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Quantification of Conidia of *Diplodia* spp. Extracted from Red and Jack Pine Cones

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ABSTRACT

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Frequency of detection and inoculum production by the conifer shoot blight and canker pathogens *Diplodia pinea* and *D. scrobiculata* on cones of red pine (*Pinus resinosa*) and jack pine (*P. banksiana*) were studied. Cones were collected from the ground and from canopies of red and jack pine trees in mixed stands at three sites in each of two different locations during two consecutive summers in Wisconsin. Conidia were extracted in water, quantified, germination tested, and the *Diplodia* species present was determined using molecular methods. At least one pathogen was detected from each tree at each site in both years. Overall, more conidia were extracted from cones from canopies than cones from the ground and from red pine cones than jack pine cones. Both total numbers of conidia extracted and proportions of cones yielding *D. pinea* or *D. scrobiculata* varied by location and pine species. Cones from either the ground or canopies can be used for surveys to detect *Diplodia* spp. at a given site but cones from canopies may be more useful to determine the relative abundance of potentially available inoculum of these pathogens.

Additional keywords: sporulation, *Sphaeropsis sapinea*

Two native pine species, jack pine (*Pinus banksiana* Lamb.) and red pine (*P. resinosa* Ait.), are ecologically important components of conifer forests in northern portions of eastern North America. Red and jack pine grow in sandy, low-fertility soils (20,21). Red pine commonly grows on level to gently rolling sand plains or on low ridges adjacent to lakes and swamps (20). Jack pine usually grows in less fertile and drier soils than those associated with other pine species (21). Each species is shade intolerant and jack pine, with serotinous cones, often invades recently burned areas (21). The ranges of both species overlap in central and eastern portions of the northern United States and Canada. However, jack pine is more widely distributed than red pine and its range in Canada extends farther northwest. Both pine species are at the southern edge of their range in Wisconsin.

Compared with presettlement forests, conifer stands in Wisconsin have been lost to poor regeneration following early logging in the 1800s (35). Although most jack pine stands are naturally regenerated, today red pine is the most planted tree species in Wisconsin. Six and a half million hectares are forested in Wisconsin, of which the red and jack pine forest types occupy 243,945 and 156,653 ha, respectively (36). Both species are harvested for pulpwood, and large red pine trees are also used for lumber (36).

Among the pathogens of red and jack pine are two recently differentiated asexual fungi: *Diplodia pinea* (Desmaz.) J. Kickx f. (syn. *Sphaeropsis sapinea*) and *D. scrobiculata* J. de Wet, Slippers & M.J. Wingf. *D. pinea* is the more frequently reported and widely distributed pathogen, causing damages including shoot blight, stem cankers, crown wilt, bluestaining of the sapwood, and seedling collar rot (18). Both native and exotic conifers in nurseries, plantations (27), windbreaks (13), forests, and those planted as ornamentals are affected. *Diplodia* spp. are frequently found sporulating on dead needles, bark, and especially mature seed cones (13,34). Conidia are dispersed by rain throughout the growing season (15) and can germinate within a few hours (5). The fungus infects through wounds and needle stomata (5) and by direct penetration of young stems (6). Shoots are particularly susceptible to shoot blight in early spring during bud break and shoot elongation (15,17). Many publications that describe disease development by *D. pinea* were written before *D. scrobiculata* was recognized as a distinct species (8), but the disease cycles are presumed to be similar. Like *D. pinea*, *D. scrobiculata* is a pathogen of red and jack pine and the two fungi co-occur in the same forests, trees, and tissues (24).

Information regarding occurrence and inoculum production of *D. pinea* and *D. scrobiculata* on jack and red pine cones is very limited. Vujanovic et al. (33) reported *D. pinea* sensu lato from a collection of red pine cones and seed from a botanical garden but neither the number of trees or cones examined nor the number yielding the fungus is clearly indicated, and jack pine was not studied. Nicholls and Ostry (13) reported that 84% of 300 2-year-old or older red pine cones from two windbreaks bore pycnidia of *D. pinea* sensu lato but no information on sampling design or numbers of conidia present were provided, nor were jack pine trees sampled. Additionally, an attempt was made to study conidial dispersal from individual red pine cones collected from windbreaks of a single nursery (15). Spores were trapped on slides beneath cones placed on screens and the authors stated that the numbers of conidia trapped varied among and within windbreaks. However, data from only five cones from one red pine tree were shown and, again, jack pine was not studied. Palmer (14) also indicated the presence of both *D. pinea* and *D. scrobiculata* among collections of cones and dying or dead red or jack pine branches in four stands in Minnesota or Wisconsin. However, no information on the frequency of detection of either fungus was provided, nor was it stated whether cones (and not branches) were the source of the particular isolates obtained.

Because the distribution and relative abundance of *D. pinea* and *D. scrobiculata* in the forests of jack and red pine in which both the pathogens and hosts co-occur has not been studied, our objective was to use cones as a tool to explore the influence of location and host species on frequency of detection and inoculum production by these fungi. Cones were collected from red and jack pine trees in mixed stands. Conidia were extracted in water and quantified, and the *Diplodia* sp. present was determined using molecular methods. The null hypotheses were (i) there is no difference between hosts in the number of conidia extracted from cones, (ii) there is no difference among sites in the number of conidia extracted from cones, and (iii) there is no difference in the frequency with which *D. pinea* and *D. scrobiculata* are detected from cones.

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