Botryosphaeriaceae and Diaporthaceae in California Forest Environments: An Annotated Bibliography and Observations

**Botryosphaeriaceae**


- “... it is now thought that most, if not all, Botryosphaeriaceae might have an endophytic phase.”
- Diseases caused by this group usually manifest themselves after the onset of tree stress factors.
- Botryosphaeriaceae appear to be mainly rain-dispersed (slimy spore masses, etc.).
- If the agent of stress is widespread, extensive losses can occur over large areas.
- *Botryosphaeria dothidea* was uncommon in native eucalyptus in Australia, but common in non-native eucalyptus in England and South Africa (Fisher et al. 1993; Smith et al. 1996)
- “Removal or treatment of diseased parts of trees is possible in intensively managed orchards (Brown-Rytlewski et al. 2000; Flowers et al. 2001). This, together with sanitation to reduce spore loads, can help to reduce disease (Brown-Rytlewski and McManus 2000; Michailides 1991; Palmer et al. 1988; Stanosz et al. 2005). But over time, new symptoms are likely to continue appearing from other endophytic infections, so the control is not absolute either.”


- This experiment tested pathogenicity of *Botryosphaeria dothidea, Neofusicoccum nonquaesitum,* and an unidentified *Dothiorella* species (named later in this paper as *Dothiorella californica*) to bay laurel branches. All the isolates were collected from bay laurel in Napa County.
- Both *B. dothidea* and *N. nonquaesitum* caused lesions on bay laurel branches that were significantly greater than control lesions (pathogenic); *D. californica* did not (endophytic or only opportunistically pathogenic).


- This experiment tested pathogenicity of *Diplodia corticola, Dothiorella iberica,* and an unidentified *Diplodia* species (named later in this paper as *Diplodia agrifolia*) on coast live oak. The isolates were all collected from coast live oak in southern California.
• *D. corticola* made by far the longest lesions on inoculated seedlings and usually killed the entire seedling. *D. agrifolia* produced lesions that were significantly larger than the controls, and *Dothiorella iberica* produced very small lesions.

• *D. corticola* aggressively colonized and killed the root tissues of the oak seedlings, and except for one very old report with another *Diplodia* species, this was the first report of any *Diplodia* colonizing roots.

• *D. corticola* is likely an introduced pathogen.

• *Dothiorella iberica* was only a weak pathogen on seedlings, but several studies are mentioned with other bot canker pathogens, *Fusarium solani*, *Biscogniauxia* sp. (cause of some Hypoxylon cankers), and some *Diaporthe/Phomopsis* species wherein subjecting the seedlings to stress increased the length of lesions caused by these pathogens.


• *Neofusicoccum australe* was originally described from *Acacia* spp. native to eastern Australia and from exotic *Sequoiadendron giganteum* in Canberra.

• It has been shown to be a fairly aggressive pathogen of *Eucalyptus* spp. including *Eucalyptus globulus*.

• It is also the dominant endophyte found in a number of native western Australian tree species, including several species of *Acacia* and *Eucalyptus*.

• The paper concludes that *N. australe* is likely native to western Australia and that it has been circulating on plant material around the world and diversifying in its new homes. [However, they did not know about the very wide occurrence of this pathogen in California on manzanita species.]

• The paper also stresses that *N. australe*’s lifestyle is like many other pathogens in this family: it normally lives as an endophyte and becomes pathogenic in response to environmental stress on the tree host.


• Samples were collected from redwoods in many areas of California, including Marin, Alameda, and San Mateo Counties.

• 5 pathogen species were identified: *Botryosphaeria dothidea*, *Neofusicoccum luteum*, *N. parvum*, *N. mediterraneum*, and *N. australe*.

• *N. australe* produced the longest lesion lengths in pathogenicity tests ($\bar{x}$=17.5 cm), followed by *N. parvum* ($\bar{x}$=11.9 cm), *N.luteum* ($\bar{x}$=10.2 cm), *N. mediterraneum* ($\bar{x}$=9.9 cm), and *B. dothidea* ($\bar{x}$=2.6 cm).
• The discussion section of this paper reviews what is known about water stress and opportunistic pathogens such as these. Prolonged water stress of -12 to -18 bars can activate opportunistic fungi to initiate infections. If the water stress lasts less than 3 to 5 days, wilting and infection are reversible/containable. If water stress is prolonged, infections and the resulting damage become irreversible.
• The authors speculate that prolonged droughts in CA between 2000 and 2016 may be the factor underlying recent increases in detection of canker-causing fungi causing significant damage in a variety of tree species throughout the state.
• All the *N. australe* isolates collected were of a single genetic type, supporting the idea of Sakalidis et al. (2011) that this may be an introduced species outside of Australia.

**Fabre, B., Piou, D., Deprez-Loustau, M.-L., and Marçais, B. 2011. Can the emergence of pine Diplodia shoot blight in France be explained by changes in pathogen pressure linked to climate change? Global Change Biology 17(10): 3218-3227.**

• Researchers were trying to evaluate whether climate change contributes to increases in Diplodia shoot blight severity by directly increasing pathogen inoculum or by increasing host susceptibility; they also wanted to determine the relative frequency of *Diplodia pinea* vs. *Diplodia scrobiculata* in France.
• Primary reservoir of *Diplodia pinea* inoculum in forest stands: infected cones. So a cone survey was their primary vehicle for determining inoculum levels. Study trees: *Pinus nigra*, *Pinus sylvestris*, and *Pinus pinaster*—they also looked at quite a few *Pinus radiata*.
• They used their current survey data to parameterize a model that related *Diplodia* prevalence to climate parameters during the preceding 15 years (1990-2005) and then used the model to “predict” *Diplodia* presence from 1960-1990 and from 2030-2060.
• *Diplodia scrobiculata* presence was negligible—only found on 3 *Pinus radiata* cones and on none of the native pine species. *Diplodia pinea* incidence ranged from 0-100% of cones collected in study sites.
• Three explanatory factors were found: Host species (*P. nigra* and *P. sylvestris* often colonized; *P. pinaster* and *P. radiata* rarely colonized), High minimum temperatures in winter (so that the pathogen was more often found at low elevations), and high rainfall in summer.
• The models predicted that the pathogen prevalence was 1.14 times what it had been from 1960-1990 and that from 2030-2060 it will be 1.05 times higher than the 1990-2005 period.
• It is important to keep in mind that the conditions for inoculum production (wet) are different than the conditions needed for disease development (drought), so the relationship between inoculum availability and disease development are not straightforward. [This probably points toward the lag times between infection and disease development (pathogen latency) we have observed in the Bay Area].

- Spore trapping studies in California vineyards have shown that Botryosphaeriaceae species (which include all the main ones that also infect forest trees) produce and disseminate the majority of their spores during winter rain events, followed very distantly by fall and early spring and almost none in late spring and summer.
- Spore release happened mostly during times when it was raining AND temperatures were between 3 °C (37 °F) and 7 °C (44 °F).


- Samples from 13 different woody hosts were subjected to a 2-locus phylogenetic analysis for identification. Four species were identified: *Dothiorella symphoricarposicola, D. iberica, D. sarmentorum,* and *D. vidmadera.* There are 23 *Dothiorella* species known as of this paper (*D. viticola,* isolated from Bay Area acacia, is not listed); most have narrow host ranges while a few have wide host ranges.


- Among several species of bot fungi recovered from diseased citrus throughout southern California, *Dothiorella viticola* produced the smallest lesions—although it was still 7 times as long as the control (non-fungal) lesions and pretty close to the lesion size produced by most of the other pathogenic isolates.

**Diaporthaceae**


- This is primarily a phylogeny, resolving 95 species in this genus. However, there are an estimated 2000 species, so this is just a beginning.
- Many species of *Diaporthe* appear to be primarily endophytes.


- Vineyards and orchards were sampled in northern California to determine *Diaporthe* species present in and around these croplands; the sampling included native willow trees in riparian areas around the croplands, as well as pear, almond, grapevine, and apricot.
• *D. foeniculina* was found on willow.
• The “generalist” *D. foeniculina* had lower genetic diversity than the grape-specific pathogen *D. ampelina*; this could be because of genetic bottlenecks after introduction to CA.


• According to the literature, *Diaporthe* is monocyclic: only one cycle of infection per season.
• The fungus overwinters as pycnidia; *D. eres* spores germinate between 5 and 36 °C but optimum is 27-29 °C.
• Active water values of 0.96-0.99, roughly corresponding to nearly 100% humidity, were best for pycnidia production, spore cirrhus generation, and spore germination.
• Hot weather (e.g., 35 °C) inactivates the pathogen, but the return of cooler temperatures along with rain will reactivate it.


• Again, it is stressed that many *Diaporthe* species are endophytes.
• Many species that were split away from *Diaporthe* because of differing morphology (they make the “wrong” spores for *Diaporthe*) are members of the genus *Diaporthe* based on DNA.


• This paper primarily establishes the pathogenicity of *D. foeniculina* on stems and shoots of young grafted sweet chestnuts.
• The authors believe that the pathogen was probably present in the natural stands of sweet chestnut where the grafted scions were collected.


• To study which members of the genus *Diaporthe* were associated with citrus trees, the authors collected samples from 90 sites in southern Europe; they recovered 79 isolates of *Diaporthe*, and 54 of them turned out to be *D. foeniculina* (identifications were based on a 5-gene phylogenetic analysis).
• However, *D. foeniculina* was more weakly pathogenic than some of the other species recovered.
• Wide host range of *D. foeniculina*, both in this study and in previous studies of other crop plants.
Recent experience with these fungi on forest trees in NW California

- *Diplodia scrobiculata* has been isolated from Monterey pine, bishop pine, shore pine, and redwood.
- *D. scrobiculata* is commonly found on dead seedlings and saplings of Monterey pine; it produces blue stain that can be found throughout the wood and pycnidia that appear on all parts of the seedling after death.
- *Diplodia scrobiculata* has been recovered in close association with the pitch canker pathogen on shore pine and bishop pine.
- When 3 bot canker fungi were tested on redwood seedlings, *D. scrobiculata* was the only one to cause lesions that were significantly larger than the controls. Even then, they were not extremely long (only up to about 2.5 cm). Also, this pathogen could not infect unwounded seedlings, only wounded ones. Lesions appeared more damaging on green stems than on woody (lignified) ones. *Diplodia mutila* and an unnamed *Dothiorella* species did not make lesions larger than the controls. The initial isolation of *Dothiorella* came from young redwoods that died without exterior symptoms, but the pathogen was isolated from discolored internal tissues.
- Many other species belonging to these fungal groups have been isolated from both conifers and hardwoods in NW CA over the past few years, including *Diplodia mutila, Diplodia pinea, Diplodia corticola, Neofusicoccum luteum, Neofusicoccum australis, Neofusicoccum parvum, Neofusicoccum nonquaesitum, Diaporthe erson, Diaporthe nothofagi, Diaporthe c.f. nobilis, Diaporthe c.f. passiflorae, Botryosphaeria dothidea, Neoscytalidium dimidiatum,* and others.
- *Neofusicoccum australis* has almost always been the single pathogen associated with samples of manzanita dieback collected by Chris Lee, CAL FIRE over the past couple years.