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DISEASE NOTES

First Report of *Little cherry virus 1* affecting European Plum (*Prunus domestica*) in Belgium

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Little cherry disease (LChD), one of the major viral diseases of cherry worldwide, can be caused by two viruses (*Little cherry virus 1* and *2*), both *Closteroviridae* members. LChD has an important impact on both yield and fruit quality in commercial sweet and sour cherry (*Prunus avium* L. and *P. cerasus* L.) (Ruiz-Garcia et al. 2016). LChV-1 (genus *Velarivirus*) is known to be graft-transmissible and is spread via infected propagated plant material, but no vector has been identified. For LChV-2 (genus *Ampelovirus*), at least two species of mealybugs (Hemiptera, Pseudococcidae) are known to transmit the virus, namely the apple mealybug (*Phenacoccus aceris* Signoret) and grape mealybug (*Pseudococcus maritimus* Ehrhorn). During two growing seasons (2013–15), intensive surveys were conducted in Belgium to monitor the incidence of LChD in sweet and sour cherries and in ornamental *Prunus* spp., revealing widespread occurrence of both LChV-1 and 2 (De Jonghe et al. 2016). In the close vicinity of a sweet cherry (*P. avium* cv. Coralise) orchard with an infection rate of 30% with LChV-1, plum (*P. domestica* L. cv. Opal) trees growing at the edge of a plum orchard and showing sporadic undetermined leaf symptoms such as premature leaf reddening and chlorosis were observed and sampled. RNA of leaves and roots collected from 50 plum trees was extracted using the Spectrum Total Plant RNA kit (Sigma-Aldrich, Machelen, Belgium) and tested using RT-PCR with LChV-1 specific primers as follows: LCUW7090 (5′-GGTTGTCCTCGGTTGATTAC-3′)/LCUWc7389 (5′-GGCTTGGTTCCATACATCTC-3′)

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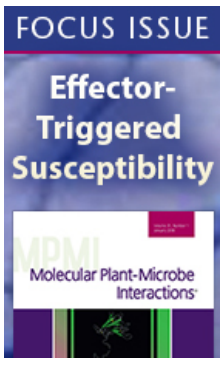

(Bajet et al. 2008), amplifying a 300-bp fragment spanning the ORF1b encoding the RNA dependent RNA-polymerase (RdRp) gene and 1LC_12776F (5′-TCAAGAAAAGTTCTGGTGTGC-3′)/1LC_13223R (5′-CGAGCTAGACGTATCAGTATC-3′) (Nagyova et al. 2015), targeting a 456-bp fragment of the coat protein (CP) gene. The presence of LChV-1 was confirmed in 12% of the samples. Bidirectional sequencing (Macrogen, Amsterdam) was done for each LChV-1 amplicon. BLAST searches of the assembled sequences revealed a distinct variability between the Belgian plum and cherry isolates (8% and 6% divergence in the amplified RdRp and CP sequences, respectively) from the respective adjacent orchards, suggesting separate introduction events. RdRp gene sequences of the Belgian plum isolates (GenBank accession nos. KY173002 and KY173004) shared 99% identity with the Greek cherry (HG792418) and peach isolates (HG792399), while the Belgian cherry isolate (KY173001) showed 99% homology with the deposited RdRp gene sequences of the Greek cherry (HG792420, HG792398). Partial CP gene sequence of the Belgian plum isolates (KY173006, KY173008) were the closest to Italian ITMAR (EU715989) and German V2356 (JX669615) cherry isolates, sharing 96% and 94% identity, respectively. Further investigation is in progress to assess the importance of LChV natural host shift among *Prunus* spp., its epidemiology in propagation material, and its association with potential vectors. To our knowledge, this description of LChV-1 associated with *P. domestica* constitutes the first report in Belgium.

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