

# First Report of *Phaeocystostroma ambiguum* Causing Maize Stalk Rot in Brazil

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## DISEASE NOTES

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## ABSTRACT

Maize stalk (*Zea mays* L.) of three hybrids (2B512PW, 2B810PW, and DKB390PRO) showing external and internal disease symptoms—light brown lesions or blackening of lower internodes, and dark brown pith tissue—were collected from the central-west region of Brazil. The samples were collected in July 2015 and submitted to the plant pathology laboratory at the National Maize and Sorghum Research Center (CNPMS-EMBRAPA) located in the Brazilian state of Minas Gerais. The symptoms were typical of maize stalk rot (SR) in Brazil, a disease mainly caused by the following fungi: *Colletotrichum graminicola*, *Macrophomina phaseolina*, *Fusarium graminearum*, *F. verticillioides* (syn. *F. moniliforme*), *Stenocarpella macrospora*, and *S. maydis*. To prepare the

isolations, stalk fragments were removed from the surface bordering the lesions and disinfested in 0.5% sodium hypochlorite for 2 min before plating on an oatmeal agar medium (OMA) with tetracycline. Initially the colonies were white, similar to members of *Stenocarpella*. However, their alpha conidia had several distinguishing characteristics: medium brown; smooth, ellipsoid, to pyriform; widest in the middle, apex bluntly rounded, base truncate; and sizes ranging from 10 to 19  $\mu\text{m}$  long and 4 to 6  $\mu\text{m}$  wide ( $n = 50$ ). These morphological characteristics are very similar to descriptions of *Phaeocystroma ambiguum* (Mont.) Petrak (Lamprecht et al. 2011). Three monoconidial isolates (CFMS 1293, CFMS 1294, and CFMS 1295) recovered from different plants and locations were deposited in the Plant Pathogens Collection of CNPMS. For these three isolates, the DNA of the internal transcribed spacer (ITS) region and that of the translation elongation factor 1-alpha gene (TEF) were sequenced (Carbone and Kohn 1999; White et al. 1990). All sequences were deposited in GenBank with accession nos. KU323506, KU323507, KU323508; and KU351846, KU351847, KU351848 for ITS and TEF, respectively. The isolates were 99% identical to *P. ambiguum*, accession nos. FR748044.1, FR748043.1 (ITS); and FR748066.1, FR748068.1 (TEF). To test pathogenicity, a toothpick immersed in the spore suspension ( $10^6$  conidia  $\text{ml}^{-1}$ ) was inserted into the third internode (disinfested with 70% alcohol) of maize plants in the tasseling stage. Three maize hybrids P3646YH, P30F53YH, and DKB390PRO were inoculated with the isolates CFMS 1292 and CFMS 1295, in addition to three plants without the fungi (control). In the evaluation performed 30 days later, the inoculated maize plants showed typical SR symptoms, and the morphological features of the fungi that were reisolated were identical to those of the *P. ambiguum* isolates used for the inoculation, thus fulfilling Koch's postulates. The experiments were performed twice, and in both cases, the control plants did not exhibit disease symptoms. Although *P. ambiguum* is reported to cause maize SR in Australia, Bulgaria, France, North America, Tanzania, and South Africa (Bobev et al. 2016; Farr and Rossman 2016), to our knowledge, this is the first report of maize SR caused by *P. ambiguum* in Brazil, which is the world's third-largest producer of maize with a planted area of 15.7 million ha. Hence, this report has great importance for the correct identification of the pathogen associated with maize SR in Brazil, and for the development of appropriate management strategies for controlling this disease.

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