

Document downloaded from:

<http://hdl.handle.net/10251/82759>

This paper must be cited as:

López- Cortés, I.; Salazar Hernández, DM. (2014). First report of *Alternaria* black spot of pomegranate caused by *Alternaria alternata* in Spain. *Plant Disease*. 98(5):689-689.
doi:10.1094/PDIS-07-13-0717-PDN.



The final publication is available at

<http://doi.org/10.1094/PDIS-07-13-0717-PDN>

Copyright American Phytopathological Society

Additional Information

First report of *Alternaria* black spot of pomegranate caused by *Alternaria alternata* in Spain. M. Berbegal, Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia, Spain; and I. López-Cortés and D. Salazar, Departamento de Producción Vegetal, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022, Valencia, Spain; and D. Gramaje, A. Pérez-Sierra, J. García-Jiménez and J. Armengol, Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022-Valencia, Spain.

Since 2010, a new foliar and fruit disease was observed in pomegranate (*Punica granatum* L.) orchards in Alicante province (eastern Spain). Symptoms included black spots on leaves and fruits, as well as chlorosis and premature abscission of leaves. Fungal isolates were obtained by surface-disinfecting small fragments of symptomatic leaf and fruit tissues in 0.5% NaOCl, double-rinsing in sterile water, and plating them onto potato dextrose agar (PDA) amended with 0.5 g/L of streptomycin sulfate. Gray-to-black colonies were obtained, which were identified as *Alternaria* sp. based on the dark, brown, obclavate to obpyriform catenulate conidia with longitudinal and transverse septa tapering to a prominent beak attached in chains on a simple and short conidiophore (4). Conidia (no. = 100) measured (12.2-) 20.2 (-27.6) x (5.7-) 9.2 (-12.0) μm , and had 3 to 6 transverse and 0 to 5 longitudinal septa. Single spore cultures were obtained and their genomic DNA was extracted. The internal transcribed spacer (ITS) region of rDNA and partial sequences of the beta tubulin gene were amplified and sequenced with primers ITS1-ITS4 and Bt1a-Bt1b, respectively (3). BLAST analysis of the sequences showed that they were 100% identical to a pathogenic *A. alternata* (Fr.) Keissl. isolate obtained from black spot disease of pomegranate in Israel (Accession no. JN247826.1, ITS and Accession no. JN247836.1, beta tubulin) (2). As all the sequences obtained showed 100% homology, ITS and beta tubulin sequences of a representative isolate (1516B) were submitted to GenBank (Accession nos. KF199871 and KF199872, respectively). In addition, a PCR reaction with specific primers (*C_for/C_rev*) designed to recognize highly virulent isolates of *A. alternata* causing black spot of pomegranate was used with all isolates (2). A characteristic fragment of ~950 bp was amplified in two isolates: 1552B and 1707B. Pathogenicity was assessed on plants and detached fruit of pomegranate cv. Mollar (1). Two-year-old pomegranate trees were inoculated with isolates 1552B and 1707B by spraying a conidial suspension (10^6 conidia/mL) onto the upper and lower leaf surfaces. Five plants per fungal isolate were used and five control plants were sprayed with sterile water. Plants were covered with transparent plastic bags and incubated in a growth chamber for 1 month at 25°C, with a 12-h photoperiod. One month old fruit were surface sterilized in 1.5% sodium hypochlorite solution for 1 min and rinsed twice in water. Two filter paper squares (5 x 5 mm) were dipped in the conidial suspensions and placed on the fruit surface. Inoculated fruit were incubated in a humid chamber in the dark at 25 °C. Ten fruit per fungal isolate were used and ten control fruit were inoculated with sterile water. Black spots were visible on inoculated leaves and fruit, 10 and 3 days after inoculation, respectively. Symptoms were not observed on controls. The fungus was reisolated from leaf and fruit lesions, confirming Koch's postulates. Leaf black spot of pomegranate caused by *A. alternata* was first described in India in 1988, and later in Israel in 2010 affecting both fruit and leaves (1). To our knowledge, this is the first report of the disease in Spain, where it could represent a threat for pomegranate cultivation due to the increasing amount of area dedicated to this crop.

References: (1) D. Ezra et al. Australas. Plant Dis. Notes. 5:1, 2010. (2) T. Gat et al. Plant Dis. 96:1513, 2012. (3) N.L. Glass and G.C. Donaldson. Appl. Environ. Microb. 61:1323, 1995. (4) E. G. Simmons. Alternaria: An identification manual. CBS Fungal Biodiversity Center, Utrecht, Netherlands, 2007.