First report of *Alternaria* black spot of pomegranate caused by *Alternaria alternata* in Israel

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Abstract. Black spot disease of pomegranate is a new disease caused by *Alternaria alternata* on pomegranates in Israel. Symptoms can be seen on the leaves and fruits but no damage to the inner edible tissue is found. The causal agent was isolated and Koch Postulates were demonstrated. This is the first report of black spot disease of pomegranate in Israel.

In 2004 a new disease was observed in pomegranate (*Punica granatum*) orchards in Israel. Black spot symptoms were noted on leaves and fruits, ranging from a single spot to more than 50% of the fruit surface (Fig. 1); some leaves became chlorotic and abscised (Fig. 2). Spots, which appeared at the start of the summer on all cultivars, were small (1–3 mm) and round on fruit and round to irregular (1–4 mm) on leaves. Each spot consisted of a green-yellow halo surrounding a necrotic lesion.

Fig. 1. *Alternaria* black spots of pomegranate fruits caused by *A. alternata*.

Damage to fruit was limited to the peel surface; the edible tissue was not damaged.

Symptomatic fruits and leaves were collected during the summer of 2007. Lesion pieces were plated on potato dextrose agar (PDA) amended with 12 µg/L tetracycline and incubated at 25°C. Fungal colonies emerging from symptomatic tissue had morphology and conidia typical of *Alternaria* spp. Sequence analysis of the rDNA ITS region and β-tubulin gene of four different pathogenic isolates (A17, D2, 49, 53) from different cultivars were found to be identical (i.e. A17 GenBank accessions GQ240306, GQ240308), exhibiting 100% identity to *A. alternata* (accessions FJ946480; EU139350). Polymerase chain reaction (PCR) specific primers, apPCR-based) revealed differences between *A. alternata* isolated from pomegranates affected by 'black rot' and *A. alternata* isolated from 'black spot' disease

Fig. 2. *Alternaria* black spots of pomegranate leaves caused by *A. alternata*.

Fig. 3. Polymerase chain reaction (PCR) on *A. alternata* from pomegranate. Specific primers were designed based on differences found in apPCR performed on pathogenic and non-pathogenic isolates from pomegranate. 1. λ HindIII/EcoRI DNA marker. 2. Isolate of *A. alternata* from black spot (displays symptoms on Pomegranate leaves and fruits). 3. *A. alternata* isolated from black rot infected Pomegranate (number 83). 4. *Alternaria* isolate from black spot. 5. H₂O control.

(Fig. 3). Other *Alternaria* isolates from non-symptomatic pomegranate and other fruits, pathogenic and non-pathogenic, were not identified by the designed primers (data not shown).

Laboratory pathogenicity was assessed by detached leaf and fruit inoculation. Drops (10 μ L) of a 10^6 conidia/mL suspension, from 50 different isolates, were inoculated on leaves (~2 weeks

Fig. 4. Detached leaf inoculation test with pathogenic (black spot) and black rot *A. alternata* isolates. The black rot isolate caused no symptoms while the pathogenic isolates caused necrosis of the inoculated area. 1. H₂O control, 2. Black rot (internal rot) *A. alternata* isolate 83. 3. Pathogenic black spot *A. alternata* isolate 48. 4. Pathogenic black spot *A. alternata* isolate 43.

old), fruit (~2 months old) and flowers, placed in a moist chamber (25°C). Symptoms appeared 48–72 h later and were similar to those observed in the field. Koch's Postulates were completed by inoculation of greenhouse plants (25°C, 14 h photoperiod). Plants (3–6 months old), cvv. Wonderful and Shami, were sprayed or drop-inoculated with a 10^6 conidia/mL suspension and covered with plastic bags for 1 month. Black spots appeared on leaves approximately 1 week after inoculation; the fungus was reisolated from infected leaves. *Alternaria alternata* from 'black rot' did not cause symptoms when inoculated on leaves (Fig. 4). This is the first report of *A. alternata* causing black spot disease of pomegranate in Israel. We believe that this is the first record of *Alternaria* black spot on pomegranate fruit while leaf spot has been previously reported in India (Madhukar and Reddy 1988).

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Reference

Madhukar J, Reddy SM (1988) Some new leaf spot diseases of pomegranate. *Indian Journal of Mycology and Plant Pathology* **18**, 171–172.

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