

1 **First Report of Gummosis Caused by *Phytophthora frigida* on Black Wattle in**  
 2 **Brazil**

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 11 Black wattle (*Acacia mearnsii*), a tree species native to Australia, is considered the main  
 12 source of bark for the tannin industry worldwide. It is the third most cultivated forest  
 13 species in Brazil. Gummosis, caused by *Phytophthora* spp., is a major disease affecting  
 14 black wattle plantations in Brazil, where the disease incidence can reach up to 43%. The  
 15 most common disease symptoms are dark brown, irregular, necrotic lesions on the  
 16 trunk, which may or may not be accompanied by gum exudation. Severe infection can  
 17 lead to plant death. *Phytophthora nicotianiae* and *P. bohemeriae* were reported as  
 18 causative agents of black wattle gummosis in Brazil (Santos et al. 2006). In South  
 19 Africa, besides these species, *P. meadii* was also recorded on black wattle (Roux and  
 20 Wingfield 1997) and *P. frigida* on green wattle (*A. decurrens*) (Maseko et al. 2007). A  
 21 survey in six-year-old black wattle plantations located in the Piratini and Cristal  
 22 counties in the state of Rio Grande do Sul in 2008 revealed the occurrence of a third  
 23 *Phytophthora* species causing gummosis on black wattle in Brazil, *P. frigida*. Isolates  
 24 were obtained from bark tissue of 24 diseased trees, and all were identified as *P. frigida*  
 25 based on morphological characteristics, and the sequence of portions of the internal

26 transcribed sequences (ITS) of ribosomal DNA, and the cytochrome oxidase subunits I  
27 (*coxI*) and II (*coxII*) genes. Morphological characterization of colonies on carrot-agar  
28 medium (CA) revealed persistent sporangia with prominent papilla, formed individually  
29 or in loose sympodium. The dimensions of sporangia ranged from 29 to 71  $\mu\text{m} \times 20$  to  
30 53  $\mu\text{m}$  (avg. 46  $\times$  33  $\mu\text{m}$ ), with length-to-breadth ratios of 1.3 to 1.5 (avg. 1.4). The  
31 sporangial shape was predominantly ovoid. The colony growth rate was 12 mm/d at 24  
32 to 30°C. The isolates produced globose chlamydospores, terminal or intercalary, and  
33 measured 21 to 55  $\mu\text{m}$  diameter (avg. 32  $\mu\text{m}$ ). All isolates tested were heterothallic and  
34 produced oospores globose, aplerotic, 18 to 31  $\mu\text{m}$  (avg. 24  $\mu\text{m}$ ) in diameter, with  
35 amphigenous antheridium. Oogonium diameter was from 22 to 37  $\mu\text{m}$  (avg. 30  $\mu\text{m}$ ).  
36 Portions of the ITS (815 bp) and the *coxI* (654 bp) and *coxII* (945 bp) were amplified by  
37 PCR. BLAST search of the GenBank database revealed that the fragments for ITS  
38 (KU570067), and *coxI* (KU570065), and *coxII* (KU570066) sequence fragments from  
39 isolate P92 were 99-100% similar with the accessions of *P. frigida* HQ261569 and  
40 HQ261316 (Robideau et al. 2011). To confirm pathogenicity, the 24 isolates of *P.*  
41 *frigida* were used to inoculate 10 one-year old black wattle plants. For inoculation, a  
42 mycelial plug from a one-week-old isolate grown on CA was placed on a stem wound  
43 made with a cork borer (6 mm diam.) and sealed with a strip of parafilm. Plants were  
44 kept under greenhouse conditions at temperatures ranging from 22 to 32 °C. Four weeks  
45 after inoculation, the stems of the control plants, inoculated with sterile CA plugs, only  
46 showed small dark brown spots at the inoculation points. The *P. frigida* inoculated  
47 stems exhibited necrotic lesions up to 4 cm in length, with presence or absence of gum.  
48 *Phytophthora frigida* was re-isolated from each infected stem. Worldwide, this is the  
49 first report of *P. frigida* occurring in *A. mearnsii*.

50 **References:**

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