P10: Survey on grapevine yellows and their vectors in the Republic of Moldova

Victor Bondarciuc[1], Luisa Filippin[2], Evghenii Haustov[1], Vally Forte[2], **Elisa Angelini**[2]*

[1]Practical Scientific Institute of Horticulture and Food Technology, Virology Laboratory, Vierul Street 59, Chisinau, Moldova
[2]CREA Research Center for Viticolture and Enology, Viale XXVIII aprile 26, Conegliano (TV) Italy
**Corresponding author: elisa.angelini@crea.gov.it*

INTRODUCTION

In the Republic of Moldova, the area of grapevine plantations is 140,000 hectares. Between 2004 and 2014, 40,000 hectares were planted with imported seedlings from European nurseries. However, in the same period, a very harmful grapevine disease appeared. Currently, the disease is common in all the vineyards. Symptomatic plants are found in autochthonous grapevine varieties such as Rara neagra, Feteasca neagra, Moldova, as well as in vineyards older than 35 years. This suggests that Moldova has very effective and mobile phytoplasma insect vectors. The aim of this work was to identify the agents associated with the disease by molecular analyses. Moreover, monitoring of vineyards was conducted to provide more detailed information on the spread of the disease. Finally, a preliminary survey of possible insect vectors was carried out.

MATERIALS AND METHODS

Visual survey. Plantations were inspected in various zones of cultivation of grapevines from July to September 2017 on seven plots. In each plot five to 40 rows of plants (400 to 10,000 plants) were observed, depending on the area of the plantation. The line of visually observed plants passed along the diagonal of the plantation.
Insect collection. The catching of insects in the vineyard was carried out using sticky yellow traps, from July to September 2017. Three traps were placed in 4 plots and replaced once a month, for a total of 36 traps.
Molecular analyses. The extraction of DNA was carried out according to Angelini *et al*. (2001). Identification of phytoplasmas was performed by nested and quantitative (q)PCR. Nested PCRs were carried out with universal primer pair P1P7 (Deng and Hiruki, 1991; Schneider *et al*., 1995))followed by 16r758f/M23Sr (Martini *et al*., 1999) or by primers R16(V)F1/R1 and R16(I)F1/R1 (Lee *et al*., 1995; 1995), whose specificities are universal, and only for 16SrV and 16SrI, 16SrIX, 16SrXII, and 16SrXV phytoplasma groups, respectively. RFLP analysis with the restriction enzymes *Taq*I and *Tru9*1 was performed to identify the phytoplasmas. The qPCR was carried out on phytoplasma ribosomal genes according to Angelini *et al*. (2007).

RESULTS AND DISCUSSION

Symptoms. In Moldova the first symptoms of the disease appear in early July. In white varieties a weak chlorosis appears on the leaves of one or several shoots of the plant. With the development of the disease, these leaves become golden yellow with metallic luster, crispy and curly downward. Along the main veins, chrome yellow spots appear, which subsequently become necrotic. By the end of the growing season, the leaves twist into a triangular shape. In varieties with red berries, redness of the leaf blades occurs. Redness often concerns only one sector, limited to two or three veins. Affected leaves, unlike healthy ones, survive the first light frosts, so in late autumn infected plants are visible from afar. Infected shoots are distinguishable by short internodes and stunted growth. On the surface of the symptomatic canes numerous pustules appear. In autumn, the affected canes show lack of lignification and with the onset of low temperatures, often die. Affected canes, as a rule, are not harvested due to the fact that the inflorescences dry up and fall off. The characteristic symptoms indicate that this disease could be associated with phytoplasma presence.
Visual survey. The inspected vineyards in the Republic of Moldova are highly affected by the disease (Table 1). Infection varies from 2% in Syrah to 69% in Chardonnay. In 4-years old vineyards, the percentage of symptomatic plants ranges from 29% in Sauvignon to 35% in Chardonnay, which poses a serious threat to the viticulture. Both international grapevine varieties and autochthonous varieties Feteasca Neagra, Feteasca Alba and Rara Neagra are affected by the disease.
Molecular analyses. In the summer 2017, 17 symptomatic plants of three varieties (one Traminer, eight Sauvignon, eight Feteasca neagra) were sampled for phytoplasma detection by molecular analyses. Nested PCR with universal primer pair 16r758f/M23Sr yielded 16 positive samples, all positive also to the R16(I)F1/R1 primer pair. RFLP analyses of the amplicons revealed that the samples were containing 16SrXII-A phytoplasmas,

'*Candidatus* Phytoplasma solani', the agent associated with grapevine "bois noir". The data were confirmed by qPCR.

Insect survey. In order to survey for the potential insect vectors of the disease, yellow sticky traps installed in the vineyards were used for insect sampling. The identification of captured insects revealed the presence in the vineyards of known and potential vectors of phytoplasmas, such as *Scaphoideus titanus, Hyalesthes obsoletus, Orientus ishidae*, and other leafhoppers, such *as Philaenus spumarius* and *Euscelidius variegatus*. *S. titanus* had been previously identified in Moldova in 2013 (Timus, 2015). The role of these insects, in particular of *H. obsoletus*, in the transmission of the BN phytoplasma under the Moldova conditions is being studied.

Table 1. Percentage of symptomatic plants observed in the 2017 survey in Moldovan vineyards.

Grapevine cultivation area	Variety	Year of planting	Total number of plants observed	Symptomatic plants (%)
Central zone	1. Chardonnay	2005	1,600	51,68
	2. Cabernet Sauvignon	2004	7,230	9,66
	3. Pinot noir	2004	4,301	11,54
Southern zone	1. Feteasca Neagra	2008	4,277	6,91
	2. Rara Neagra	2008	4,600	14,31
	3. Syrah	2008	3,382	1,99
	4. Malbec	2008	3,700	7,91
	5. Chardonnay	2013	1,180	35,4
	6. Sauvignon	2013	1,180	29,2
	7. Feteasca Alba	1982	400	22,7
South-eastern zone	1. Cabernet Sauvignon	2004	8,512	19,55
	2. Pinot noir	2004	6,920	16,21
	3. Merlot	2004	10,240	6,48
	4. Chardonnay	2004	3,800	68,71

REFERENCES

Angelini, E., Clair, D., Borgo, M., Bertaccini, A., Boudon-Padieu, E. 2001. "Flavescence dorée" in France and Italy — occurrence of closely related phytoplasma isolates and their near relationships to Palatinate grapevine yellows and an alder yellows phytoplasma. Vitis, 40: 79-86.

Angelini, E., Bianchi, G.L., Filippin, L., Morassutti, C., Borgo, M. 2007. A new TaqMan method for the identification of phytoplasmas associated with grapevine yellows by real-time PCR assay. Journal of Microbiological Methods, 68: 613-622.

Deng, S., Hiruki, C. 1991. Genetic relatedness between two non culturable mycoplasma like organisms revealed by nucleic acid hybridization and polymerase chain reaction. Phytopathology, 81: 1475-1479.

Lee, I-M., Gundersen, D.E., Hammond, R.W., Davis, R.E. 1994. Use of mycoplasmalike organism (MLO) group-specific oligonucleotide primers for nested-PCR assays to detect mixed-MLO infections in a single host plant. Phytopathology, 84: 559-566.

Lee, I-M., Bertaccini, A., Vibio, M., Gundersen, D.E. 1995. Detection of multiple phytoplasmas in perennial fruit trees with decline symptoms in Italy. Phytopathology, 85: 728-735.

Martini, M., Murari, E., Mori, N., Bertaccini, A.1999. Identification and epidemic distribution of two "flavescence dorée"-related phytoplasmas in Veneto (Italy). Plant Disease, 83: 925-930.

Schneider B., Seemüller, E., Smart, C.D., Kirkpatrick B.C., 1995. Phylogenetic classification of plant pathogenic mycoplasma-like organisms or phytoplasmas, pp. 369-380. In: Molecular and Diagnostic Procedures in Mycoplasmology (Razin, S., Tully, J.G., Eds).- Academic Press, San Diego, CA, USA.

Timus, A.M. 2015. The invasive entomofauna of the hemimetabola group for Republic of Moldova. Current Trends in Natural Sciences, 4: 32-40.