First Report of Tomato Black Ring Virus Infecting Raspberry and Blackberry in Poland

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Plant Dis. 105:3310, 2021; published online as <https://doi.org/10.1094/PDIS-11-20-2321-PDN>. Accepted for publication 27 April 2021.

Raspberry (*Rubus idaeus* L.) and blackberry (*Rubus fruticosus* L.) are infected by at least 29 viruses, including tomato black ring virus (TBRV) (Martin et al. 2013). TBRV belongs to the genus *Nepovirus* (subgroup B) of the family *Secoviridae* and is listed as a plant pathogen in over 40 countries. TBRV infects a wide range of herbaceous and woody plants. In Poland, TBRV has been described on the plants of the following species: *Tagetes patula*, *T. erecta*, *Cucumis sativus*, *Cucurbita pepo*, *Lactuca sativa*, *Solanum tuberosum*, *S. lycopersicum*, *Sambucus nigra*, and *Robinia pseudoacacia* (Hasiów-Jaroszewska et al. 2015; Jończyk et al. 2004). To this date, there is no information on the incidence of TBRV in raspberry and blackberry in Poland. In the spring of 2019, 52 blackberry leaf samples and 408 raspberry leaf samples were collected from four plantations located in central Poland. None of the raspberry plants (cvs. Glen Ample, Polka, Sokolica), nor the blackberry plants (cvs. Thornfree, Polar, Gaj, Kotata) exhibited viral symptoms. Enzyme-linked immunosorbent assay was carried out for extracts from the 460 collected leaf samples to detect TBRV using commercial antisera (Loewe Biochemica, Germany). The results indicated that nine samples (four blackberry, five raspberry) were infected with TBRV. The isolates of the virus were transferred by sap inoculation and maintained in *Nicotiana tabacum* cv. Xanthi. Systemic ringspot, necrosis, and patterned lines were observed on tobacco leaves. The presence of the virus in tobacco leaf samples was confirmed by reverse transcription PCR (RT-PCR). Total RNA was extracted from all nine

samples using the silica capture method described originally by Boom et al. (1990) and adapted to the detection of plant viruses by Malinowski (1997). Part of the CP gene was amplified with the CPF (5'-GCCTGTCTCTCTCGCAATG-3') and CPR (5'-AAGGAGCCAACTGAAATGT-3') primer pair (Hasiów-Jaroszewska et al. 2015). Amplicons of the expected size (763 bp) were obtained for each sample. The amplified products were purified, sequenced in both directions, deposited in GenBank, and assigned accession numbers: MT507387 to MT507390 and MT507394 for the isolates from *R. idaeus* and MT507391 to MT507393 and MN954654 for the isolates from *R. fruticosus*, respectively. The nine newly obtained TBRV CP gene sequences, together with the 25 isolates deposited in GenBank, were aligned by ClustalW. The isolates obtained in this study showed a 99.0 to 100% nucleotide (nt) and a 98.7 to 100% amino acid (aa) identity in the part of the CP, respectively. Comparison of the part of the CP of the four blackberry and the five raspberry TBRV isolates with 25 TBRV isolates available in GenBank showed an 80.6 to 97.8% nt and an 87.9 to 99.5% aa identity, respectively. The results of the phylogenetic analysis revealed that the TBRV isolates obtained in this study are closely related to three Polish isolates (AY157994, KR139941, KR139951) and one Bioreba ctrl isolate from Switzerland (KT923164). These findings are of epidemiological significance because TBRV was detected on symptomless *Rubus* plants, which therefore represent a reservoir of the virus and a threat in case of a symptomatic infection of sensitive cultivars. Accordingly, the results will assist in using appropriate strategies for reducing TBRV incidence in *Rubus*-growing areas. Moreover, this is, to the best of our knowledge, the first report of TBRV in raspberry and blackberry in Poland.

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The author(s) declare no conflict of interest.

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Keywords: *Rubus* sp., TBRV, *Nepovirus*, RT-PCR, genetic diversity

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