Bulletin of Insectology ***64*** *(Supplement): S133-S134, 2011*
ISSN 1721-8861

Preliminary investigations on *Graminella nigrifrons* as a potential vector for phytoplasmas identified at the Canadian Clonal Genebank

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Abstract

Prunus and *Pyrus* species from the Canadian Clonal Genebank infected with putative phytoplasma diseases, as well as leafhopper species collected from *Prunus* and *Pyrus* fields were molecularly tested for phytoplasma presence. Preliminary results showed that *Graminella nigrifrons* may be a potential vector for phytoplasma group 16SrI ('*Candidatus* Phytoplasma asteris'), 16SrVII ('*Candidatus* Phytoplasma fraxini') and 16SrX-C ('*Candidatus* Phytoplasma pyri'). Results also showed that *G. nigrifrons* may be able to transmit multiple phytoplasmas within the same location. *G. nigrifrons* appears to have a complex ecology therefore further transmission trials are required to verify its phytoplasma vector role in fruit trees.

Key words: phytoplasma, *Graminella nigrifrons*, phytoplasma vector, ecology.

Introduction

Graminella nigrifrons (Forbes), the black-faced leafhopper is widely distributed in North America. This member of Membracoidea, Cicadellidae, Deltocephalinae is the known vector of the maize bushy stunt phytoplasma, ('*Candidatus* Phytoplasma asteris' group, subgroup 16SrI-B), and of several maize viruses (Beirne, 1956). Recently, phytoplasmas of groups 16SrI, 16SrVII ('*Candidatus* Phytoplasma fraxini') and 16SrX-C ('*Candidatus* Phytoplasma pyri') have been reported affecting *Prunus* and *Pyrus* species in Ontario, Canada, and are included in the Canadian Clonal Genebank (CCG) (Hunter *et al*., 2010, Zunnoon-Khan *et al*., 2010a, 2010b). Vector management is an effective control strategy for phytoplasma diseases (Weintraub and Beanland, 2006). Surveys to identify potential vector species for *Prunus* and *Pyrus* phytoplasma diseases at CCG were conducted to assess the importance of insect management in phytoplasma spreading.

Materials and methods

During June-August/2010, over 500 leafhopper specimens were collected by sweep net in *Prunus* and *Pyrus* in four CCG fields: T4 Centre-apricot, T4 East-peach, T3 West plum and cherry, and T4 West-pear (Table 1), and subjected to identification and PCR testing. Random leaf samples from peach and apricot trees from each field showing symptoms of decline, leaf reddening, witches' broom and peach rosette-like (Zunnoon-Khan *et al*., 2010a, 2010b) were also collected and tested (table 1). Total DNA was extracted from leafhopper specimens and plant samples (FastDNA Spin kit, MP Biomedicals, USA). Nested PCR was performed with primers specific for phytoplasma 16S rRNA, R16mF2/R1 (Gundersen and Lee, 1996) and fU5/rU3 (Lorenz *et al*., 1995). PCR products were subjected to RFLP with *Mse*I and *Alu*I restriction endonucleases. Representative PCR products of the expected size (880 bp) from samples from each field were purified (EZNA Cycle Pure kit, Omega Bio-Tek, USA), cloned (pGEM-T Easy Vector, Promega), and sequenced (Princess Margaret Hospital, Toronto, Canada).

Results

The four fields surveyed yielded specimens of *Balclutha impicta* (Van Duzee), *Delphacodes campestris* (renamed *Muirodelphax arvensis* in 2010), and *Graminella nigrifrons*. Only *G. nigrifrons* produced PCR amplicons for phytoplasmas; 263 out of 322 (81.7%) samples collected (table 1). PCR/RFLP confirmed phytoplasmas in plant samples as members of groups 16SrI-B (peach-almond acc. PRU0382, cultivar Kando; peach acc. PRU0445, cultivar HW271; apricot acc. PRU0134, cultivar Harglow; apricot acc. PRU0142, cultivar Sundrop); 16SrVII-A (peach acc. PRU0164, cultivar Harcrest; peach acc. PRU0168, cultivar Harrow Diamond; peach acc. PRU0176, cultivar Siberian C; peach acc. PRU0180, cultivar Vanity; plum acc. PRU0406, cultivar Pembina); 16SrX-C (peach acc. PRU0336, cultivar Redhaven; apricot acc. PRU0147, cultivar Wescot).

Table 1. Phytoplasma groups identified in *G. nigrifrons* specimens and in plant samples randomly collected from *Prunus* and *Pyrus* fields at the CCG.

Field	Accession No.	*G. nigrifrons* PCR positive/tested	Phytoplasma 16S ribosomal groups
T4 East-peach	PRU0168, PRU0176, PRU0180, PRU0445, PRU0382, PRU0336	79/89	16SrI, 16SrVII, 16SrX
T4 Centre-apricot	PRU0134, PRU0142, PRU0147	39/85	16SrI, 16SrVII, 16SrX
T3 West-plum and cherry	PRU0406	81/81	16SrI, 16SrX
T4 West-pear	PYR0190	63/66	16SrI, 16SrX

Figure 1. *G. nigrifrons*. Picture taken by Tom Murray. (In colour at www.bulletinofinsectology.org)

BLAST analysis of partial 16S rDNA sequences of representative phytoplasmas detected in *G. nigrifrons* samples showed 99% of sequence identity with those from groups 16SrI, 16SrX and 16SrVII at NCBI and those from plant samples, including those of phytoplasmas previously reported at CCG in peach (16SrI-B, HQ450211 and 16SrVII-A, GU223903), as well as, a 98.8% of sequence identity with that of the phytoplasma reported in pear associated with pear decline in Ontario (16SrX-C, GU565960).

Discussion

More than 75% of all confirmed phytoplasma vector species are monophagous to polyphagous members of Deltocephalinae, and transmit one or more phytoplasma taxa (Weintraub and Beanland, 2006).

Three different phytoplasma groups have been identified in *G. nigrifrons* that we collected from four fields planted with peach, apricot, plum and pear, suggesting that this leafhopper is polyphagous and may contribute to disease spread by moving from field to field. Phytoplasma of groups 16SrI and 16SrVII were simultaneously detected in *G. nigrifrons* from the field T4-Centre apricot; while those from groups 16SrI and 16SrX were found in fields T3-West plum and cherry; T4-East-peach, and T4-West pear, which suggest that *G. nigrifrons* populations possess a complex ecology.

The 16SrX and 16SrI were the predominant phytoplasma groups identified in *G. nigrifrons*. This supports previous findings of *G. nigrifrons* as a vector for group 16SrI; however this is the first report of group 16SrX being carried by *G. nigrifrons* and affecting *Prunus* species in Ontario, Canada.

A highest 16S rDNA sequence identity was found between phytoplasmas identified in insect and plant samples. This suggests that *G. nigrifrons* may be a potential vector for 16SrI, 16SrVII and 16SrX phytoplasmas. Transmission trials are required to determine its vector role and ecological factors influencing transmission of phytoplasma diseases in *Prunus* and *Pyrus* at the CCG.

Acknowledgements

We thank Dana Gagnier for assisting with leafhoppers collections, Amanda Drouillard (Federal Student Youth Internship Program) for database management, and Dr. Lorna Woodrow for critically reviewing of the manuscript.

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