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Research Note

Diversity of grapevine yellows in Germany

M. Maixner[1]), Maria Rüdel[2]), X. Daire[3]) and E. Boudon-Padieu[3])

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Introduction: Different phytoplasmas have been found associated with grapevine yellows (GY) diseases in various viticultural areas of the world (Caudwell 1993). 'Vergilbungskrankheit' (VK) is widespread in Germany and the only type of GY detected so far (Maixner *et al.* 1995). Restriction fragment length polymorphism (RFLP) analysis indicated a close relationship between VK in Germany, Bois noir in France and GY of various other countries (Daire *et al.* 1993 a). VK has been assigned to the stolbur-subgroup of the aster yellows strain cluster on the basis of the sequence of its 16S rDNA (Seemüller *et al.* 1994). Flavescence dorée (FD) on the other hand, the most important GY of the Mediterranean area, is related to phytoplasma diseases of woody plants such as elm yellows and ash yellows (Daire *et al.* 1993 b; Seemüller *et al.* 1994). This paper reports the first detection of a non-VK grapevine yellows in Germany and its identification as FD.

Materials and methods: Grapevines from the Palatinate viticultural area and from one location in Rheinhessen were checked for symptoms of GY from August to October 1994. Symptom expression of leaves, shoots, and berries was monitored on seven cultivars (Tab. 1).

Table 1

Type of symptoms (details in text) exhibited by grapevine yellows infected cultivars of *Vitis vinifera* collected in the Palatinate area, and type of phytoplasmas detected by PCR. VK: Vergilbungskrankheit; FD: Flavescence dorée

Sample	Type of symptoms		vines	type of yellows	
	I	II	tested	VK	FD
Riesling	6	0	6	6	0
Müller-Thurgau	1	1	2	1	1
Kerner	0	1	1	1	0
Scheurebe	1	9	10	0	10
Chardonnay	1	0	1	1	0
Portugieser	1	0	1	1	0
Pinot noir	2	0	2	2	0

DNA was isolated from leaf petioles of 24 symptomatic grapevines as described by Maixner *et al.* (1995). Polymerase chain reaction (PCR) amplification of DNA was performed using an OmniGene TR3 thermocycler (Hybaid Ltd.). The phytoplasma specific primers fU3/rU5 (Ahrens 1994) were used to detect phytoplasma infections unspecifically. VK was detected with primers fStol/rStol (Maixner *et al.* 1995), and primers fFD9/rFD9 were used to detect FD infected vines (Daire *et al.*, unpublished). Amplification products were analysed by electrophoresis of 5 µl of reaction mixtures in 1.2 % agarose gels in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0; 5 V/cm).

A sandwich-ELISA procedure was applied according to Caudwell and Kuszala (1992). Test antigens were prepared from either frozen (healthy and FD-positive controls) or freeze-dried leaf petioles of five grapevines from which DNA had been amplified with primers FD9. Plates were coated with polyclonal rabbit anti-FD antibodies and a cocktail of four monoclonal anti-FD antibodies was used as second antibodies followed by alkaline phosphatase anti-mouse conjugate. Each sample was tested in four replicates and the OD_{405} was recorded after 150 min of incubation with enzyme substrate.

Results and discussion: Two different types of symptom expression of infected vines could be distinguished. Type I that is typical for VK was characterised by the partial expression of symptoms by some shoots while others appeared healthy. Symptomatic shoots did not lignify, leaves showed discoloration patterns including necrotic areas, and berry symptoms developed early. Symptom type II was mainly exhibited by cv. Scheurebe. Typical for this type was a systemic expression of symptoms, severe rolling of uniformly discolored leaves, an almost complete lignification of primary shoots, and late occurrence of berry symptoms. Rows of black pustules along the internodes were observed in both types.

PCR amplification of DNA with primers U3/U5 revealed phytoplasma infections of all symptomatic vines (Figure). VK was detected with primers fStol/rStol in 11 vines assigned to symptom type I and one vine of cv. Kerner exhibiting type II symptoms (Tab. 1). We

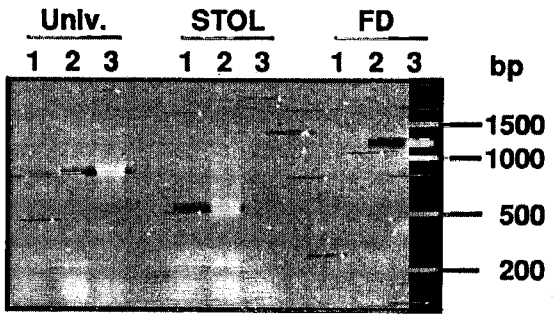

Figure: Polymerase chain reaction amplification of DNA isolated from grapevine exhibiting symptoms of grapevine yellows. Primers: Univ. - U3/U5, "universal" phytoplasma detection; STOL - fStol/rStol, detection of Vergilbungskrankheit; FD - fFD9/rFD9, detection of Flavescence dorée. Samples: 1 - Healthy control; 2 - Field-grown grapevine (Dittelsheim) cv. Scheurebe; 3 - Field-grown grapevine (St. Martin) cv. Scheurebe.

[1]) Biologische Bundesanstalt für Land- und Forstwirtschaft, Institut für Pflanzenschutz im Weinbau, Brüningstraße 84, D-54470 Bernkastel-Kues, Germany. Fax: (06531) 4936.
[2]) Staatliche Lehr- und Forschungsanstalt, Neustadt/W., Germany.
[3]) Station de Recherches sur les Mycoplasmes des Plantes, INRA, Dijon, France.

achieved positive results using the FD specific primer set FD9 with 10 of 11 vines of symptom type II and one vine of type I. These results hint at a correlation between symptom expression and the type of yellows. However, more vines of different cultivars need to be tested to exclude varietal variation of susceptibility as a possible cause of the differences in symptoms.

Samples of vines, from which DNA was amplified with primers FD9, were tested by ELISA with FD-specific antibodies (Tab. 2). Two of five samples revealed uncertain results, which are probably due to the use of freeze-dried material. The OD_{405} of the other samples, however, clearly exceeded the healthy control and even surpassed the reading of the FD-positive control sample.

Table 2

ELISA readings (average of 4 replicates) of samples prepared from grapevines which revealed positive PCR results with FD-specific primers

Sample	OD_{405}
Healthy control	0.037
FD infected control	0.151
Scheurebe (St. Martin I)	0.080
Scheurebe (St. Martin II)	0.163
Müller-Thurgau (St. Martin)	0.181
Scheurebe (Burrweiler)	0.050
Scheurebe (Appenhofen)	0.288

The data of this study confirm that a second type of grapevine yellows beside VK is present in German vineyards. PCR amplification of DNA with FD-specific primers as well as the positive results of ELISA with antibodies to FD lead to the conclusion, that this disease is similar, or very close to FD though additional investigations are necessary to characterise the German isolates and their relationship to FD in other European countries. Both types of GY were found in the same area, sometimes in adjacent vineyards. This observation is similar to vineyards in Southern France and Northern Italy, where Bois noir and FD were found within the same areas (CARRARO *et al.* 1994; DAIRE 1994).

The detection of FD in Germany raises questions regarding its epidemics. In contrast to the areas mentioned above, *Scaphoideus titanus,* the natural vector of FD in southern Europe, is not present in Germany and probably is not able to complete its generation cycle under the specific climatic conditions of the northern viticultural areas. Therefore, FD has either been introduced by infected grapevines, most likely rootstocks (CAUDWELL *et al.* 1993), or the disease is endemic in Germany. In this case, the presence of another vector besides *S. titanus* has to be proposed.

Phytosanitary measures to detect and control yellows diseases in Germany are also hampered by the presence of a second type of yellows beside VK. It will therefore be necessary to investigate the reasons for the recent occurrence and increase of grapevine yellows in the Palatinate and other German viticultural areas in order to prevent further spreading and thereby increasing damage to viticulture.

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