

1 **First report of stalk rot of maize caused by *Phaeocystostroma ambiguum* in the**
2 **Iberian Peninsula**

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10 Maize (*Zea mays* L.) with symptoms of premature leaf senescence and wilting was
11 observed in two commercial fields, in Santarem (Portugal) and Almodovar del Río
12 (Spain), in the summer of 2014. Similar symptoms including brown lesions of lower
13 internodes and disintegrated stalk pith tissues occurred again in two commercial fields
14 in Almacelles and Biota (Spain), in the summers of 2016 and 2017, respectively.
15 Depending on the field, estimated disease incidence varied between 10 and 30%.
16 Lodging was observed on 10-20% of maize plants in the two commercial fields
17 following rain and strong wind. Four symptomatic plants were collected from each of
18 the four fields. Their first internodes were excised, surface sterilized for 5 min in 10%
19 sodium hypochlorite solution, rinsed in water, and air dried. Cross sections were
20 incubated in potato-dextrose-agar (PDA) at 25°C in darkness. Fungal colonies with
21 similar morphological features were isolated from all the samples. One colony from
22 each location was selected and two single spore isolates obtained. Colonies grown on
23 PDA were white-beige, flat with floury appearance at the top and light grey turning to
24 irregular shapes of black with age at the bottom. Globose pycnidia similar to those of

25 *Stenocarpella maydis* (de la Riva et al. 2019) were observed. The fungus was
26 tentatively identified as *Phaeocystroma ambiguum* (Mont.) Petr. in Petr. & Syd. (syn.
27 *Phaeocytospora zea* G. L. Stout) (White 1999). The region consisting of the 5.8S
28 ribosomal DNA and internal transcribed spacers (ITS) 1 and 2 was amplified with the
29 primer set ITS5/ITS4. PCR products were sequenced and sequence data were
30 deposited in GenBank (acc. MK249746). The query sequence was 99% identical to the
31 *P. ambiguum* (acc. FR748048.1) in the NCBI database. The pathogenicity was
32 confirmed in an open-air enclosure from March to July of 2018. In order to enhance
33 root infection by this soil-borne fungus, four-day-old seedlings of the hybrid MO1501
34 (Monsanto Spain SL) were used as plant material. They were transplanted in 5 l pots
35 filled with sand:silt:peat moss (2:1:2) (SSP) homogeneously infested with *P. ambiguum*
36 (Biota-2017 isolate) colonized wheat grains (Ortiz-Bustos et al., 2016). Seedlings of the
37 controls were transplanted in non-infested SSP. In a different group of plants, another
38 inoculation method was assayed by immersing a toothpick in a suspension of 10^6
39 conidia per ml or in deionized water (control) for 6 hours and then inserting it into the
40 third internode of each plant in the tasseling stage (Aguar et al. 2016). For each
41 inoculation method, the experimental unit consisted of one plant (pot) and six
42 replications were established for each treatment according to a complete randomized
43 design. Greater percent leaf senescence (LS) per plant was observed 9 weeks after
44 transplanting in infested soil (11.7% compared to 4.2% in the controls) ($P = 0.0276$). At
45 the end of the experiment, significant differences (*sd*) of LS were again observed: 55%
46 in inoculated maize as compared to 30.1% in the controls ($P < 0.0001$), as well as *sd* of
47 height ($P = 0.0050$) (178 and 242 cm in inoculated and control plants, respectively).
48 With toothpick insertion, *sd* of LS only occurred at final time (30.8 and 21.7% in

49 inoculated and control plants, respectively). Symptoms were reproduced with either
50 inoculation method but, after transplant to infested soil, they appeared sooner and
51 were greater at the end of the experiment. The fungus was re-isolated from tissues of
52 the first internode of three plants inoculated by each method, fulfilling Koch's
53 postulate. In Europe, *P. ambiguum* has been reported as causing stalk rot of maize in
54 Bulgaria and France (Farr and Rossman 2016), but this is the first report on the
55 pathogen shift to warmer areas (Spain and Portugal), and suggests that appropriate
56 measures must be taken for controlling the disease.

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58 **References**

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Phaeocystostroma ambiguin maize



A. Lodged plants following rain and strong wind; **B.** Morphological features of *P. ambiguum* colonies on PDA (top and bottom of the plates) **C.** Symptoms caused by *P. ambiguum* in maize plants 12 weeks after inoculation (control to the left, plants inoculated by transplant in infested soil in the center, plants inoculated by insertion of colonized toothpick to the right).