New Disease Reports

First report of '*Candidatus* Phytoplasma rubi' on blackberry in Belgium

K. De Jonghe* and T. Goedefroit

Flanders Research Institute for Agriculture, Fisheries and Food (ILVO), Burgemeester Van Gansberghelaan 96, 9820 Merelbeke, Belgium

*E-mail: kris.dejonghe@ilvo.vlaanderen.be

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In May 2018 stunted blackberry plants were observed on a small-scale organic farm producing several small fruit types in West Flanders, Belgium. Besides severe stunting, leaf yellowing and distortions could also be observed (Figs. 1-2). Only *Rubus fruticosus* plants of the cultivar Obsidian showed symptoms but disease incidence was estimated to be almost 100%, of which almost half the plants were stunted. Other cultivars did not seem affected.

Seven symptom-bearing plants, of which four had severe stunting, and three only leaf symptoms, were sampled and tested. Additionally, three symptomless plants from neighbouring rows and belonging to different cultivars, were tested. All samples consisted of leaves and roots, and tests were conducted on a pooled leaf/root sample. Total genomic DNA was extracted by a CTAB protocol. The 16S rRNA gene was partially amplified using the phytoplasma universal primer pair P1/P7 (Deng & Hiruki, 1991; Schneider *et al*., 1995) followed by a nested PCR with R16F2/R2 primers (Lee *et al*., 1993) as a diagnostic test, and P1/Tint primers (Smart *et al*., 1996) on positive samples for sequencing. All of the diseased plants tested positive for phytoplasma in nested PCR tests. Additionally, specific phytoplasma group V primers targeting the *tuf* (FDTUF-F1/R1 & FDTUF-F2/R2; Malembic-Maher *et al*., 2011), and *secY* (primer sets FD9f2L/FD9r & FD9f3L/FD9r2L; Arnaud *et al*., 2007) genes were used in nested PCRs and yielded fragments of 998 and 1174 bp respectively.

The obtained 16SrRNA (P1/Tint), *tuf* and *secY* fragments were gel purified (SmartPure, Eurogentec), and sequenced directly (Genewiz, Leipzig, Germany). BLAST analysis of the 16SrRNA sequence, revealed the highest identity (99.85%) with a '*Rubus* stunt phytoplasma' strain from blackberry (*R. fruticosus*) in Italy (isolate RuS400; GenBank Accession No. AY197649). Partial sequence of the 16S ribosomal RNA (1607 bp), and *tuf* (835 bp) and *secY* (1053 bp) genes were submitted to GenBank (MH801133, MH809672 and MH809673, respectively). Phylogenetic analysis was undertaken on all three fragments, and the phytoplasma was identified as *'Candidatus* Phytoplasma rubi', 'elm yellows' group 16SrV-E (Figs. 3-5). To our knowledge, this is the first report of '*Ca.* P. rubi' on blackberry in Belgium. An additional nationwide survey is needed to assess the phytopathological impact of this outbreak. Although a growing number of reports indicate the increased importance of *Rubus* stunt, associated with '*Ca*. P. rubi', unlike the related '*Ca*. P. ulmi', associated with elm trees, no regulatory actions are currently taken against this pathogen.

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Figure 1

Figure 2

Figure 3

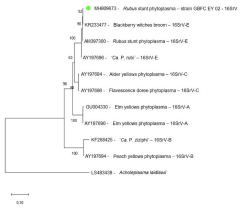

Figure 4

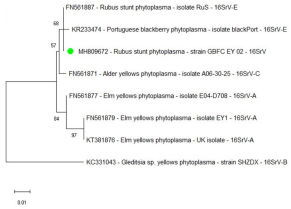

Figure 5

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