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

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Plant Disease Note

Erwinia rhapontici is an opportunistic bacterial plant pathogen which can cause two types of symptoms, i.e., pink seed or crown, 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 peaches cv. "Champion" and one 8-year-old peach cv. "Hope" trees (see photos in the Supplement) in a private garden in Budakeszi, Hungary, on the 30th of 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 agars and incubated at 28°C for 24-30 hours in order to reveal the aetiological 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 V3.2.0 database, using manufacturer's instructions. All colonies were identified as *E. rhapontici*, based on high confidence scores (2.03-2.18). For whole genome sequencing bacterial DNA was isolated (PureLink™ Genomic DNA Mini Kit, Thermo Fisher Scientific, USA) from the subculture of one colony according to the instructions of the manufacturer. The whole genome sequencing and de novo assembly occurred as it was 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 (GenBank accession GCA_004364855.1) confirming that the isolate was *E. rhapontici* (the ANI was 98.81%).

To verify the aetiological role of *E. rhapontici,* experiments were performed based on the Koch postulates. Ten one-year-old „Champion" peach trees were treated. Trees were planted in 10 l dishes and cultivated under artificial conditions in plant chambers (temperature 22 ± 1 ^{o}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 ($7x10^7$ CFU/ml; cells were pelleted with centrifugation at 6000 g and resuspended in sterile PBS) was sprayed on the injured leaves of five trees. Sterile PBS was applied to five control trees. The first symptoms of infection (Supplement) were detected four 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 (Supplement). 54-78% of the leaves on the *E. rhapontici*-infected trees showed signs of infection 14 days after treatment, while no leaves on the control trees showed any symptom (Supplement). 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

Huang, H. C., Hsieh, T. F., and Erickson, R. S. 2003. Biology and epdemiology of Erwinia rhapontici, causal agent of pink seed and crown rot of plants. Plant Pathology Bulletin 12:69-76.

Wang, D., Yang, X., Chen, H., Kan, Y. Y., Yao, J. X., Li, Q., Liu, Y., Gong, G. S., and Yang, H. 2017. First Report of Erwinia rhapontici Causing Bacterial Leaf Spot on Kiwifruit in China. Plant Disease.

Infected trees in Budakeszi, Hungary

Experiment with one-year-old trees

Control trees



Erwinia rhapontici treated trees