Vitis **46** (1), 101–102 (2007)

Research Note

Temporal shifts of Bois Noir phytoplasma types infecting grapevine in South Tyrol (Northern Italy)

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K e y w o r d s : Grapevine yellows, phytoplasma, vector, Vergilbungskrankheit, *Vitis vinifera.*

Introduction: During the last years in the vineyards of South Tyrol (Northern Italy) an increased incidence of grapevine yellows has been observed. Grapevine yellows can be induced by several different phytoplasma strains, the most important in Europe being Flavenscence dorée (16SrV or Elm yellows group) and Bois noir (BN; 16SrXII-A or Stolbur group) (Lee *et al.* 1998). Both phytoplasmas are present in the northern part of Italy and in some vine-growing areas they occur simultaneously (Bianco *et al.* 2002). Although the two diseases considerably differ in their epidemiology and thus require different phytosanitary measures, they can not be distinguished on behalf of the symptoms they induce. For this reason, in South Tyrol a continuous monitoring program has been carried out since summer 2002. In the course of this program vines with yellows symptoms were tested by a nested PCR-RFLP procedure in order to determine the phytoplasma involved (Pasquini *et al*. 2001, Maixner *et al*. 1995). So far, in South Tyrol primarily BN was identified, which is transmitted to grapevine occasionally by the polyphagous Cixiid planthopper *Hyalesthes obsoletus* from vineyard weeds (Weber and Maixner 1998, Alma *et al.* 2002, Sforza *et al.* 1998). Recently, the existence of three different sub-types of BN was reported, which can not only be distinguished by molecular traits, but also by their association with different host plant species of the insect vector *H. obsoletus* (Langer and Maixner 2004). The objective of the present study was to identify the BN-types infecting grapevine in South Tyrol in order to assess the possible reservoirs of this dangerous grapevine disease. By analysing samples collected over a 4-year period it was even possible to gain insight into the stability of distribution patterns over time of the BN-types in the investigated grapevine-growing region.

Material and Methods: The present survey is based on DNA isolated from leaf midribs of *Vitis vinifera* with yellows symptoms, which were collected in the period from 2002 to 2005 in the framework of the "Grapevine Yellows Monitoring Program" of the Autonomous Province of South Tyrol. All DNA extracts employed herein were previously tested positive for BN in a nested PCR with 16SrXII-A group specific primers (Maixner *et al.* 1995) and a nested PCR-RFLP procedure using universal primers (Pasquini *et al.* 2001), and stored at -20 °C for possible further analyses. Within the scope of this study nucleic acid isolates from 185 grapevine plants were analysed by amplifying an approximately 950 bp-fragment of the elongation factor Tu gene using primer pair fTufAy/rTufAy (Schneider *et al.* 1997). Amplification products were digested with restriction enzyme *Hpa*II and resolved on 2 % MetaPhore agarose gels (Cambrex Corporation, USA). Restriction enzyme patterns were compared to the three reference strains VK-Type I, VK-Type II and VK-Type III, kindly provided by M. Maixner (Langer and Maixner 2004).

Results and Discussion: Two different restriction enzyme profiles of the Bois noir (BN) phytoplasma, corresponding to VK-Type I and VK-Type II (Langer and Maixner 2004), were found in South Tyrol (Table). Both these BN-types were previously detected in German viticultural areas affected by grapevine yellows. While in Germany also a third BN-type was described (VK-Type III), which is exclusively restricted to the Mosel valley, this strain was not found in South Tyrol.

Each BN-type has been associated with a different herbaceous host plant species of the vectoring insect *H. obsoletus*: VK-Type I with *Urtica dioica*, VK-Type II with *Convolvulus arvensis*, and VK-Type III with *Calystegia sepium* (Langer and Maixner 2004). Consequently, it can be assumed that at least two different life cycles of the BN phytoplasma might play a role in the propagation of the disease in South Tyrol. Although in Germany three natural cycles of BN are known so far, the epidemiological system *C. arvensis* - *H. obsoletus* - *V. vinifera* was shown to be the predominating one there (Maixner *et al*. 1995), and VK-Type II the most abundant and most widespread BN-type (Weber and Maixner 1998). On the contrary, in northern Italy the stinging nettle (*U. dioica*) represents the principal host plant of *H. obsoletus* (Alma *et al*. 2002), an observation that is also supported by the present study, in which VK-Type I was identified as the prevalent BN-type. Moreover, this BN phytoplasma strain was the only which was detected in diseased grapevines in the years 2002 and 2003 in our survey area (Table). VK-Type II appeared as recently as in 2004 in 12.2 % of the samples investigated, and in 2005 it even displayed an incidence of 22.6 %. Although a sampling bias can not be completely excluded, our data point to a recently increased spread of the bindweed-associated BN phytoplasma type, VK-Type II, in South Tyrol. It is not yet clear what factors could have triggered a change in the distribution patterns of the BN phytoplasma strains and whether in the future the epidemiological cycle involving *C. arvensis* will become of major importance. Thus, further field studies and laboratory surveys involving grapevine, vector populations and its major host plants are being undertaken in order to develop efficient disease management strategies.

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T a b l e

Sample size and results of Bois noir phytoplasma typing, which were detected in *Vitis vinifera* in different viticultural areas of South Tyrol (Northern Italy) from 2002 to 2005

		N tested	VK-Type I	VK-Type II
2002	Northern zone[1]	12	12	-
	Southern zone[2]	17	17	-
	Total	29	29 (100 %)	-
2003	Northern zone	8	8	-
	Southern zone	23	23	-
	Total	31	31 (100 %)	-
2004	Northern zone	5	5	-
	Southern zone	36	31	5
	Total	41	36 (87.8 %)	5 (12.2 %)
2005	Northern zone	4	2	2
	Southern zone	75	58	17
	Western zone[3]	5	5	-
	Total	84	65 (77.4 %)	19 (22.6 %)

[1] Northern zone: viticultural area around Klausen/Chiusa and Brixen/Bressanone.
[2] Southern zone: viticultural area around and south of Bozen/Bolzano.
[3] Western zone: viticultural area around Meran/Merano.

Acknowledgements

We thank M. Maixner for providing DNA extracts of the three VK-Types, K. Mair and A. Morandell for supplying grapevine samples and C. Kerschbamer for technical assistance. The work was funded by the Autonomous Province of Bozen/Bolzano, Italy.

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Received September 14, 2006