

1 **First report of *Neocosmospora falciformis* causing wilt and root rot of Muskmelon**
2 **in Spain**

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20 ‘Cantaloupe’ and ‘Piel de Sapo’ are melon (*Cucumis melo* L.) varieties cultivated in
21 Spain. In 2018, during a pathogens survey in experimental fields of Valencia and
22 Alicante provinces (SE Spain), wilt and root rot of melon plants were detected in
23 grafted and ungrafted plants. Disease incidence ranged from 10% (Alicante) to 45%
24 (Valencia). Symptoms included yellowing and wilting of leaves, rotting at the stem base
25 and upper root and collapse of the entire plant. Samplings were conducted from severely
26 decayed and dead plants. Fragments (0.5-1 cm) from rotted lower stems and roots were
27 surface disinfected for 1 min in 1.5% NaOCl, washed twice with sterilized distilled
28 water and plated onto potato dextrose agar (PDA) with streptomycin sulphate (0.5 g L⁻¹).
29 Plates were incubated at 25°C in the dark for 3-5 days. Mycelia resembling *Fusarium*
30 were isolated and characterized by morphological and molecular methods. Based on
31 their adpressed beige mycelia, growth in concentric rings and the absence of

32 sporodochia, colonies growing on PDA and Spezieller Nährstoffarmer Agar (SNA)
33 were preliminary identified as belonging to the *Fusarium solani* species complex
34 (FSSC). On PDA, colonies were white-greyish to pale-cream growing in concentric
35 rings with beige reverse after 6 days. No sporodochia were observed. Macroconidia
36 were slender, falcate, hyaline, 3-5 septate 43 (38-47) x 4.5 (3.8-5.2) μm ; aerial
37 microconidia were abundant, borne on short, undifferentiated monophialides, ellipsoidal
38 to reniform, sometimes with truncate base, 0-1 septate 10 (9.2-11.4) x 3.5 (2.5-6) μm .
39 Chlamydospores were globose, single or in chains, intercalary and thin to thick-walled.
40 Sequencing of the ITS region, a fragment of translation elongation factor-1 α (TEF-1 α)
41 and RNA Polymerase II (RPB2) partial genes was done using ITS1/ITS4 (White et al.,
42 1990), EF1/ EF2 (O'Donnell et al., 1998) and fRPB2-7cF / fRPB2-11aR (Reeb et al.,
43 2004) primers, respectively. After comparisons using BLASTn and Fusarium ID
44 Database (<http://www.westerdijkinstituut.nl/fusarium/>), eight isolates were identified as
45 *Neocosmospora falciformis*. The ITS, EF-1 α and RPB2 sequences of isolate CRR 2-6
46 showed 99% homology with *Neocosmospora falciformis* EU329691 (ITS), AB817158
47 (EF-1 α) and EU329650 (RPB2). Sequences were deposited in GenBank with accession
48 numbers MN086327 (ITS), MN509809 (TEF-1 α) and MN509810 (RPB2). For
49 pathogenicity tests, isolate CRR 2-6 was grown in 250 ml flasks containing potato
50 sucrose medium for 3 days at 25°C in the dark with constant agitation. Roots of ten 15-
51 day-old 'Piel de Sapo' seedlings grown 6 days in trays with sterilized substrate were
52 submerged into a suspension of 5 x 10⁶ conidia/ml for 2 min, and transferred to the
53 plastic. Three plants submerged in sterile water served as controls. Plants were
54 incubated in a growth chamber (25°C; 16/8 h photoperiod). Scarce development, wilting
55 and yellowing, followed by plant death were observed 15 days post-inoculation. Non-
56 inoculated controls remained asymptomatic. The fungus was re-isolated from all the

57 inoculated plants and identified using ITS, TEF-1 α and RPB2. *Neocosmospora*
58 *falciformis* belongs to the *Neocosmospora (Fusarium) solani* species complex
59 (O'Donnell et al., 2008). To our knowledge, this is the first report of *N. falciformis*
60 causing wilt and root rot of melon in Spain. The adoption of molecular-based
61 identification methods should lead to a more precise determination on incidence of the
62 pathogen in this Mediterranean area.

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