

O.46 - Phytoplasma diseases in Canadian vineyards

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Abstract

Phytoplasma are non-culturable wall-less prokaryotes belonging to the class Mollicutes that are transmitted by phloem-feeding insects, mostly leafhoppers. Phytoplasmas have been associated with grapevine yellows and have been detected in grapevine worldwide. A phytoplasma disease survey was performed in 2006 and 2007 in vineyards from British Columbia, Ontario and Québec to detect and identify phytoplasma present in Canadian vineyards. Grapevines and insects from an average of 20 vineyards in BC and ON and 5 vineyards in QC were sampled and tested for the presence of phytoplasma using nested PCR. Phytoplasma belonging to group 16Srl (Aster Yellow group (AY)) was found in vineyards from the three provinces. Percentages of grapevines infected with phytoplasma were 1.2% and 1.7% in 2007, in BC and ON, respectively and 6.1% and 0.9% in 2008, in ON and QC respectively. Phytoplasma DNA was detected in *Erythroneura comes*, *Latalus* sp. and *Scaphoideus titanus* in ON, in *Erythroneura tricincta*, and *Erythroneura vitis* in QC, and in *Ceratagalia humilis*, *Colladonus germinatus*, *C. torneellus*, *Endria inimica*, *Exitianus exitiosus*, *Gyponana hasta*, *Hecalus viridis*, *Macrosteles quadrilineatus*, *Neokolla confluens*, *Psammotettix lividellus* and *Scaphytopius acutus* in BC. The most numerous cicadellid species in ON and QC vineyards were *Erythroneura* sp showing a population infected at 2.6% with phytoplasma. In BC, cicadellids that phytoplasma were most commonly isolated from were *M. quadrilineatus* and *N. confluens* which populations were infected with phytoplasma at 10.3% and 8.5% respectively. DNA sequencing showed that phytoplasma found in insects and grapevines sampled in BC, ON and QC vineyards belong to 16Srl-A or 16Srl-B.

Introduction

Phytoplasma are non-culturable wall-less prokaryotes belonging to the class Mollicutes that are transmitted by phloem-feeding insects, mostly leafhoppers. Phytoplasmas have been detected in grapevines worldwide (Boudon-Padiou et al., 2003). Typical symptoms include leaf chlorosis and rolling, flower abortion or berry withering, uneven or total lack of lignification of canes, as well as reduced vitality and wine quality. Phytoplasma diseases are very difficult to control in grapevine, as there is no registered chemical to control the pathogen. Spraying for the insect vectors, when known, destruction of all infected vines and establishment of a quarantine area around infected plants are methods commonly used to control phytoplasma diseases. The Canadian wine industry imports a large number of grafted vines yearly from France and Germany where two phytoplasma diseases, Flavescence dorée (FD) and Bois Noir (BN), are spreading despite strong compulsory control measures. Phytoplasmas belonging to the Aster Yellow (AY) and X-disease groups were also found in grapevine in the USA. In 2006, BN was found in the provinces of Ontario and British Columbia (Canada), in imported grapes from France (Rott et al., 2007). In order to establish the identity and frequency of phytoplasma diseases in Canadian vineyards, a phytoplasma disease survey was performed in vineyards from British Columbia, Ontario and Québec in

2006 and 2007. The goals of this project were: 1) to identify the phytoplasma strains present in Canadian vineyards, 2) to estimate the frequency of the diseases, and 3) to identify potential insect vectors.

Material and methods

Plant sampling: Grapevine were sampled in an average of 20 vineyards in BC and ON in 2006 and 2007 and in 5 vineyards in QC in 2007. In each vineyard, leaves were sampled from 50 grapevines (4 leaves per grapevine, 10 grapevines per site, 5 sites per vineyards) showing symptoms resembling those of phytoplasma diseases. In the absence of symptoms, grapevines were randomly sampled. Two discs per leaf were sampled using a 3/4" hole punch. Leaf discs were freeze-dried and stored at -20C before being tested for phytoplasma DNA presence using a PCR test.

Insect sampling: Insects were sampled at the same site where leaf collection took place. Insects were sampled with sweep nets in BC and ON (25 sweeps inside and 25 sweeps from vegetation adjacent to the vineyard). In QC, insects were sampled using the beating methods where vines were shaken and insects collected on sheets placed below the vines. Insects were placed in plastic bags and stored at -20C before being identified and tested for phytoplasma DNA presence using a PCR test.

PCR test: Phytoplasma DNA was extracted from leafhoppers and leaf tissues of all harvested plants and amplified using nested PCR technology with universal phytoplasma-specific primer pairs P1/P6 and R16R2/R16F2 (Tanne et al., 2001). Species of leafhoppers that were known to be phytoplasma vectors or carriers in the literature were tested individually, while other species were tested in groups of 5 or 10, depending on their size. DNA sequencing was conducted on DNA extracts that were PCR-positive. Insects that tested positive were sent to Dr. A. Hamilton (AAFC-Ottawa) for identification.

Results – discussion

Grapevine: In BC, 2 grapevines out of 170 tested positive for phytoplasma in 2006 and no grapevine phytoplasma DNA was detected in 2007. None of the plants that tested positive in 2006, tested positive in 2007. This might be due to the remission phenomenon already observed (Boudon-Padieu et al, 2003). In ON, 1.7% and 6.1% of the tested grapevines tested positive in 2006 and 2007 respectively. In QC, 0.9% of the grapevines were infected with phytoplasma. DNA sequencing showed that most plants in the three provinces were carrying phytoplasma belonging to the 16Srl group (*Ca. P. asteris* or Aster Yellow group). Phytoplasma belonging to 16Srl were also found in *Vitis* sp sampled in Pennsylvania and Virginia (Boudon-Padieu et al., 2003). Most of the infected grapevines in QC showed strong symptoms of yellow diseases, while in ON and BC, most of the grapevines that tested positive for phytoplasma DNA did not show symptoms. This absence of symptoms in infected grapevines has already been observed and is dependant on the cultivars and the susceptibility of the cultivars to the disease (Boudon-Padieu et al., 2003).

Leafhoppers: In BC, 22 species of leafhoppers were identified in vineyards among which 11 tested positive for phytoplasma DNA: *Ceratagalia humilis*, *Colladonus germinatus*, *C. torneellus*, *Endria inimica*, *Exitianus exitiosus*, *Gyponana hasta*, *Hecalus viridis*, *Macrosteles quadrilineatus*, *Neokolla confluens*, *Psammotettix lividellus* and *Scaphytopius acutus*. Among those 11 species, the more numerous were *M. quadrilineatus* and *N. confluens* and the percentage of infection in the sampled population was 10.1% and 8.5% respectively. *M. quadrilineatus* is known to be a vector of phytoplasma belonging to the group Aster yellow, stolbur and clover proliferation and *N. confluens* has previously been found on grapes at densities sufficiently high to require insecticide controls. In ON, 22 species were identified in vineyards and PCR tested positive for phytoplasma DNA in *Erythroneura vitifex*, *Erythroneura* sp and *Scaphoideus titanus*. *Erythroneura* sp carried phytoplasma strain 16Srl (AY group) while *S. titanus* carried the Elm

Yellow phytoplasma strain. *S. titanus* is the FD vector. The population of *Erythroneura* sp was infected at 4.2%, while only 1 specimen of *S. titanus* was found positive for phytoplasma DNA. In QC, 5 leafhopper species were identified and phytoplasma DNA was detected in 5.4% of the sampled *Erythroneura vitis* and 3.7% of the sampled *Erythroneura tricincta*. *Erythroneura* sp are not known to be phytoplasma vectors. The presence of phytoplasma DNA in *Erythroneura* sp might be due to the presence of phytoplasma in the sap contained in the insect midgut.

Conclusion

Phytoplasma DNA belonging to the AY group was detected in grapevines in ON, BC and QC. Phytoplasma incidences in vineyards were 1.1% and 0% in BC in 2006 and 2007, 1.7% and 6.1% in ON in 2006 and 2007, respectively, and 0.9% in QC in 2007. The presence of AY phytoplasma as well as the recent detection of BN in ON vineyards mean that phytoplasma diseases are present in Canadian vineyards and should be monitored.

Phytoplasma DNA was also detected in several species of leafhoppers known to be phytoplasma vectors as well as in leafhopper species not previously known to be vectors, suggesting that infection risks, especially of AY phytoplasma, are high in Canadian vineyards. Insect vectors of AY such as *M. quadrilineatus* were present in high number in the vineyards of the three provinces. *M. quadrilineatus* is also known to be a vector of phytoplasma belonging to the groups stolbur and clover proliferation. Other insect species known to be AY vectors, such as *Endria inimica* and *Scaphoideus titanus* were also present in ON vineyards. No known vector of BN was found in the ON vineyards. However, *Fieberiella florii*, a stolbur vector, was found in high number in BC.

Detection of phytoplasma in grapevines and in insects showed that it is important and timely to establish a checklist of the leafhopper and psyllid species present in grapevine-growing areas in Canada and to investigate the presence and the identity of phytoplasma in the leafhopper and psyllid species, as well as in plants of *Vitis* sp. Additional research will be required to determine which species of leafhopper are able to transmit phytoplasmas to grapevines in Canada.

References

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