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Detection of *Blueberry red ringspot virus* in different stages of *Parthenolecanium corni* in Poland

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Abstract: *Detection of* Blueberry red ringspot virus *in different stages of* Parthenolecanium corni *in Poland*. European fruit lecanium scales (*Parthenolecanium corni*) appeared in a large mass on highbush blueberry plants in central Poland. Some of the highbush blueberry plants growing in the studied plantation were infected by *Blueberry red ringspot virus* (BRRSV). Since no insect vector of BRRV is known so far, attempts were made to establish whether the soft scale pests were able to uptake the virus with the sap of the infected plants during feeding. The virus was detected and identified using the PCR and the DNA-DNA dot blot hybridisation technique in almost all the tested larval stages (56/60) and adult females (18/20) of *P. corni* feeding on BRRSV-infected highbush blueberry plants.

Key words: *Blueberry red ringspot virus*, *Parthenolecanium corni*, virus acquirement, *Vaccinium corymbosum*

INTRODUCTION

Blueberry red ringspot virus (BRRSV) is one of the most widespread pathogens of highbush blueberry (*Vaccinium corymbosum* L.). It causes red ringspots in leaves and older stems as well as circular blotches or pale spots on the fruits of many commercial cultivars. Fruit yield is earlier, but it is decreased (Cline et al. 2009). The virus was first reported in the United States and then in several countries in Europe (the Czech Republic, Slovenia, Poland, Serbia) and in Asia (Japan, South Korea) (Jevremović et al. 2016). The *Blueberry red ringspot virus* is a member of the genus *Soymovirus* (*Caulimoviridae*). Virions are isometric (42–46 nm in diameter) and contain a single molecule of non-covalently closed circular dsDNA (ICTV 2017). BRRSV is not mechanically transmissible; however, spread of the virus is observed in the field. Up to now, there is no evidence of the virus vector occurrence. Aphids and mealybugs (*Coccoidea* spp.) are the possible transmission agents of the pathogen (Cline et al. 2009, Polashock et al. 2009). All the data indicates that the major mode of BRRSV spread has been, at least in the past, infected planting material. European fruit lecanium scales (*Parthenolecanium corni* Bouche) (Hemiptera, Coccidae) were observed on a highbush blueberry plantation in central Poland (M.S. Szyndel personal observations). Soft lecanium scales are sucking pests and they feed on plant sap. The female scales overwinter

as immature forms on highbush blueberry plants, mature in spring and develop a hard, turtle-shaped hemispherical brown or dark red shell of approx. 5 mm in diameter. Females reproduce parthenogenetically and eggs are laid beneath the female's body. The eggs hatch into 1st--instar nymphs (crawlers) which move to feed on leaves. In autumn the 2nd-instar nymphs migrate from leaves to branches and twigs, on which they overwinter (Marrota and Tranfaglia 1997, Herrbach et al. 2016). The aim of the research was to determine whether the pest acquires the virus while feeding on the sprouts of the BRRSV-infected plants.

MATERIAL AND METHODS

Parthenolecanium corni insects occurred massively on the sprouts of the BRRSV-infected highbush blueberry plant cultivar Darrow over the period 2017–2019. In 2018, three sprouts were taken from 10 different BRRSV-infected plants on which the scale 1st--instar nymphs (crawlers) or adult females were feeding. Research material was collected according to the scheme: April – sprouts with crawlers (30 insect samples) and settled 1st-instar nymphs – 30 samples (it was impossible to obtain the 2nd-instar nymphs due to the spraying conducted in the second half of the summer); early May – highbush blueberry sprouts with adult females (20 specimens). Crawlers (10 specimens) and adult females (10 specimens) sampled from virus-free Bluecrop cultivar plants growing on the same plantation were used as a negative control. Insect samples were stored in 1.5 ml tubes at a temperature of –80°C for further testing. The occurrence of the *Blueberry red ringspot virus* in scale insects was investigated by the PCR and the DNA-DNA dot-blot hybridisation technique. The extraction of the total DNA was carried out according to the procedure described by Boom et al. (1990), modified by the use of the DNeasy® Plant Mini Kit QIAGEN Inc. (USA). In order to identify BRRSV, the polymerase chain reaction was performed using the Taq PCR Core Kit QIAGEN Inc. (USA), and a specific primer pair RRSV3/RRSV4 (Polashock et al. 2009), responsible for the amplification of a fragment of a transcriptional activator (TA) gene, was used with the sequence (5'→3'):

RRSV3 ATCAGTCCCAGAAGAAAAGAAGTA (F)

RRSV4 TCCGAAAAATAGATAGTGTCAGC (R)

The reaction mixture comprised 20 ng of the extracted DNA, 1.25 U of Polimeraza Taq and mixed primers (F+R), 0.4 μM each. The electrophoretic separation of the PCR reaction products was conducted by using the apparatus for the horizontal electrophoresis Easy-Cast^{TM} Horizontal System model B1.A (Owl Separation Systems, USA).

For the DNA-DNA dot-blot hybridisation, the DIG High Prime DNA Labelling and Detection Starter Kit II (Roche, Basel, Switzerland) was used. The hybridised probes were immuno -detected with an anti-digoxigenin-AP conjugate and visualised with the chemiluminescence substrate CSPD® using the ChemiDoc^{TM} MP System BIO-RAD (Berkeley, CA, USA).

RESULTS AND DISCUSSION

Parthenolecanium corni has previously been shown to vector *Grapevine leafroll-associated virus 1* and *Grapevine leafroll-associated virus 3* (*Ampelovirus*, *Closteroviridae*) as well as *Grapevine virus A* (*Tymovirales*, *Beteflexiviridae*, *Trivirinae*, *Vitivirus*) (Sforza et al. 2003, Hommay et al. 2008, Bahde et al. 2013). The results of the study indicated that soft lecanium scales could also acquire the *Blueberry red ringspot virus*. In almost all the conducted PCR analyses, specific products of the expected approximation of 450 bp (including a fragment of the gene coding the translation transactivator (TA) of BRRSV) were obtained from specimens processed out of both the 56 1st-instar nymphs and the 18 adult females of European fruit lecanium (the figure). No products were observed for the negative control (20 specimens). All the obtained results were entirely confirmed by DNA-DNA dot-blot hybridisation.

To this date, there is no evidence that any stage (juvenile or adult) of *P. corni* is capable of transmitting BRRSV from highbush blueberry to highbush blueberry plants. However, the presented results are the first known reported evidence that *P. corni* acquires the virus while feeding on the sprouts of the BRRSV-infected plants. Additional studies could confirm whether European fruit lecanium can act as an efficient vector of the *Blueberry red ringspot virus*. The epidemiological significance of the presented discovery is not yet known.

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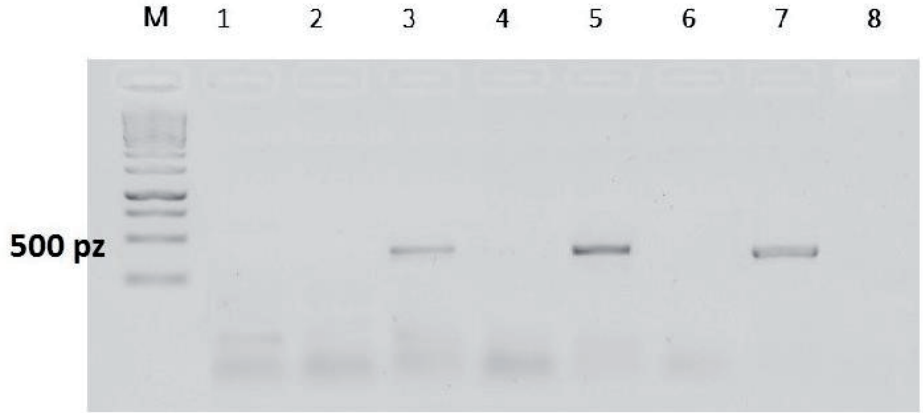

Lane M – size marker O'GeneRuler 1 kb Plus DNA Ladder (Thermo Fisher Scientific); different stages of Parthenolecanium corni: lane 1 – 1 crawler, lane 2 – a negative control for crawlers, lane 3 – 1 settled 1st-instar nymph, lane 4 – a negative control for settled 1st-instar nymphs, lanes 5 and 7 – 1 adult female, lanes 6 and 8 – a negative control for adult females.

FIGURE. Agarose gel electrophoresis analysis of PCR-amplified products of BRRSV

Conflicts of Interest: The authors declare no conflict of interest.
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Streszczenie: *Wykrycie* Blueberry red ringspot virus *w różnych stadiach rozwojowych misecznika śliwowego* Parthenolecanium corni *w Polsce*. Na plantacji borówki wysokiej (*Vaccinium corymbosum*) znajdującej się w centralnej Polsce zaobserwowano masowe pojawy szkodnika – misecznika śliwowego *Parthenolecanium corni.* Część roślin borówki była porażona przez wirus czerwonej pierścieniowej plamistości borówki wysokiej (*Blueberry red ringspot virus* – BRRSV). Żaden wektor tego wirusa nie jest znany do tej pory wśród owadów, próbowano więc ustalić, czy podczas żerowania szkodliwy pluskwiak jest w stanie pobrać wirusa z sokiem porażonych roślin. Wirus został wykryty i zidentyfikowany przy użyciu technik PCR oraz DNA-DNA dot blot w prawie wszystkich badanych larwach (56/60) oraz w dorosłych samicach (18/20) *P. corni* żerujących na porażonych przez BRRSV roślinach borówki wysokiej.

Słowa kluczowe: *Blueberry red ringspot virus*, *Parthenolecanium corni*, pozyskiwanie wirusa, *Vaccinium corymbosum*

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