

A Brief History of *Diplodia sapinea* on Red Pine in Minnesota's State Forest Nurseries

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Abstract

Diplodia sapinea is a common fungal pathogen that has caused sporadic issues on red pine seedlings in Minnesota nurseries since the mid-1970s. Despite significant improvements in cultural controls made during the early 2000s, the Badoura State Forest Nursery in Akeley, MN, still experienced an unexpected resurgence of *Diplodia* problems in 2016 that resulted in the destruction of an entire field of 3–0 seedlings due to unacceptably high disease incidence. To address concerns about the possibility of additional *Diplodia* infections elsewhere in the nursery, Minnesota Department of Natural Resources forest health program and nursery staff have reinitiated annual testing and outplanted an experimental plot to monitor long-term mortality of potentially infected red pine seedlings. This paper was presented at the Joint Annual Meeting of the Northeast Forest and Conservation Association, the Southern Forest Nursery Association, and the Intertribal Nursery Council (Walker, MN, July 31–August 3, 2017).

Background

Diplodia sapinea (syn. *Diplodia pinea*, syn. *Sphaeropsis sapinea*), hereafter referred to simply as *Diplodia*, is a fungal pathogen most commonly associated with shoot blight on red pine (*Pinus resinosa* A.). Although shoot blight is the most recognizable form of *Diplodia* infection, *Diplodia* also causes collar rot, which can lead to extensive seedling mortality in both nurseries and forest plantations (Stanosz and Carlson 1996). In Minnesota, elevated levels of shoot blight and seedling mortality first became apparent in State forest nurseries around the mid-1970s. Once *Diplodia* was determined to be the cause, State nurseries adopted a fungicide treatment regimen that resulted in a noticeable decrease in

the prevalence of shoot blight on red pine seedlings. Considerable mortality continued, however, throughout the 1980s and 1990s of outplanted red pine seedlings sourced from State nurseries. In the absence of conspicuous shoot blight symptoms, mortality was unknowingly attributed to excess stress from drought or handling. It was not until the late 1990s that research revealed *Diplodia* can also exist as a latent infection within seedling stems, meaning it is nonactive and asymptomatic at times (Stanosz et al. 1997, 2001).

Diplodia Infection Levels 2002 to 2010

The potential for prolific latent infections led to renewed interest in *Diplodia* and inspired indepth investigations at Minnesota's State-operated nurseries to better understand disease levels. In 2002, an estimated 65 percent of out-planted red pine seedlings died in the field statewide, with latent *Diplodia* infection as the prime suspect. Subsequent testing revealed that as much as 88 percent of the 2002 nursery stock had harbored latent infections. In 2003, formal surveys revealed latent infection rates ranging from 40 to 71 percent in fields of red pine seedlings at both the Badoura State Forest Nursery in Akeley, MN and the now-decommissioned General Andrews State Forest Nursery in Willow River, MN. As a control measure, all windbreaks containing mature red pines on the nursery grounds were promptly removed to prevent spreading of *Diplodia* spores from mature trees onto the seedlings below (figure 1). Latent *Diplodia* infections decreased to only 2.5 percent in 2004 following removal of these mature trees (figure 2). Annual laboratory testing continued through 2010 (except 2009), followed by visual monitoring by nursery inspectors once the problem finally appeared to be largely resolved.



Figure 1. All mature red pines on Badoura State Forest Nursery grounds were removed from the windrows designated in yellow between 2003 and 2005. (Photo by Michael Parisio, 2017)

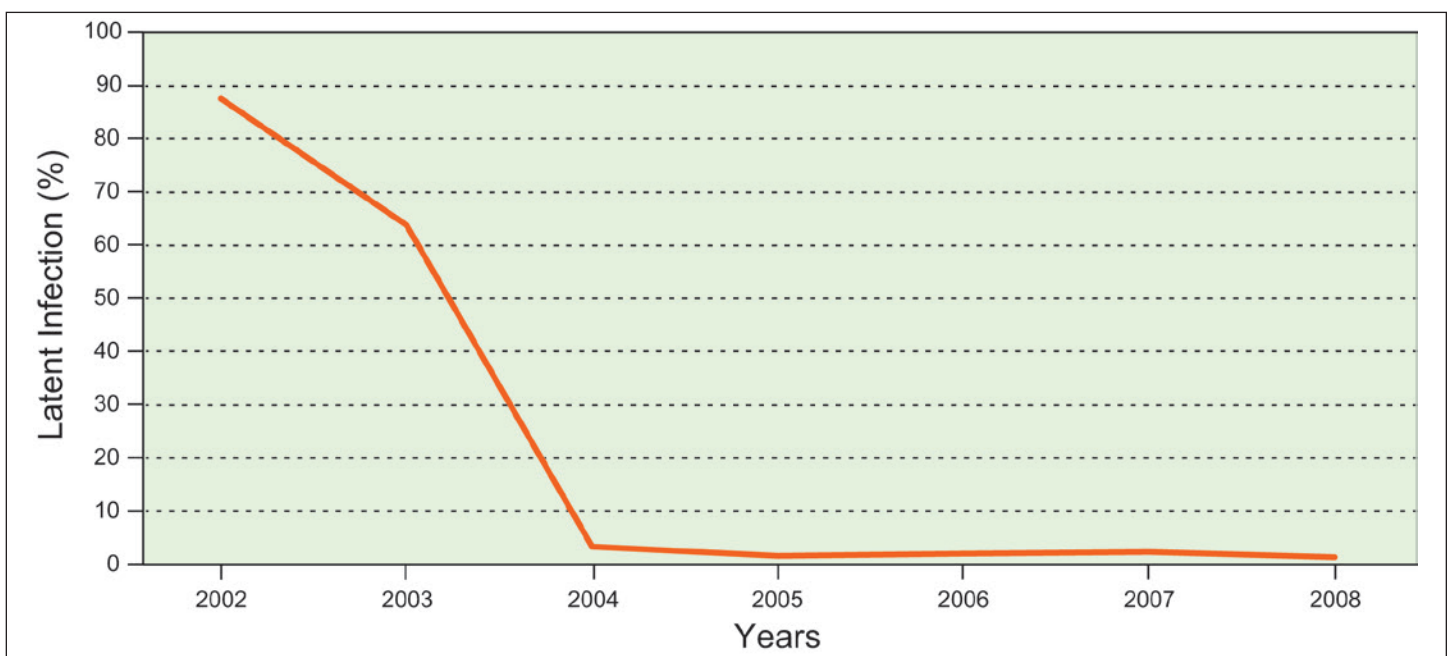


Figure 2. Following removal of red pine from windrows on Badoura State Forest Nursery grounds, latent *Diplodia* infection levels dropped dramatically and remained stable at very low percentages until 2016.



Figure 3. Red pine seedlings exhibiting classic symptoms of *Diplodia* shoot blight in field E6 at the Badoura State Forest Nursery. (Photo by M. Parisio, 2016)

Diplodia Resurgence 2016 to Present

In July 2016, reports of abundant shoot blight on red pine seedlings once again revealed an unexpected resurgence of elevated *Diplodia* levels at the Badoura State Forest Nursery (figure 3). This resurgence prompted an investigation to quantify the percentage of seedlings afflicted with visible shoot blight and to what degree latent *Diplodia* infections were present on asymptomatic seedlings throughout the affected fields. Of three affected fields, the most severely affected field contained 3–0 stock (table 1). It is thought that frequent, heavy rainstorms with high winds and above-average seasonal precipitation enabled the spread of spores throughout beds of densely growing 3–0 seedlings, perhaps also negating the effectiveness of fungicide treatments applied immediately prior to heavy storms by nursery staff. Elevated levels of shoot blight and latent infections in other forest nurseries in neighboring Wisconsin during 2016 also suggests that the weather played an important role in more widespread *Diplodia* flurries across the region.

In addition to obvious shoot blight, laboratory testing determined that the 3–0 field contained levels of latent infection above the Minnesota Department of Natural Resources’ (MNDNR) acceptable threshold of 10 percent. To prevent widespread outplanting of red pine seedlings predisposed to mortality from latent *Diplodia* infection in our forests, the entire 3–0 crop of 400,000 to 500,000 seedlings was destroyed. The other two affected nursery fields contained 2–0 stock and tested well below the threshold, with none of the seedlings testing positive in field A8 and only 4.4 percent testing positive in field A7. After all visibly affected seedlings were culled from these fields, another larger sample of 784 asymptomatic seedlings was tested in 2017, and only 2 seedlings (0.26 percent) tested positive for latent infection.

Latent *Diplodia* Effects on Outplanted Seedlings

To better understand the consequences of outplanting red pine seedlings with latent *Diplodia* infections, a sample of 616 asymptomatic 3–0 seedlings from field E6 was transplanted into a decommissioned area in General Andrews State Forest Nursery in April 2017 for long-term mortality monitoring. To compare mortality of potentially infected versus uninfected stock, 628 containerized red pine seedlings from a private nursery were planted adjacent to bareroot seedlings from Badoura State Forest Nursery.

MNDNR forest health staff monitored seedlings for mortality throughout the growing season and completed the second assessment in October 2017. Laboratory results at the time of planting indicated that about 15 percent of the bareroot seedlings could be positive for latent *Diplodia* infection, but more than 60 percent bareroot seedling mortality was documented through the end of the first season.

Table 1. Summary of test results indicating the number of asymptomatic seedlings that tested positive for latent *Diplodia* infection.

Field identification	Seedling age	Sample size	Number positive seedlings	Percent positive seedlings
A7	2–0	45	2	4.4 %
A8	2–0	35	0	0.0 %
E6	3–0	85	13	15.3%

Although cause of death could not be definitively determined on some bareroot seedlings, we estimate that more than two-thirds of the mortality was due to *Diplodia* collar rot, likely stemming from pre-existing latent infection at the time of planting. All containerized stock tested negative for *Diplodia*, but we still observed 24 percent mortality by the end of the first season. Another pathogen, *Cylindrocarpon* sp., was possibly already present in the unsterilized beds, and we suspect it was a major cause of mortality for containerized stock after submitting several dead seedlings for laboratory diagnosis. The forest health program plans to continue monitoring these plantings through 2018, although preliminary results indicate certain laboratory methods might sometimes underestimate levels of latent *Diplodia* infection.

Future Directions

Because past *Diplodia* infection at the nursery clearly pointed to mature red pines in windrows as the primary source of spores, the fact that these trees were removed long ago begs the question of where significant spore sources still remain. Evidence suggests that viable *Diplodia* spores can exist on pine debris (dropped needles or cones) on the forest floor for as long as 5 years (Oblinger et al. 2011). However, no pine species capable of carrying *Diplodia* had been planted in the same field as the affected 3–0 red pine for at least a decade. Also, it is possible that *Diplodia* may persist on or within seeds sourced from infected cones collected throughout the State. Although seeds are treated with fungicide at the nursery, research has shown that a small percentage of treated seeds can still harbor *Diplodia* spores (Smith et al. 2014).

Despite the best efforts of nursery staff to prevent larger *Diplodia* outbreaks, background levels of *Diplodia* will seemingly never fully be eliminated from nursery grounds. Future management will continue to rely on important cultural and chemical controls, paying special attention to the timing of these chemical applications in the upcoming years. Fortunately, test results were favorable in 2017 and allowed for all remaining red pine stock to be safely

offered for sale. Until the definitive cause for the recent outbreak is determined, annual testing will be reinstated to ensure outgoing pine seedlings are vigorous enough for successful outplanting.

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