1	First report of stalk rot of maize caused by Phaeocytostroma 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 tentatively identified as *Phaeocytostroma ambiguum* (Mont.) Petr. in Petr. & Syd. (syn. 26 Phaeocytosporella zeae G. L. Stout) (White 1999). The region consisting of the 5.8S 27 ribosomal DNA and internal transcribed spacers (ITS) 1 and 2 was amplified with the 28 primer set ITS5/ITS4. PCR products were sequenced and sequence data were 29 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 root infection by this soil-borne fungus, four-day-old seedlings of the hybrid MO1501 33 (Monsanto Spain SL) were used as plant material. They were transplanted in 5 l pots 34 35 filled with sand:silt:peat moss (2:1:2) (SSP) homogenously infested with P. ambiguum 36 (Biota-2017 isolate) colonized wheat grains (Ortiz-Bustos et al., 2016). Seedlings of the controls were transplanted in non-infested SSP. In a different group of plants, another 37 inoculation method was assayed by immersing a toothpick in a suspension of 10<sup>6</sup> 38 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 (Aguiar 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

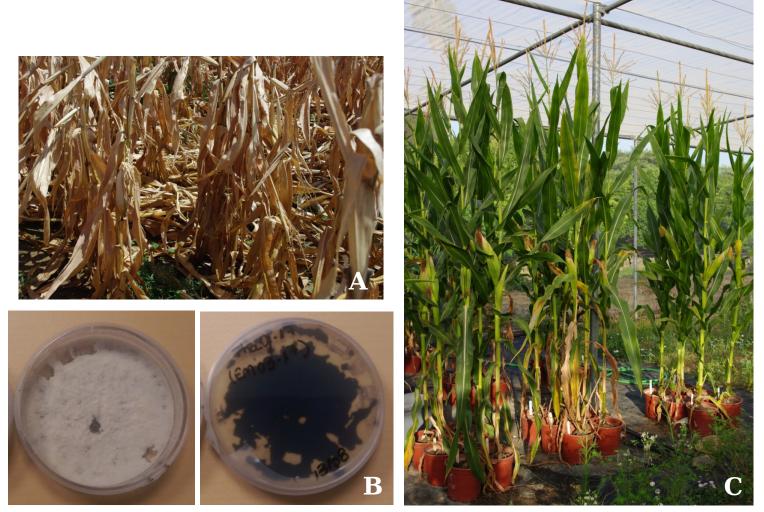
49 inoculated and control plants, respectively). Symptoms were reproduced with either inoculation method but, after transplant to infested soil, they appeared sooner and 50 were greater at the end of the experiment. The fungus was re-isolated from tissues of 51 the first internode of three plants inoculated by each method, fulfilling Koch's 52 postulate. In Europe, P. ambiguum has been reported as causing stalk rot of maize in 53 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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## Phaeocytostroma ambiguumin maize



**A.** Lodged plantsfollowing rain and strongwind; **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).