Diseases Caused by Bacteria and Phytoplasmas

First Report of *Erwinia rhapontici* Causing Bacterial Rot on Peach, Detected in Hungary

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Plant Dis. 104:3248, 2020; published online as <https://doi.org/10.1094/PDIS-04-20-0868-PDN>. Accepted for publication 10 July 2020.

Erwinia rhapontici is an opportunistic bacterial plant pathogen that can cause two types of symptoms: pink seed or crown, and soft, bulb, and blossom rot (Huang et al. 2003). It has been shown to cause disease in numerous plant species including kiwifruit (Wang et al. 2017), wheat, onion, cereal, pea, bean, rye, hyacinth, and tomato (Huang et al. 2003). However, *E. rhapontici* has not yet been reported to cause disease on the peach (*Prunus persica*). We observed typical bacterial rot, shriveled stems, a characteristic shepherd's crook, and bacterial ooze on two 5-year-old cultivar Champion and one 8-year-old cultivar Hope peach trees (see photos in the supplement) in a private garden in Budakeszi, Hungary, on 30 May 2019 during wet weather conditions. The garden is located in a calm suburban setting far from bigger plantations, where only these peach trees were planted and no similar symptoms were observed on these trees earlier. These were isolated cases in this area. Nine samples were taken from the oozes, inoculated on sucrose-peptone agar, and incubated at 28°C for 24 to 30 h in order to reveal the etiological agent of the infection. Outgrown colonies produced pink pigment, and three of them were chosen for identification using MALDI-TOF MS by comparing

the sample's spectra against the VITEK MS version 3.2.0 database, using the manufacturer's instructions. All colonies were identified as *E. rhapontici*, based on high confidence scores (2.03 to 2.18). For whole-genome sequencing, bacterial DNA was isolated (PureLink Genomic DNA Mini Kit, Thermo Fisher Scientific, U.S.A.) from the subculture of one colony according to the instructions of the manufacturer. The whole-genome sequencing and de novo assembly occurred as described in GenBank (accession GCA_012271765.1). We performed an ANIb analysis at JSpecies (<http://jspecies.ribohost.com/jspeciesws/#analyse>) against the GenBank reference strain of *E. rhapontici* BIGb0435 (accession GCA_004364855.1), confirming that the isolate was *E. rhapontici* (the ANI was 98.81%). To verify the etiological role of *E. rhapontici*, experiments were performed based on the Koch's postulates. Ten 1-year-old Champion peach trees were treated. Trees were planted in 10-liter dishes and cultivated under artificial conditions in plant chambers (temperature 22 ± 1°C, illumination: 14 h/day). Infection was carried out when at least five leaves were present on each tree. One-third of the leaves were injured using a sterile rubber, and 20 ml of *E. rhapontici*-containing suspension (7 × 10⁷ CFU/ml; cells were pelleted with centrifugation at 6,000 × g and resuspended in sterile phosphate-buffered saline [PBS]) was sprayed on the injured leaves of five trees. Sterile PBS was applied to five control trees. The first symptoms of infection were detected 4 days after the treatment on injured leaves. Symptoms also spread to the uninjured leaves, indicating the systemic nature of the infection. For example, brown spots appeared first along the midrib and veins, later also on other parts of the leaves, and larger lesions could be detected as well. Fifty-four to 78% of the leaves on the *E. rhapontici*-infected trees showed signs of infection 14 days after treatment, whereas no leaves on the control trees showed any symptoms. Colonies were reisolated from three infected leaves, and their identities were confirmed with MALDI-TOF MS as *E. rhapontici*. To our knowledge, this is the first report on *E. rhapontici* causing disease in peaches.

References:

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Wang, D., et al. 2017. Plant Dis. 101:1315.

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Keywords: *Erwinia rhapontici*, peach, host, blossom rot

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