New Disease Reports

First report of *Pseudomonas syringae* pv. *alisalensis* causing bacterial blight of arugula (*Eruca vesicaria* subsp. *sativa*) in Greece

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In March of 2007, leaf symptoms were observed on two commercial fields of arugula (*Eruca vesicaria* subsp. *sativa*) grown in Heraklion, Crete. Approximately 20% of plants were affected. Likewise, similar symptoms were observed on samples which were collected from freshly packeted arugula leaves in the market, and plants originating from the areas of West Peloponnese (Ilia) and Attica (Marathonas and Megara) in Greece. In the early infection stage, small angular water-soaked spots (2-5mm) were observed on leaves, often surrounded by chlorotic haloes visible on both leaf surfaces. At later stages these water-soaked lesions became necrotic with a brown to tan centre, remaining angular in shape, expanding progressively and resulting in leaf collapse (Fig. 1 A, C). Pure cultures of blue-green fluorescent bacteria (aerobic Gram negative rods) were consistently isolated on King's medium B from leaf lesions of infected plants. In LOPAT assays, arugula isolates exhibited the phenotype [+ - - - +] of Lelliot's Ia group, which includes *Pseudomonas syringae* pathovars. Biochemical tests were performed as described by Goumas *et al.*(1999). All the arugula isolates tested were positive for D(-) tartrate utilisation while negative for erythritol. Trigonelline and ascorbic acid tests were able to differentiate those isolates from *Pseudomonas syringae* pv. *maculicola*. Based on these results, we hypothesized that the isolates correspond to *Pseudomonas syringae* pv. *alisalensis*, recently renamed as *P. cannabina pv. alisalensis* (Cintas *et al.,* 2002; Bull *et al.,* 2004; Bull *et al*., 2010).

To confirm our hypothesis, we performed both BOX and ERIC-PCR, as described by Weingart & Völksch (1997) and Marques *et al.,* (2008), on two out of ten isolates (R1-3 and R6) using *P. s.* pv. *maculicola* (CFBP 1657), *P. s.* pv. *tomato* (DC3000)and *P. s.* pv. *alisalensis* (CFBP 6866 and CFBP 6870) as controls. The BOX and ERIC-PCR patterns obtained with *P. s.* pv. *alisalensis* CFBP 6866 and CFBP 6870 and R1-3 and R6 isolates were identical, while those obtained with *P. s.* pv. *maculicola* and *P. s.* pv. *tomato* were clearly different (Fig. 2). Cultures were grown on nutrient agar for 24h at 27oC and adjusted in sterile water to 1 x 106 cfu/ml. Arugula leaves of two to three-week-old plants were inoculated by spraying until runoff with a hand mister. Inoculated plants were incubated in plastic polyethylene bags with high humidity for 48h at 22-2°C. Sterile distilled water was used as control. Symptoms were visible one week after inoculation and similar to those observed under natural infection (Fig.1B-C, Fig 3). To fulfil Koch's postulates we re-isolated *P. s.* pv. *alisalensis* from symptomatic tissue of arugula plants. Control plants remained symptomless. Using similar inoculation and incubation methods (except for spot inoculating detached Brussels sprouts heads with 10 µl of bacterial suspension at 1x108 cfu/ml in sterile water), these isolates were found to be pathogenic on broccoli (*Brassica oleracea* var. *italica*), brussel sprouts (*B. oleracea* var. *gemmifera*), turnip (*B. napus*) and broccoli raab (*B. rapa* var. *rapa*), radish (*Raphanus sativus*), oats (*Avena sativa*) and tomato (*Solanum lycopersicum*). Based on morphological, biochemical, physiological, molecular and pathogenicity tests, we conclude that the arugula isolates identified here belong to *P. s.* pv. *alisalensis*. To our knowledge this is the first report of *P. s.* pv. *alisalensis* in the European area (Bull *et al.,* 2010). Two isolates (R1-3 and R6) isolates have been deposited in the Benaki Phytopathological Institute Collection, Greece with the reference numbers BPIC 2127 and BPIC 2128 respectively.

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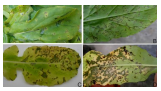

Figure 1

Figure 2

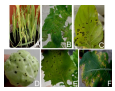

Figure 3

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