks to an insect vector in California (ambrosia beetles or the gold spotted oak borer) have been inconclusive (Lynch et al., 2013).

In grapevine, *Vitis vinifera*, the disease can spread through grafts between rootstocks and scions, and cankers are known to develop from pruning wounds (Urbez-Torres, 2011).

Mechanism of Host Disease and Death

Cork oaks and holm oaks artificially infected with *D. corticola* exhibited significant reduction in net photosynthetic rate and stomatal conductance (Linaldeddu et al., 2009; Luque et al., 1999). Because the impact of the infection on gas exchange rates was independent of stem lesion length in both species, Linaldeddu et al. (2009) suggested the cause was "diffusible toxins."

Other pathogenic Botryosphaeria species were known to produce a veritable smorgasbord of plant toxins as metabolites (Venkatasubbaiah et al., 1991). Bearing the prediction out, *D. corticola* was found to produce a compound named diplopyrone, toxic to cork oak (*Q. suber*) at concentrations from 0.01 to 0.1 mg/mL (Maddau et al., 2008). On tomato cuttings at 0.1 to 0.2 mg/mL, diplopyrone caused collapse of internal stem tissue (Maddau et al., 2008). A toxin specific to *D. quercivora* has also now been identified--diplopimarane--described as exhibiting "remarkable phytotoxicity" (Andolfi et al., 2014). Both *D. corticola* and *D. quercivora* also produce the metabolites sphaeropsidins A and C and sapinofuranone B. (Sphaeropsidin A has been shown to exhibit cytotoxicity against human cancer cell lines, Lallemand et al., 2012). In a study conducted at the University of Florida from 2011–13, 382 trees with 34 genotypic constitutions were inoculated with *D. corticola* or *D. quercivora*. None formed callus over the inoculation wound (although all controls that were wounded but not inoculated did form callus) (Mullerin et al., in press). All of the 382 inoculated trees were highly susceptible to both pathogens, developing long lesions and girdling over the 3- to 4-month study period, with several deaths. The absence of callus indicates that both fungi interfere with wound healing in oaks, probably by virtue of the phytotoxins. Pycnidia formed only on dead tissue, and the average lesion length in response to both pathogens in white oaks was smaller than that in red oaks.

Red oaks (Section Lobatae) are more affected than white oaks (Section Quercus) by *D. corticola*, and small stem diameter is a risk factor for infection by *D. corticola* (Mullerin et al., in press).

Fungal Morphology

Different lighting conditions or temperatures have different effects on the development of *D. corticola* and *D. quercivora* in culture, and members of Botryosphaeriaceae are notoriously similar one to another, with several species often inhabiting the same plant. However, only these two species in Botryosphaeriaceae are known to cause disease in oaks. The appearance of branch cankers and twig death is a major step to identifying these pathogens.

In culture on potato dextrose agar, *Diplodia corticola*, when viewed from above, initially appears fluffy white, turning to dark gray after about five days. The underside turns first olive-green, then black (Alves et al., 2004; Dreaden et al., 2011). *Diplodia quercivora* is distinguishable because it pulls back from the edge of the plate, retaining a definite wavy edge and remaining appressed to the surface, while *D. corticola* fills the plate and then piles mycelia aerially (Mullerin et al., in press). Figure 5 shows the differences in development between the two fungi in culture.

Microscopic identification was, until recently, based on features of conidia (asexual spores), since the sexual ascospores are only rarely found in nature. It has proved to be problematic even for experts, however, because features of conidia (such as shape, size, septation, and pigmentation) can change dramatically during conidial maturation, under different environmental conditions, or on different hosts (Slippers et al., 2013). The easiest and most reliable way to identify and distinguish these two pathogens is by culturing them, as described above, which can be followed by DNA sequencing for additional confirmation.

Origin, Host Range, and Classification

The source of *D. corticola* (and *D. quercivora*) in the United States is unresolved. The ITS ribosomal DNA sequence of the strain of *D. corticola* isolated in Florida (from Marion County) has 100% homology to the strains from oaks in California and Europe, as well as from *Vitis vinifera* in Texas. The ITS rDNA sequence of the *D. quercivora* strain from Alachua County, FL, has 99% homology to *D. quercivora* isolated in Tunisia. There are substantial indications in the literature, dating back to 1912, that *D. corticola* (or *D. quercivora*, or both) may be native to North America (Vajna, 1986).

The University of Florida study mentioned above was conducted to test the susceptibility to both *Diplodia corticola* and *Diplodia quercivora* of 34 species (or cultivars) of members of Fagaceae, both native to Florida and not. All host species or genotypes tested were highly susceptible to both fungi (Mullerin et al., in press).

The classification of these organisms is:

Kingdom: Fungi

Phylum: Ascomycota

Class: Dothidiomycetes

Order: Botryosphaeriales

Family: Botryosphaeriaceae

Genus: *Diplodia*

Species: *corticola* (or *quercivora*)

Management Options

Oak

No control measures for *D. corticola* or *D. quercivora* have yet been found to be effective in oaks. Remedial surgery has been successful in grapevine (see the “Grapevine” section below), and may prove to be the best treatment for oaks as well. When pruning, dip tools in 10% bleach solution not simply before moving from tree to tree, but before moving from branch to branch within the same tree. Do not prune during periods of high rainfall (when most spores are released), and remove wood debris afterwards to destroy persisting inoculum sources.

Stress makes oaks susceptible to bot canker, so the best way to ensure they stay healthy is to provide optimum growing conditions. Because such conditions will depend on soil type, soil chemistry, and drainage, consult with a local certified arborist or Extension office for detailed advice tailored to site-specific conditions. Allow for proper root room when planting: restriction of roots (in median plantings and parking lot islands, for instance) appears to increase trees’ susceptibility to the disease.

Luque et al. (2008) evaluated fourteen commercial fungicides to control *D. corticola* on cork oak. The fungicides were effective sprayed on debarked areas after cork removal, so should be equally effective sprayed on pruning wounds. Although none provided complete protection, three of the fungicide treatments (carbendazim, benomyl, and thiophanate-methyl) resulted in significant reduction of canker incidence and canker area in *Q. suber* after only one application. All of the three most effective compounds are members of the same chemical family (benzimidazoles). Both thiophanate-methyl and benomyl are converted to carbendazim within the plant.

The Environmental Protection Agency no longer registers benomyl for use in the United States, which means it cannot be sold or distributed. Thiophanate methyl (TM) is registered for use on lawns and ornamentals, so homeowners may use it on oaks. This compound is a known developmental and reproductive toxicant, hormone disrupter, and suspected human carcinogen, however, so it is not a remedy for large-scale use.

Biocontrol via antagonistic endophytic fungi, such as *Trichoderma citrinoviride* Bissett, other species of *Trichoderma*, and *Fusarium tricinctum* (Campanile et al., 2007; Maddau et al., 2009), holds promise, although these species must colonize a pruning wound before having inhibitory effect . *T. citrinoviride* and *F. tricinctum* dramatically inhibit the growth of *D. corticola* both in culture and in the plant.

Grapevine

Twenty-one different species of Botryosphaeriaceae are known to be pathogenic on grapevines or grapes worldwide. (Table 2 in Urbez-Torres 2011.) Because these fungi occur under widely variable geographic and climatic conditions, "remedial surgery" is recommended generally for infected trunks, along with removal and destruction of diseased wood. Urbez-Torres (2011) provides a table of fungicides tested on other species of Botryosphaeriaceae in grapevine without specific mention of *D. corticola*; thiophanate-methyl has been 80% protective against Botryosphaeriaceae species when painted on pruning wounds. (Before using thiophanate methyl, consult the EPA fact sheet on this potentially dangerous chemical: http://www.epa.gov/oppsrrd1/REDs/factsheets/tm_red_fs.pdf. Thiophanate methyl can have serious adverse effects on human health.)

The possibility that grapevines might have been the source for infection of oaks by *D. corticola* in California has not been investigated. Sprinkler irrigation triggers spore release in vineyards just as rain does (Urbez-Torres et al., 2010a, 2011), possibly increasing nearby oaks' exposure to infection, and oaks are used in the wine industry for both corks and casks. Cross-infection would seem to be within the realm of possibility.

Figure 1. Live oak *(Q. virginiana* Mill.*)* showing dieback
Credits: Jason Smith, Univ. of Florida

Figure 2. Cankers on naturally infected live oak branch.
Credits: Tyler Dreaden, Univ. of Florida

Figure 3. Pycnidia on artificially infected branch.
Credits: Jason Smith, Univ. of Florida

Figure 4. Stem of *Q. muehlenbergii* wound inoculated with *D. corticola*, showing phloem necrosis (localized black spot) and xylem staining extending up the branch.
Credits: Adam Black, Univ. of Florida

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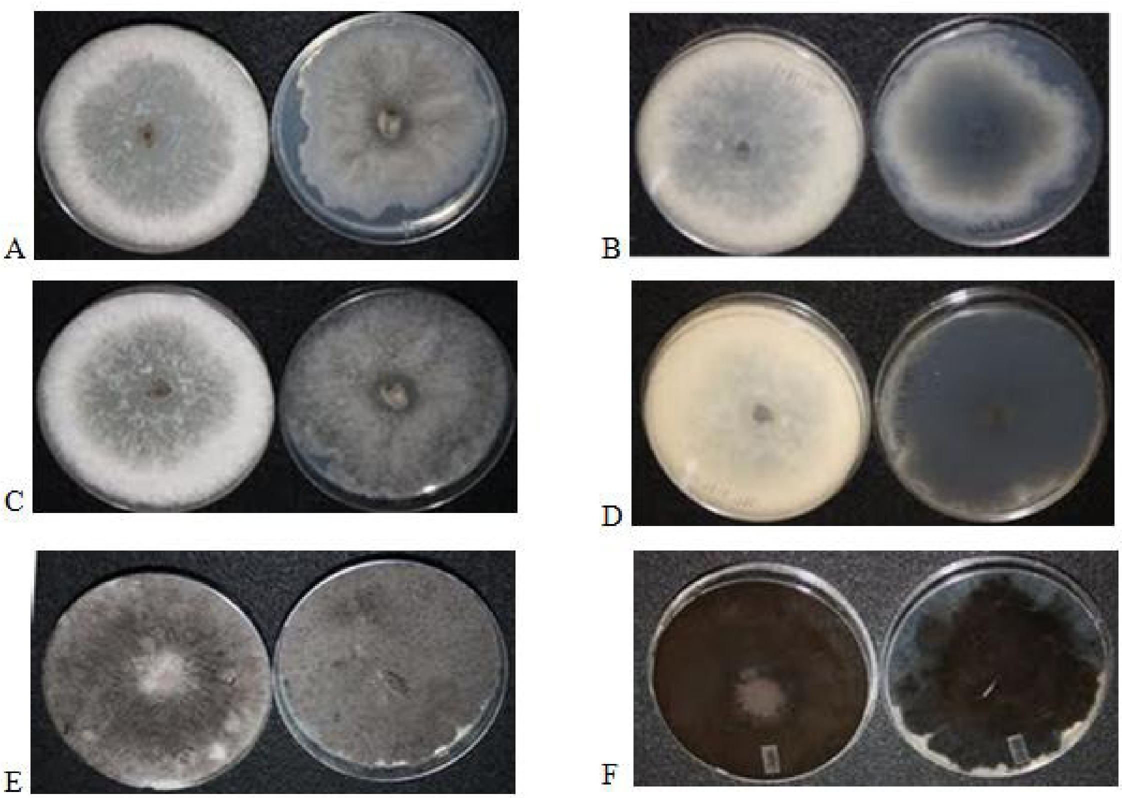

Figure 5. Isolates of *D. corticola* (left, in each picture) and *D. quercivora* (right, in each picture) in culture. A) 5 days old, top; B) 5 days old, bottom; C) 9 days old, top; D) 9 days old, bottom; E) 30 days old, top; F) 30 days old, bottom (appearing browner in photo than it was).
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