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DISEASE NOTES

First Report of Root Rot on *Rosmarinus officinalis* Caused by *Ceratorhiza fragariae* (Binucleate *Rhizoctonia*) in Spain

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Citation

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Rosemary (*Rosmarinus officinalis* L.) (*Labiatae*) is an evergreen, woody shrub natural to the Mediterranean basin, and is widely cultivated for ornamental and medicinal purposes. In winter of 2016, garden beds in a shopping center of Zaragoza (northeast Spain) planted with 6-year-old rosemary plants (both semierect and prostrate cultivars) exhibited up to 60% of plants diseased with clear symptoms of plant stunting, reflected in a progressive drying of loose branches and eventually the collapse of the whole plant. Infected individuals did not respond to fertilization and were uprooted. At the root level, there was little development with presence of blackish secondary roots and the central core showing a grayish to rusty-brown coloration. Progress of symptoms was seen in both upright and prostrate cultivars. To isolate the responsible fungus, leaf, stem and root fragments from five plants were taken from the diseased tissues, surface sterilized for 30 s in 70% ethanol, 3 min 5% NaCl, rinsed in sterile water, and then placed on potato dextrose agar (PDA, Difco). Isolates obtained from all root fragments exhibited the typical morphology described for the so-called binucleate *Rhizoctonia* taxa, such as their light to dark brown, radially zonate colonies, hyphal branching pattern, growth rate, lack of any reproductive structures, and the presence of loose clusters of monilioid cells forming sclerotium-like structures of 1 to 2.2 mm diameter (Roberts 1999). ITS region of the fungus was amplified using the primers ITS1/ITS4 and sequenced (GenBank Accession No. KY624588). BLASTn comparison (Altschul et al. 1997) of the 630-bp product obtained showed a 98% similarity with several sequences of *Ceratobasidium* /*Rhizoctonia* sp. AG-G (e.g., JF519837). To confirm identification, ITS sequence was introduced in a multiple alignment including sequences from most AG testers of the *Ceratobasidiaceae* (multi- and binucleate species). After performing a maximum-likelihood phylogeny of these sequences, isolates from rosemary plants were determined to belong to *Rhizoctonia fragariae* (*Ceratorhiza fragariae* AG-G). For pathogenicity tests, fungal inoculum was prepared by colonizing wheat grains

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previously autoclaved for 3 h and grown in petri dishes for 10 days at 25°C in the dark. Eighteen one-year rosemary plants (12 infested with *R. fragariae*, and three positive and three negative controls), were grown in 0.2-liter pots with an autoclaved substrate of moss peat and vermiculite (3:1), and treated with colonized wheat grains at 5 g/liter. Plants were covered with plastic bags and cultured in a growth chamber at 25°C in a 12-h photoperiod for 20 days. After 12 to 15 days, symptoms similar to those reported from diseased gardening plants were observed in the test group, and binucleate *Rhizoctonia* were reisolated from all these plants. Controls remained healthy and asymptomatic. Although *Rhizoctonia solani* has been previously reported on *R. officinalis* in other continents including Europe (Garibaldi et al. 2013), to our knowledge this is the first report of a root rot caused by a pathogenic binucleate *Rhizoctonia* species on rosemary. This fungus is associated with black root rot of strawberry (Martin 1988), and its presence on *R. officinalis* should be checked due to the shrub's importance both as an ornamental and natural taxon worldwide.

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Section:

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