TECHNICAL INNOVATION FACTSHEET

UPDATE ON GRAPEVINE YELLOWS DISEASE EPIDEMIOLOGY IN ITALY
Phytoplasma detected in selected Italian viticultural environments

■ THE PROBLEM ADDRESSED

Diversity of phytoplasmas detected in grapevine plants

The descriptions of severe epidemics of grapevine yellows in Italy date back to the 1990s, when severe losses mainly due to the presence of "flavescence dorée" were reported especially in the northern regions. In the following years in other regions the presence of "bois noir" and erratically of other phytoplasmas was also described. The economic impact of the grapevine yellows disease had been under control over the past 20 years, until recently scattered outbreaks and localized epidemics started to re-emerge. Surveys were carried out in the frame of the TROPICSAFE project in selected Italian vineyards located in Veneto, Emilia-Romagna, Tuscany, Marche, and Abruzzo regions and performed by sequencing the 16S ribosomal RNA gene of phytoplasmas amplified by specific polymerase chain reaction (PCR) or nested-PCR. The testing allowed identification of the presence of '*Candidatus* Phytoplasma solani', '*Ca.* P. fraxini', '*Ca.* P. prunorum', '*Ca.* P. asteris' and other '*Candidatus* Phytoplasma' species that were erratically identified. Moreover, in Veneto and Emilia-Romagna regions diverse strains of "flavescence dorée" phytoplasmas were identified by multigenic molecular testing followed by sequencing and/or restriction fragment length polymorphism (RFLP) analyses. As occurs worldwide, the main infected variety was Chardonnay, but several other popular varieties such as Sangiovese, Lambrusco, Barbera, Cabernet Sauvignon, Pinot were also infected and showed typical symptoms. A few varieties such as Glera and Nebbiolo showed no symptoms or had non-specific symptoms in presence of phytoplasma epidemics since they are tolerant to their presence.

• Grapevine yellows symptoms include leaf yellowing or reddening, downward curling very often with a triangle shape to the leaves, shortened internodes, death of tips and shoots, lack of lignification, aborting flowers, shrivelling and early drying of berries. The infected symptomatic cultivars above are from top left: Sangiovese, Lambrusco and Chardonnay.

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■ THE PRACTICE/INNOVATION PROPOSED BY TROPICSAFE

Diversity of phytoplasmas detected in Italian vineyards in alternative host species

While the recognition of GY symptoms in grapevines is easily achieved by knowing the typical symptoms, the identification of alternative plant hosts involves molecular testing because in most of the cases these do not show symptoms. Moreover, finding alternative insect vectors includes collection of the insects and molecular identification of phytoplasma presence followed by testing to demonstrate their vector ability. In Italy the TROPICSAFE surveys have allowed detection and identification of diverse phytoplasmas in plants and insects collected inside or in the surroundings of the grapevine infected vineyards.

Among the 300 plant samples belonging to a number of species tested, 16 species were shown to be hosting phytoplasmas. *Parthenocissus quinquefolia* (Vitaceae), *Calystegia sepium* and *Convolvolus* spp. (Convolvulaceae), *Fraxinus excelsior* (Oleaceae), *Quercus* spp. (Fagaceae), *Robinia pseudoacacia* (Fabaceae), *Rosa canina* and *Rubus ulmifolius* (Rosaceae), *Conyza canadensis* and *Tagetes patula* (Asteraceae), and *Skimmia* sp. (Rutaceae) were infected with '*Ca.* P. solani' (16SrXII-A phytoplasmas). *Clematis vitalba* (Ranunculaceae), *Sorghum halepense* (Pooideae), *Hedera helix* (Araliaceae), *Rubus* sp. (Rosaceae) and *Morus* spp. (Moraceae) were positive for '*Ca.* P. asteris' (16SrI phytoplasmas) presence. In addition, a phytoplasma belonging to the 16SrX group was identified in *Sorghum halepense* and one of the 16SrV group was detected in *Clematis vitalba*; both were however not identified at the '*Ca.* Phytoplasma' species level. Only the *P. quinquefolia*, *T. patula* and *Skimmia* sp. plants showed symptoms of leaf reddening and flower virescence that indicate phytoplasma presence.

In Italy the most abundant insect species captured in and arount the surveyed vineyears on yellow sticky traps or by sweep netting were *Orientus ishidae, Schapoideus titanus, Hyalesthes obsoletus* and *Hishimonus hamatus*. Others insect species were collected and identified: *Reptalus* spp., *Anaplotettix* spp., *Anaplotettix fuscovenosus, Aphorophora alni, Centrotus cornutus, Neoaliturus fenestratus, Philaenus spumarius* and *Jikradia olitoria*. Three leafhopper species, *O. ishidae, S. titanus* and *N. fenestratus*, one cixiid, *H. obsoletus*, and *P. spumarius*, tested positive for different phytoplasmas. *O. ishidae* was the most abundant species with 48% of positive samples (69 out of 213 specimens) and had the highest number of infected individuals. The phytoplasma groups and subgroups 16SrI-B, 16SrVI, 16SrVII-A, 16SrX and 16SrX-B were detected for the first time from insects in Italian vineyards. Further phytoplasmas were detected in *Reptalus* spp. (16SrII), *Anaplotettix* spp. (16SrII-D), *A. fuscovenosus* (16SrII, 16SrX-B), *A. alni* (16SrII), *C. cornutus* (16SrII) and *J. olitoria* (16SrXI). Trials to verify their ability to transmit the detected phytoplasma must be carried out to verify their relevance in the diverse areas infected by grapevine yellows.

■ HOW IS TROPICSAFE IMPLEMENTING IT?

Management of alternative grapevine yellows phytoplasma hosts

The epidemiology of grapevine yellows is complex, and the disease management methods include rouging symptomatic grapevines and control of host-plant reservoirs and insect vectors. Alternative plant hosts have an important role as a reservoir from which the insect vectors can transmit the phytoplasmas to grapevines. Phytoplasma insect vectors are primarily leafhoppers, planthoppers and psyllids. The advantage of their identification in a specific agroecosystem is that it allows their environmentally-friendly and targeted removal reducing both the infected material and the labour costs. The biology and epidemiology of the most frequently associated phytoplasmas ('*Ca.* P. solani', 16SrXII-A and "flavescence dorée", 16SrV-C/-D) are available; however, climate changes and the recent introduction of alien species of both potential phytoplasma insect vectors and weeds has increased the risk of new phytoplasmas

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or new strains spreading. The TROPICSAFE monitoring identified an increase in the number of plant species infected with 16SrXII-A phytoplasmas as possible source of infection of this phytoplasma in vineyards and in several other agricultural and wild environments. TROPICSAFE also demonstrated that some leafhoppers and planthoppers were infected with grapevine yellows-associated phytoplasmas. Although the presence of the phytoplasmas was confirmed, their presence in an insect is not proof of vector status, which needs to be additionally tested by their experimental transmission to grapevines under controlled conditions.

• Top row from left: symptomatic plants of *P. quinquefolia* and of *T. patula* infected with '*Ca.* P. solani' (16SrXII-A) in areas in which the vineyeards are infected with grapevine yellows in Italy. Bottom a vineyard with symptoms surrounded by diverse weeds and plant species hosting some of the diverse phytoplasmas detected in the TROPICSAFE surveys.

■ HOW IS IT WORKING?

Phytoplasma host plants and insect vectors: how to manage them in a sustainable manner?

During TROPICSAFE, the surveys of new alternative host plants of grapevine phytoplasmas, together with possible insect vectors of these phytoplasmas, revealed a long list of different species especially in Italy. The collected plants in and around vineyards were mostly asymptomatic, representing a hidden reservoir with the potential for further spread of the disease. Therefore, it is recommended to test or preferably remove the most widespread species detected positive for phytoplasma presence near vineyards for prevention of possible infections. It appears clear that only a constant monitoring will allow for the prompt detection of phytoplasmas that may infect the studied crops. Appropriate management is linked to the diverse geographical location and agro-ecosystem conditions but with the appropriate epidemiologic knowledge can be applied as a sustainable tool to reduce economic losses and the environmental pollution.

One of the agricultural practices that could be adopted is to use phytoplasma free grapevine materials when planting new vineyards as demonstrated in the TROPICSAFE project for other insect transmitted prokaryotes such as '*Candidatus* Liberibacter' species in citrus in Cuba. Parallel research carried out by Chilean partners in grapevine in separate projects and in the past by the authors has shown possibilities to produce phytoplasma-free grapevine plants. A sustainable control of infected plants in the fields was demonstrated by using plasma activated water (PAW) that is however a time consuming and laborious procedure, but produced encouraging results as grapevine berry production and symptom reduction. The use of phytoplasma-free grapevine plants avoids the dissemination of the pathogens in the environment and the acquisition of them from potential insect vectors present in the environment or incidentally introduced, reducing the risk of grapevine yellows epidemic outbreaks without the use of specific insecticides. The use of insecticides, although specific for the insect vectors, is not eliminating the risk of phytoplasma transmission since it is limited by the economic threshold it has biological problems in reaching all the individuals and it is reducing the biodiversity, the resilience and the safety of the vineyard agroecosystems.

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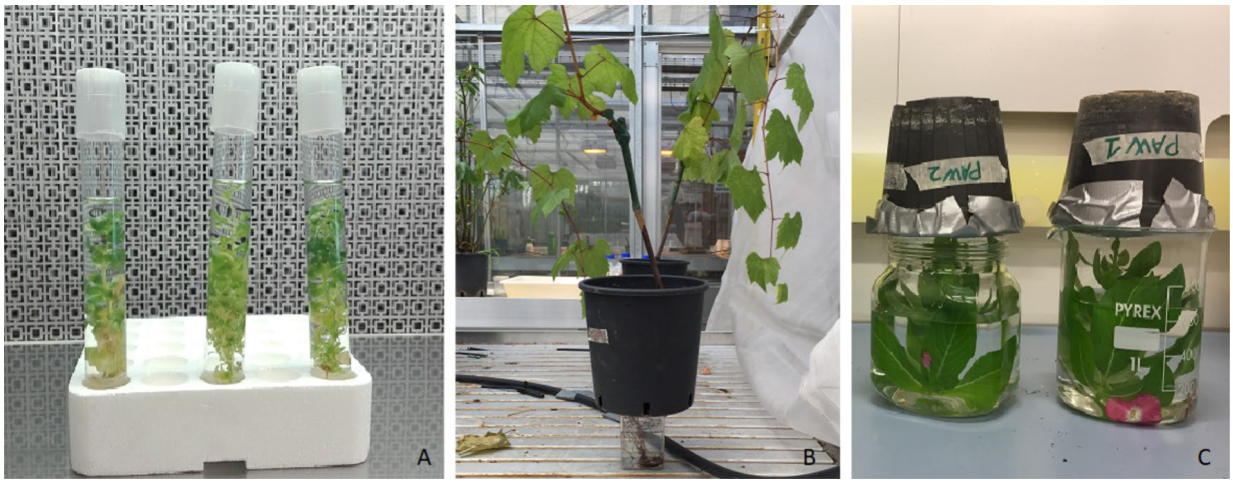

• Plasma activated water (PAW) treatments to improve the fitness of plants and induce some resistance reducing the pathogen presence. (A) Micropropagated periwinkle shoots PAW-treated by submersion; (B) grapevine plants cv. Chardonnay PAW-treated by root drenching; (C) periwinkle plants treated by upside down submersion. Scientific Reports. 2020, 10:19211 https://doi.org/10.1038/s41598-020-76247-3

KEY WORDS

Grapevine yellows, phytoplasmas, symptomatology, alternative host species, phytoplasma free plant production

FURTHER INFORMATION

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