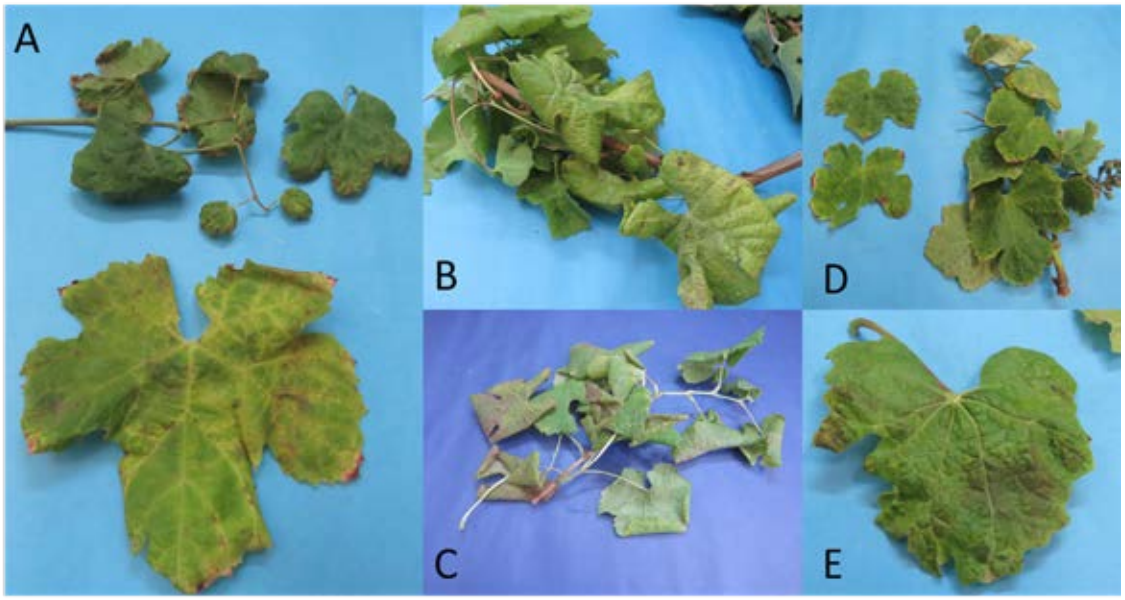
DETECTION OF PHYTOPLASMAS IN CHILE, ITALY AND SOUTH AFRICA VINEYARDS
A priority measure to focus the diseases management

■ THE PROBLEM ADDRESSED

Update on phytoplasmas detected in grapevines

In the different viticultural areas in the world, phytoplasmas cause losses that range from 13 up to 100%, depending mainly on the virulence of the pathogen and the varietal susceptibility. Control is essentially based on the prevention of spread of the pathogen. The most efficient management tools are, therefore, the use of phytoplasma-free propagation material, the control of insect vectors and the elimination of inoculum sources of the pathogen, including alternate host plants. With this aim the identification of phytoplasmas present in the vineyards is of the highest priority and importance in order to focus the disease management efforts in the countries involved in the project, since it will provide the appropriate information about target insect vectors to control and alternate host plants to eliminate in order to reduce presence of the pathogen and also reduce pesticide usage.



• Symptoms in leaves of five grapevine samples positive for phytoplasmas in Chile. A) VN17- variety Chardonnay with downward rolling and yellowing of the veins. B) VN12- variety País with downward rolling and yellowing. C) VN29- variety Tintorera with downward rolling and reddening. D) VN69- variety Semillón with downward rolling and small leaves. E) Leaf of VN32-variety Sauvignon blanc with downward rolling and deformation.

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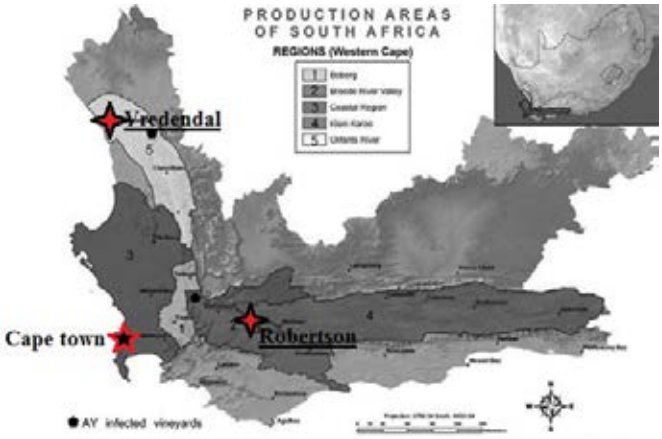
■ LATEST RESEARCH RESULTS

What is known about the identity of phytoplasmas associated with grapevine yellows in Chile, Italy and South Africa?

The grapevine yellows agents are generally well known in Europe, while less data and information are available for other countries, such as Chile and South Africa. In South Africa the agent and its main insect vector have been discovered recently (Map 1), while in Chile different grapevine phytoplasmas and insects harbouring some of these phytoplasmas have been identified (Map 2). In the majority of the European countries, the phytoplasmas associated with the disease are known as "bois noir" and "flavescence dorée", the last one being a quarantine organism (Angelini *et al.*, 2018). The surveys in Italy allowed the detection of new phytoplasmas (Map 3) and new potential insect vectors in some of the main grapevine growing regions (Zambon *et al.*, 2018). It is therefore clear that only a constant monitoring will allow for the prompt detection of phytoplasmas or new phytoplasmas that may infect grapevine plants. Furthermore, monitoring is necessary to determine if the endemic phytoplasmas are spreading. This information is the basis for control measures. Finally, knowledge of the phytoplasma strains associated with grapevine yellows in the three countries is the basis for improving the detection techniques.



• Map 1. Areas in South Africa where the '*Candidatus* Phytoplasma asteris' associated with grapevine yellows has been identified.

• Map 3. Italian areas where new phytoplasmas associated with grapevine yellows were detected.

• Map 2. Chilean regions surveyed and locations where the phytoplasmas were detected in the vineyards.

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■ THE TROPICSAFE RESEARCH AND DEVELOPMENT ACTIVITY

Techniques for the molecular identification of the grapevine yellows phytoplasmas

Samples from grapevine grown commercially in affected areas within the three countries were collected during the summer/autumn seasons in 2017 and 2018 and stored at -80°C for subsequent testing. The presence of phytoplasmas was detected after nucleic acid extraction and polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) and sequencing. The primers used for the PCR amplification are given in Table 1 for the three areas, Table 2 for Chile and Table 3 for Italy and South Africa.

In **Chile**, the survey was carried out in the Maule and Valparaiso Regions. Ninety samples were collected and analysed by nested PCR using 50S ribosomal subunit gene primers, designed by the "Laboratorio de Fitovirología" of the University of Chile (Table 2). The amplification products from four samples were sequenced to identify the phytoplasmas present. Results were confirmed using nested PCR with P1/P7 primers, followed by R16F2n/R2 primers (Table 1). In **Italy**, total nucleic acids were extracted with a chloroform/phenol method from 103 symptomatic grapevine samples collected in diverse areas and used in PCR assays with phytoplasma universal primer pair P1/P7, followed by nested-PCR with primers R16F2n/R2. Additional nested-PCR assays were carried out using primers R16(I)F1/R1 (Table 3). Phytoplasma identity was detected by RFLP analyses. Selected samples were then sequenced to confirm phytoplasma identity by virtual RFLP and phylogeny. In **South Africa**, sampling was carried out in the white cultivar Colombard. Samples from asymptomatic (41) and symptomatic (39) plants were collected and processed by removing phloem tissues from which DNA was extracted using a CTAB-based protocol. The extracted DNA was quantified by nanodrop analysis and quality parameters for the samples ranged between A260/280: 1.73-2.02 and A260/230: 0.80-1.86, while concentrations ranged from 69 to 352 ng/µL. The DNA quality was further assessed by agarose gel electrophoresis. The aster yellows phytoplasma diagnostics were performed using the PCR assay reported in Table 3.

Table 1. General primers used for phytoplasma detection in grapevine in Chile, Italy and South Africa

Assay	Primer set	Amplicon size (bp)	Literature
PCR	P1/P7	1,792	Deng and Hiruki, 1991; Schneider *et al.*, 1995
PCR nested 1	R16F2n/R16R2	1,244	Gundersen and Lee 1996
PCR nested 2	U5/U3	800	Lorenz *et al.*, 1995

Table 2. General primers used for phytoplasma detection in grapevine in Chile

Assay	Primer set	Amplicon size (bp)	Literature
PCR	LSu2pF/LSu2pR	1,700	Zamorano and Fiore, unpublished
PCR nested	LSu2pFn/LSu2pRn	1,280	

Table 3. Primers used for phytoplasma detection in grapevine in Italy and South Africa

Assay	Primer set	Amplicon size (bp)	Literature
PCR	P1/P7	1,792	Deng and Hiruki, 1991; Schneider *et al.*, 1995
PCR nested 1	R16F2n/R16R2	1,244	Gundersen and Lee 1996
PCR nested 2	R16(I)F1/R16(I)R1	1,100	Lee *et al.*, 1994

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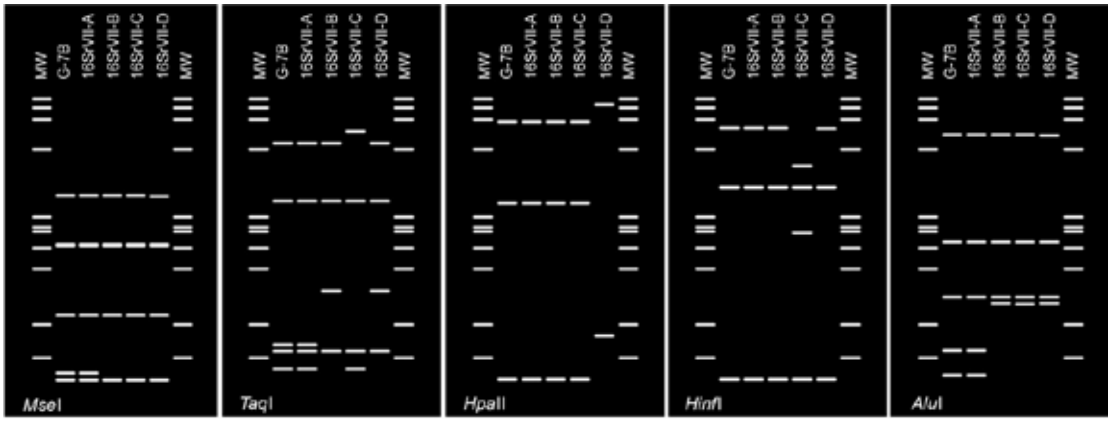
■ SCIENTIFIC DATA AND FIRST RESULTS

Identification of phytoplasmas associated with grapevine yellows diseases

The surveys carried out in the three countries confirmed the presence of diverse prevalent phytoplasmas. In **Chile** the samples examined were infected with a '*Candidatus* Phytoplasma pruni'-related strain classified in the 16SrIII-J subgroup; with a '*Ca.* P. ulmi'-related strain (16SrV-A); a '*Ca.* P. fraxini'-related strain (16SrVII-A); and a '*Ca.* P. solani'-related strain (16SrXII-A). The situation in Chile, with the constant presence of these phytoplasmas in the surveyed vineyards, remained unchanged. In **Italy** the main phytoplasmas detected were '*Ca.* P. solani'-related and '*Ca.* P. asteris'-related (16SrI-B). Others phytoplasmas were also detected: '*Ca.* P. fraxini'-related (16SrVII-A); "flavescence dorée" (16SrV-C and –D); '*Ca.* P trifolii'-related (16SrVI); '*Ca.* P. phoenicium'-related (16SrIX); '*Ca.* P. pruni'-related (16SrIII); '*Ca.* P. prunorum'-related (16SrX-B). Samples with mixed infection with two of these phytoplasmas have also been recorded. The increasing presence of '*Ca.* P. asteris'-related phytoplasma needs monitoring through specific detection tools application in order to be able to manage the possible epidemic increasing of the dissemination of the phytoplasma. In **South Africa** the presence of '*Ca.* P. asteris'-related strains (16SrI-B and 16SrI-C) was confirmed in the area where the disease was first reported several years ago, confirming the severity of the presence of this phytoplasma associated with the grapevine yellows diseases.



• Virtual restriction fragment length polymorphism analyses on grapevine R16F2n/R2 amplicon (GenBank accession number KY454858) and reference strains using the interactive online tool *i*PhyClassifier (from Zambon *et al.*, 2018).

KEY WORDS

Nested-PCR, RFLP, phytoplasmas identification, phytoplasmas control

FURTHER INFORMATION

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