

Report RCSC-20-07

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## Aggressiveness of *Diplodia* isolates from Wyoming

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*Diplodia sapinea* was recently reported in Wyoming<sup>1</sup>. This disease causes shoot blights and cankers in pines and some other conifers throughout the world.<sup>2</sup> The fungal pathogen affects seedling to mature trees and damage can be severe. Symptoms range from dead needles, new-shoot branch mortality, extensive branch and top death, to tree mortality. New infections often result in short, light brown, wilting needles that fade to gray and remain attached to stems. Needles and stems are often stunted or crooked.

In August 2018, approximately 4,100 acres of ponderosa pine (*Pinus ponderosa*) in Crook County, Wyoming had *Diplodia*-like symptoms, based on a USDA Forest Service aerial detection survey<sup>3</sup>. On May 8, 2019, *Diplodia* was confirmed to genus using traditional mycology. Denise R Smith (Plant Pathology, University of Wisconsin–Madison) confirmed the species as *D. sapinea* using DNA sequence-based diagnostics (ITS with species-specific primers).

To confirm the isolates collected from WY are aggressive and are the likely cause of the observed damage, Koch's postulates were performed using methods previously described<sup>4</sup> (**Fig. 1**). Tests were conducted from March to April 2020 at the Charles E Bessey Nursery, Halsey, NE. Three-year-old ponderosa pine seedlings were inoculated 2 to 2.5 cm from bud-tips with one of three molecularly confirmed *D. sapinea* isolates grown on 1.5% water agar. Wounded controls were treated with sterile water agar plugs. Two trials were conducted in different greenhouses with five replications per treatment.

Four weeks after inoculation, symptoms were recorded and *D. sapinea* was isolated from seedlings. Stem sections (2 cm long) were cut from canker margins (or 2 cm below wounds for controls), surface disinfected for 30 sec in 70% ethanol followed by 5 min in 1.05% sodium hypochlorite, and placed in petri plates containing tannic acid agar<sup>5</sup>.

Symptoms first appeared 5 days after inoculation (**Fig. 2**). All seedlings inoculated with all three isolates produced typical *D. sapinea* symptoms; controls had no symptoms (**Fig. 2**). Cankers averaged  $8.7 \pm 3.0$  cm (standard error); controls  $0.0 \pm 0.0$  cm (**Fig. 3**).

<sup>1</sup> Blodgett, J. T. 2019. First Report of *Diplodia* in Wyoming. USDA For. Serv., Rocky Mountain Region, For. Health Mgt., Rpt. RCSC-20-03.

<sup>2</sup> Blodgett, J. T. and Stanosz, G. R. 2016. *Diplodia* shoot blight and canker disease. pp. 180-183. In Bergdahl, A. D. and Hill, A., tech. coords. *Diseases of trees in the Great Plains*. Gen. Tech. Rep. RMRS-GTR-335. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. 229 p.

<sup>3</sup> Anonymous. 2018. Aerial Detection Survey. USDA For. Serv., Rocky Mountain Region, For. Health Protection and partners.

<sup>4</sup> Blodgett, J. T. and Stanosz, G. R. 1997. *Sphaeropsis sapinea* morphotypes differ in aggressiveness, but both infect nonwounded red or jack pines. *Plant Dis.* 81:143-147.

<sup>5</sup> Blodgett, J. T., Bonello, P., and Stanosz, G. R. 2003. An effective medium for isolating *Sphaeropsis sapinea* from asymptomatic pines. *Forest Pathology* 33:395-404.



After 3 weeks on tannic acid agar, *D. sapinea* nearly filled the plates (**Fig. 4**), and was present in all inoculated treatment plates and no control plates. An *Aspergillus* species (likely an endophytic fungus) was the only other species growing on the agar, and was present in all control plates and in 53% of the inoculated treatment plates. Based on growth-rate measurements at 3 weeks, the *Aspergillus* species grew 9.4 times slower than *D. sapinea* in inoculated treatment plates and 2.8 times slower in control plates (*i.e.*, plates with no *D. sapinea* competition; **Fig. 5**).

### Management recommendations

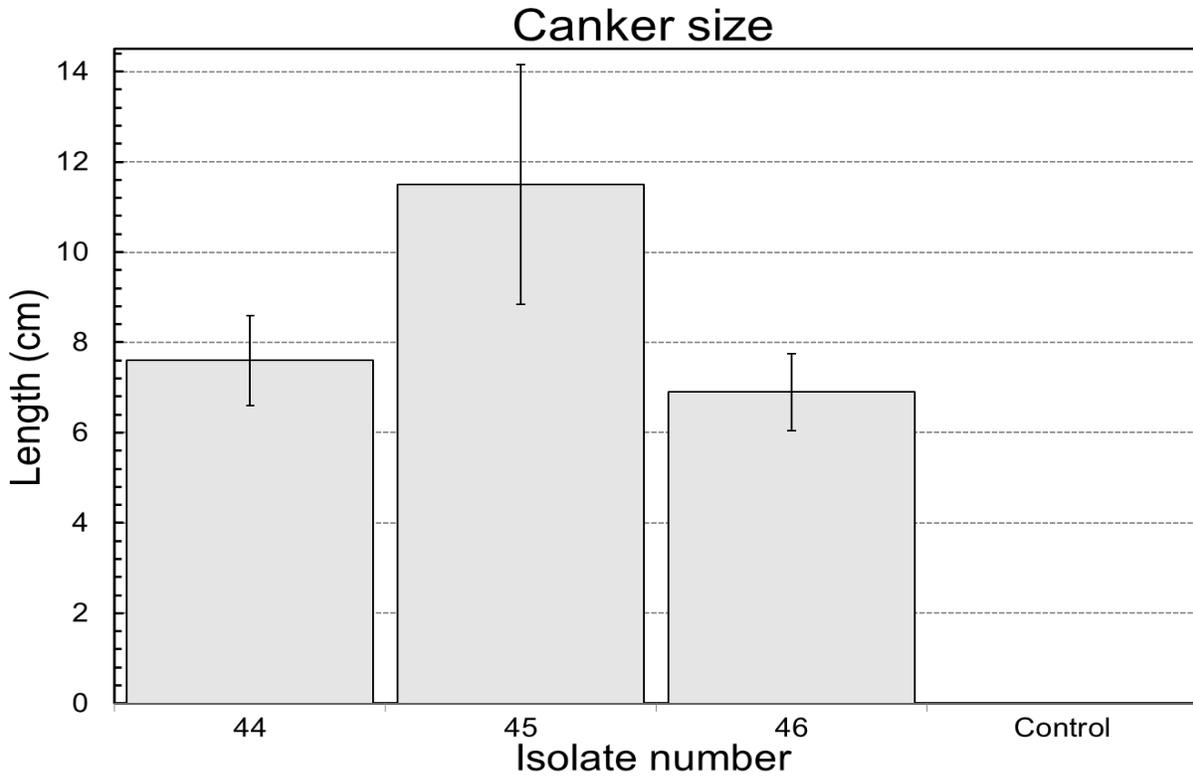
*Diplodia sapinea* is present in Wyoming (**Fig. 6**) and the Wyoming isolates are aggressive pathogens of ponderosa pine. Reducing water stress and maintaining tree vigor are the best options to control *Diplodia* shoot blight and canker disease. In forested sites, this can be done by stand thinning and/or managing competing vegetation. The selection of non-host species might be an option in some areas.



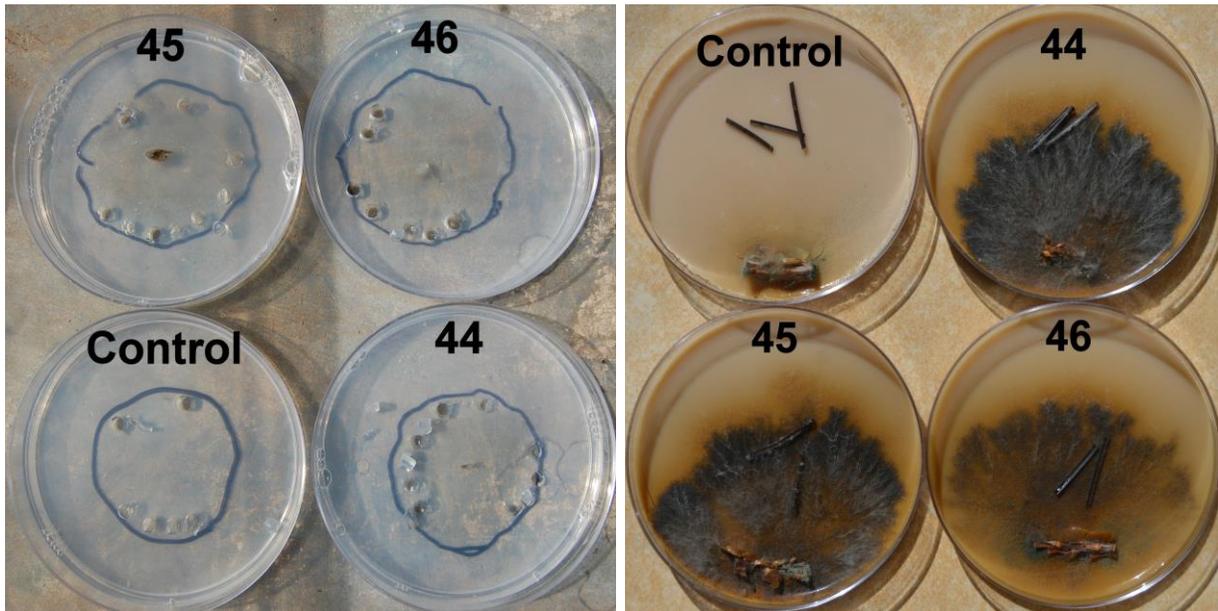
**Figure 1.** From left-to-right: unwounded ponderosa pine, trees wounded by removing a single needle fascial with a scalpel blade, a colonized agar plug was placed over the wound (for controls a sterile agar plug was used), and Parafilm was then wrapped around the shoot centered at the wound site.



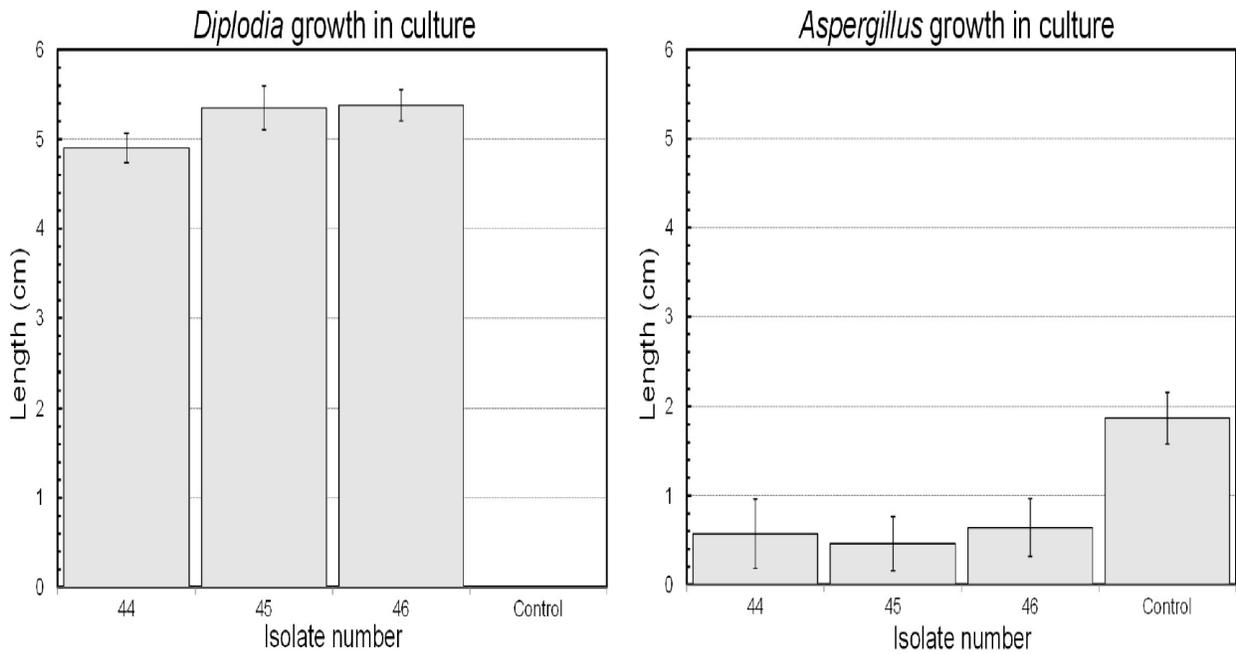
**Figure 2.** From left-to-right: *Diplodia* shoot blight symptoms at 5 days, shoot blight and canker symptoms at 4 weeks (2 photos inoculated with different isolates), and a control at 4 weeks. Far-left photo by Bailey Maca, USDA-Forest Service.



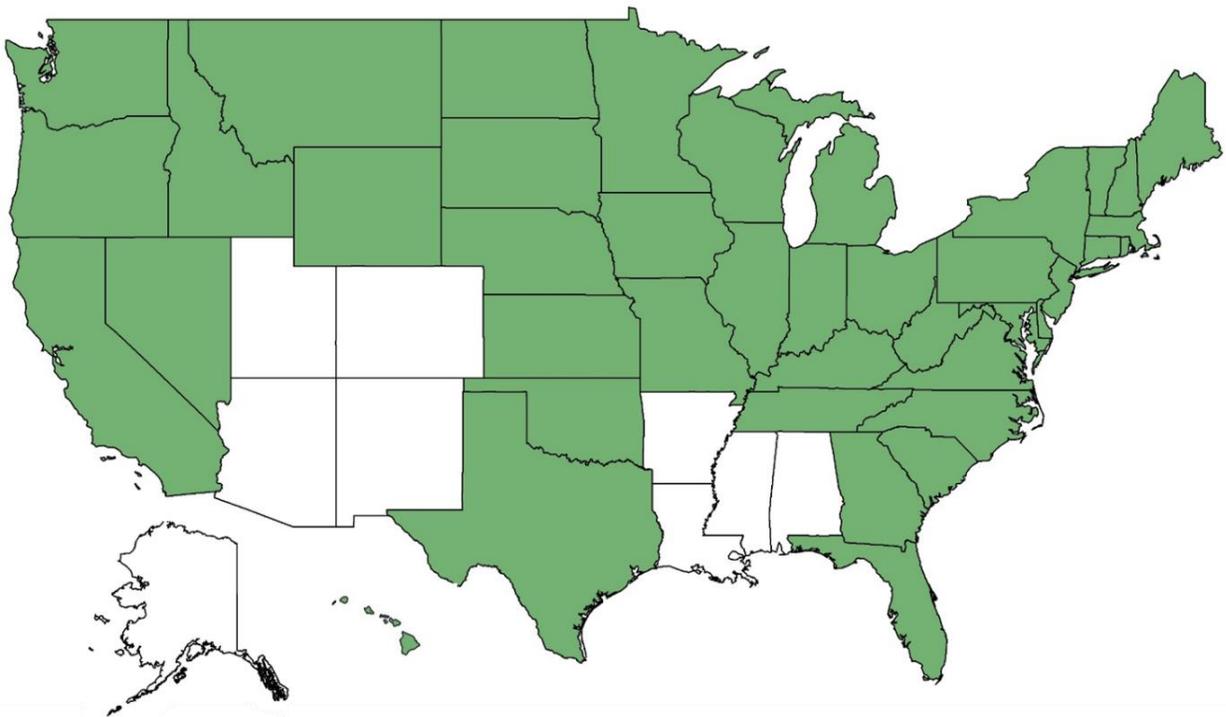
**Figure 3.** Mean *Diplodia sapinea* canker size at 4 weeks measured from the inoculation site down the stem (one direction). All trees inoculated with three Wyoming isolates developed symptoms and none of the controls developed symptoms. All inoculation treatments significantly differed from controls ( $P < 0.001$ ). Error bars are one standard error.



**Figure 4.** From left-to-right: Water agar plates used during inoculations and tannic acid agar plates used for isolations at 3 weeks. Numbers are the *Diplodia sapinea* isolate numbers and controls contain no *Diplodia sapinea*.



**Figure 5.** From left-to-right: mean *Diplodia* and *Aspergillus* growth in one direction after 3 weeks on tannic acid agar. Error bars are one standard error.



**Figure 6.** *Diplodia sapinea* is present in states with green shading.