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Research Note

High occurrence of *Flavescence dorée* phytoplasma early in the season on grapevines infected with grapevine yellows

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Summary: A survey for the presence of phytoplasmas associated with grapevine yellows in about 500 Italian vineyards was conducted from 1999 to 2004. Grapevines with the earliest symptoms were mostly infected with *Flavescence dorée* type C phytoplasma (FD-C). As the season advanced, a steady relative decrease in the occurrence of FD-C coincided with a clear relative increase in Bois noir phytoplasma. The relative occurrence of *Flavescence dorée* type D phytoplasma remained stable.

K e y w o r d s : grapevine yellows, *Flavescence dorée*, PCR/RFLP, phytoplasma, symptoms.

Introduction: Grapevine yellows (GY) are very serious *Vitis vinifera* diseases and are caused by phytoplasmas. The most important GY in Europe are *Flavescence dorée* (FD) and *Bois noir* (BN). FD disease is the most dangerous, as it is epidemic, and FD phytoplasma is listed as a quarantine organism in the European Community. Pathogens associated with FD belong to '*Candidatus* Phytoplasma vitis', ribosomal group 16SrV, subgroups C and D (Irpcm 2004; Lee *et al*. 2004). It is possible to distinguish FD from BN by means of an analysis of the DNA obtained from leaves displaying GY symptoms. It is widely held that field symptoms do not contribute to distinguish between GY phytoplasmas.

This study aims to analyse the occurrence of the different GY phytoplasmas in relation to the environment and to the time course of symptom appearance. It will also provide useful suggestions for a preliminary distinction between FD and BN in vineyards.

Material and Methods: From 1999 to 2004, from May to November, 747 leaf samples from symptomatic grapevines were collected in about 500 Italian vineyards and tested by PCR/RFLP assay. Most of the collected samples (563) came from the Treviso area in Northeast Italy, where BN, FD-C and FD-D phytoplasmas are present. Nucleic acids were extracted from vein tissues, as described elsewhere (Prince *et al*. 1993; Angelini *et al*. 2001). Ribosomal DNA from phytoplasmas was amplified by a nested-PCR assay employing primer pairs P1/P7 (Smart *et al.* 1996) and 16r758f/M23Sr (Gibb *et al.* 1995; Padovan *et al*. 1995), according to PCR conditions described by Schaff *et al.* (1992). PCR products were digested with *Taq*I endonuclease, following the manufacturer's instructions (New England Biolabs). PCR products were analysed with 1 % agarose gel electrophoresis and RFLP products with 10 % polyacrylamide gel electrophoresis, then stained with ethidium bromide and visualised by UV transillumination.

Results and Discussion: Three types of GY phytoplasmas were identified on the basis of the RFLP patterns in the 16r758f/M23Sr products: BN, FD-C and FD-D. On the whole, BN and FD-C phytoplasmas were the most widespread, being present in 47 % and 35 % of the total samples, respectively. Only 18 % of the examined grapevines were infected with FD-D phytoplasma. 184 grapevines from Italian areas other than the province of Treviso showed a prevalence of BN (76 %), while the presence of FD-C and FD-D phytoplasmas was detected in 7 % and 17 % of the samples, respectively. The difference in the occurrence of FD-C and BN phytoplasmas depends on both GY distribution in Italy and the fact that the majority of the samples came from vineyards located in the Veneto region, particularly in the province of Treviso, where FD-C phytoplasma is widespread. FD-C phytoplasma occurred in Veneto, western Friuli, Lombardy, Piedmont, Liguria and northern Tuscany, while FD-D was widespread in all northern Italian regions, in particular in Lombardy and Emilia Romagna. BN phytoplasma was present in samples from all Italian viticultural areas.

All PCR/RFLP results were then grouped according to the month of sample collection, in order to analyse the relative occurrence of the different phytoplasmas during the vegetative period (Fig. 1). It is worth noting that 80 % of the grapevines with the earliest symptoms, which became visible at the beginning of June (about 3 % of the total samples, *i.e.* 21 out of 747), were infected with FD-C phytoplasma, while FD-D and BN phytoplasmas were detected in only a few samples. As the season advanced, a steady

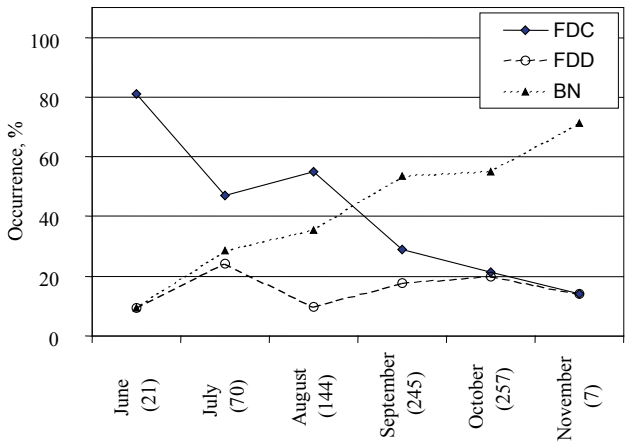

Fig. 1: The occurrence (%) of FD-C, FD-D and BN phytoplasmas from June to November. The results were obtained from 1999 to 2004 in different Italian vineyards. In May only three samples were collected (not shown); they all came from the province of Treviso. ( ) - number of samples tested per month.

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decrease in the relative occurrence of FD-C phytoplasma was observed, coinciding with a clear relative increase in BN phytoplasma. The relative occurrence of FD-D phytoplasmas roughly did not vary from June to November. In the province of Treviso the trends were quite similar (Fig. 2). Remarkably, all 18 samples collected in May and June from symptomatic plants were only infected with FD-C phytoplasma.

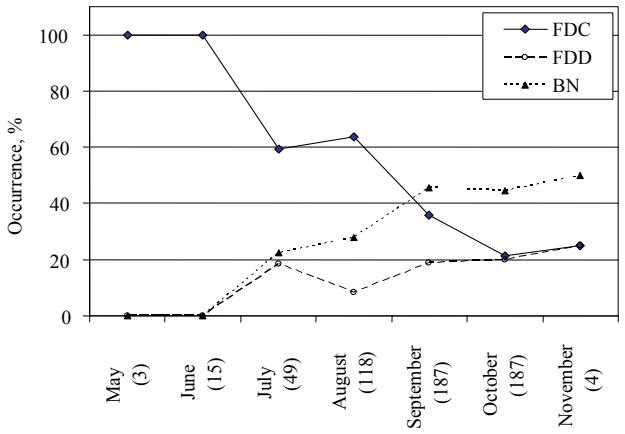

Fig. 2: The occurrence (%) of FD-C, FD-D and BN phytoplasmas from May to November in the province of Treviso, where all three phytoplasmas were found to be present simultaneously in several years. Data obtained from 1999 to 2004. ( ) - number of samples tested per month.

The results, obtained in viticultural areas where BN and FD are present, clearly show that most of the BN-infected vines only displayed GY symptoms in the middle to late period of vegetation and grapevines with early symptoms of GY were mostly infected by FD. Thus, the different behaviour of the three GY phytoplasma strains in terms of appearance of symptoms in different periods of the season could be exploited for GY monitoring, both in areas where FD is present and in those which are still free of it.

Therefore, in viticultural areas where FD is not yet present, but which are close to FD-infected regions, field observations should focus on the period before flowering, when the presence of BN symptoms is still low. This would allow to identify FD-infected grapevines more easily. If the aim is to provide a global description of GY infection, vine samples should be collected in autumn, when most of the infected plants display disease symptoms.

In conclusion, the results of this research may be useful to provide a preliminary means of distinguishing FD from BN in vineyards for those working on GY surveys and to enable them to detect FD already at the beginning of the season. It is very important to confirm the presence of FD by means of biomolecular assays on vine samples with early symptoms in order to direct agronomic and control strategies in vineyards.

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