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# Epidemiological aspects of phytoplasmas in Chilean grapevines

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## Abstract

Some Auchenorrhyncha specimens were captured, identified and tested to verify phytoplasma presence in Chilean vineyards; many of them belong to the subfamily Delthocephalinae and Agalliinae (family Cicadellidae) and to the families Cixiidae and Delphacidae, all known as potential phytoplasma vectors. Several individuals were positives to phytoplasma presence, in particular *Amplicephalus curtulus* Linnavuori & De Long, in which were detected phytoplasmas belonging to subgroup16SrI-B and 16SrXII-A, and *Paratanus exitiosus* (Beamer) positive to phytoplasmas of the subgroups 16SrI-B, 16SrVII-A and 16SrXII-A. Phytoplasmas belonging to subgroup 16SrI-B and 16SrVII-A were identified in *Convolvulus arvensis* L. and *Polygonum aviculare* L.; to subgroup 16SrXII-A in *C. arvensis*; and to subgroup16SrVII-A in *Galega officinalis* L. In three cases grapevine samples, weeds and insects collected in the same vineyard were positives to phytoplasmas of the same subgroup.

**Key words:** Auchenorrhyncha, grapevine yellows, nested-PCR, phytoplasmas, RFLP, sequencing, weed.

## Introduction

Phytoplasmas found in Chilean grapevines showing yellows symptoms were identified as belonging to the ribosomal subgroups 16SrI-B and 16SrI-C ('*Candidatus* Phytoplasma asteris'), 16SrVII-A ('*Ca.* P. fraxini') and 16SrXII-A (stolbur or "bois noir") (Gajardo *et al*., 2009). The presence of these pathogens in the plants depends on both propagation of infected plants and spreading by different insect species which feed on grapevine and also on the weeds growing near and/or in vineyards. There is no evidence of epidemic spread of yellows symptoms in the inspected vineyards so far; however a survey to verify the presence and identity of weeds and potential insect vectors was carried out.

## Materials and methods

During 2009 and 2010 surveys were carried out in thirteen vineyards in four regions of Chile where phytoplasmas were detected: 4 vineyards in Valparaíso (V); four vineyards in Metropolitana de Santiago (RM); 1 vineyard in Libertador General Bernardo O'Higgins (VI); 4 vineyards in Maule (VII). From each vineyard weeds and insects (leafhopper and planthopper) were collected.

The insects were captured from December to March by sweeping with an entomological net. They were separated by the morphological characteristics and sex. From each individual photos were taken. For determination at genus and species level the male genitalia were examined under a stereo-microscope. Weeds infected by phytoplasmas were identified at the species level.

Insects and weeds were tested in order to identify the phytoplasma presence. Total nucleic acids (TNA) was extracted with CTAB or chloroform/phenol methods, dissolved in Tris-EDTA pH 8.0 buffer, and maintained at 4°C; 20 ng/µl of nucleic acid were used for amplification. After direct PCR with primer pair P1/P7, nested PCR with R16F2n/R2 primers (Gundersen and Lee, 1996) was performed. PCR and nested PCR reactions were carried out following published protocol (Schaff *et al*., 1992). Identification of detected phytoplasmas was done using RFLP analyses on amplified ribosomal DNA fragments with *Tru*I, *Rsa*I, *Hha*I, *Tsp509*I, *Taq*I, *Alu*I (Fermentas, MBI, Vilnius, Lithuania) restriction enzymes. Selected R16F2/R2 amplicons identified after RFLP analyses were purified using Concert Rapid PCR Purification System (Life Technologies, Gaithersburg, MD, USA). DNA fragments were ligated into T/A cloning vector pTZ57R/T following the instructions of the InsT/Aclone PCR Product Cloning Kit (Fermentas, MBI, Vilnius, Lithuania). Putative recombinant clones were analysed by colony-PCR, sometimes in combination with the insert-specific primers. Amplicons obtained from three colonies per cloned fragment were subjected to RFLP analyses, as described above. Selected fragments from cloned DNAs were sequenced in both directions using the BIG DYE sequencing terminator kit (PE Biosystems, Warrington, UK). The sequences were then aligned with BLAST engine for local alignment (version Blast N 2.2.12).

## Results and discussion

From all the vineyards a total of 50 different weed samples and 200 specimens of leafhoppers and planthoppers were collected. Positive results were obtained only after nested amplification on P1/P7 amplicons. The phytoplasmas detected in weeds and insects were assigned by RFLP analyses to three different ribosomal subgroups and sequence analyses were performed for corroborate pathogen identification.

The most common weed species found positive to phytoplasmas were *Convolvulus arvensis* L. and *Polygonum aviculare* L.. Phytoplasma assigned to 16SrI-B and 16SrVII-A subgroups were identified in both species; 16SrXII-A subgroup phytoplasmas were identified in *C. arvensis*, and 16SrVII-A phytoplasmas were detected in one sample of *Galega officinalis* L.

Several insects belonging to the subfamily Deltocephalinae and Agalliinae (family Cicadellidae) and to the families Cixiidae and Delphacidae were found positives to phytoplasmas. The most common were *Amplicephalus curtulus* Linnavuori & De Long in which phytoplasmas of 16SrI-B and 16SrXII-A subgroups were detected, and *Paratanus exitiosus* (Beamer) found positive to the subgroups 16SrI-B, 16SrVII-A and 16SrXII-A. In three cases the grapevine samples, weeds and insects collected in the same vineyard were positives to the same phytoplasma: Petit Syrah grapevine, *P. aviculare* and *P. exitiosus* infected by phytopasmas belonging to the subgroup 16SrI-B; Carménère grapevine, *C. arvensis* and *P. exitiosus* by 16SrVII-A; Pinot noir grapevine, *C. arvensis* and *A. curtulus* by 16SrXII-A (table 1).

**Table 1.** Phytoplasmas detected in grapevines, weeds and insects from each of the three different vineyards.

| Grapevine cultivar (Region) | Detected phytoplasmas | | |
|---|---|---|---|
| | grapevine | weed | leafhopper |
| Petit Syrah (RM) | 16SrI-C<br>16SrI-B | 16SrI-B (*P. aviculare*)<br>16SrVII-A (*P. aviculare*, *C. arvensis*) | 16SrI-B (*P. exitiosus*)<br>16SrVII-A (*P. exitiosus*) |
| Carménère (RM) | 16SrVII-A<br>16SrXII-A | 16SrVII-A (*C. arvensis*) | 16SrVII-A (*P. exitiosus*)<br>16SrXII-A (*P. exitiosus*) |
| Pinot noir (V) | 16SrVII-A<br>16SrXII-A | 16SrXII-A *(C. arvensis)* | 16SrXII-A (*A. curtulus*)<br>16SrVII-A (*P. exitiosus*) |

Since many individuals of *P. exitiosus* and *A. curtulus* were captured on the weeds it is very likely that they only occasionally feed on the grapevine (perhaps after weeding), transmitting phytoplasmas. In Chile *P. exitiosus* was found between the regions of Los Lagos (X) to Valparaiso (V), especially in the Bío Bío region (VIII) in sugarbeet crops (Casals *et al.*, 1999; Klein Koch and Waterhouse, 2000). *A. curtulus* was found only in the region of Los Lagos (X) (Zanol, 2007). In this study, the presence of *A. curtulus* in VII, VI, V and RM regions was observed for the first time.

*P. aviculare* and *C. arvensis* have been repeatedly found positive to different phytoplasmas, so we can conclude that these weeds represent a reservoir of phytoplasmas for grapevine in Chilean vineyards.

Assays to verify the phytoplasma transmission ability of the leafhoppers *A. curtulus* and *P. exitiosus* are in progress.


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