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First report of bacterial spot caused by *Xanthomonas cucurbitae* on pumpkin in Italy

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In 2018, a disease outbreak was observed on pumpkin (*Cucurbita moschata* cv. Violina) in the municipality of Reggio Emilia (Northern Italy). Symptoms were observed on leaves and fruits. On leaves, chlorotic spots appeared in July, after a period of rainstorms, later turning necrotic and coalescing (Fig. 1). On fruits, small, brown spots developed (Fig. 2); some necrotic spots became infected by saprophytes and developed rots (Fig. 3). In the affected field, around 90% of fruits were diseased, leading to a total loss of production. In summer 2019, the disease was again observed in the same and neighbouring municipalities affecting two pumpkin varieties (*C. moschata* cv. Violina and *C. maxima* cv. Mantovana), displaying the same symptoms as in the previous year (Fig. 4). Symptoms were also observed on watermelon; small necrotic spots developed on leaves, only rarely confluent in large necrotic areas (Fig. 5) whereas, on fruits, no symptom was noted.

Isolation from symptomatic tissue was attempted on glucose yeast chalk agar medium. Bright yellow and mucoid colonies, with entire margins, developed after 48-60 hours at 28°C. Individual colonies were further purified for identification. Pathogenicity of isolates was confirmed on young pumpkin plants (*C. moschata* and *C. maxima*, 4 to 6 true leaves) by spraying a bacterial suspension (10^7 cfu/ml) on the canopy; inoculated plants were then kept in a glasshouse and observed for symptom development for up to three weeks. Re-isolated bacteria were identical to the original strains as determined by rep-PCR, thereby fulfilling Koch's postulates. Simplex-PCR with *Xanthomonas cucurbitae* genus-specific primers (RST2/RST3) produced a 1.4 kb amplicon, similar to *X. cucurbitae*, type strain CFBP 2542. Isolate identity was confirmed by sequencing of the *gyrB*, *rpoD*, *fyuA* and *dnaK* housekeeping genes (GenBank Accession Nos.: *gyrB*: MT110622, *rpoD*: MT215599, *fyuA*: MT110623 *dnaK*, MT227561). BLAST comparison of representative isolates in GenBank showed 99.76-100% identity with *X. cucurbitae* strains (ICMP 2299, ICMP 2179, ICMP 4767). Therefore, the pathogenic xanthomonads isolated in the province of Reggio Emilia were identified as *Xanthomonas cucurbitae*.

Prior to 1980, a few unconfirmed records of the pathogen were reported in the former Soviet Union (Kiryukhina, 1980). More recently, bacterial spot of pumpkins was reported in India, China and Nepal (Lamichhane *et al.*, 2010). Bacterial spot of pumpkin is also present in other countries, such as the USA, where it may cause up to 90% crop loss in infected fields (Babadoost & Ravanlou, 2012) and in Canada (Trueman *et al.*, 2014). Recently, the cause of leaf spot of pumpkin affecting fields in Styria (Austria) was attributed to a set of phytopathogenic bacteria, including *X. cucurbitae* (Huss, 2011), although no official identification was completed. This is the first confirmed report of bacterial spot of pumpkin, followed by isolation, identification and characterisation of its causal agent, *Xanthomonas cucurbitae*, in Italy and Europe.

The geographical origin of the pathogen is unknown, but its introduction might be connected with the international trade of pumpkin seed. Our observation of *X. cucurbitae* leaf spot on watermelon supports the report of Dutta *et al.* (2013). Further research is needed to assess the host range of the Italian isolates of *X. cucurbitae* amongst cucurbits.



Figure 1: Yellow to brown and angular spots observed on pumpkin leaves, later coalescing in large, necrotic lesions.



Figure 2: Circular, water-soaked, sunken spots developing on *Cucurbita moschata* cv. Violina; spots vary in size and had a pale-brown and cracked centre.



Figure 3: On several fruits, bacterial spots were infected by pectolytic saprophytes, causing large and deep tissue rots.



Figure 4: Bacterial spot symptoms on fruit of *Cucurbita maxima* cv. Mantovana.



Figure 5: Bacterial spot on leaves of watermelon, *Citrullus lanatus* cv. Crimson Sweet.

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