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Occurrence of Stolbur phytoplasma in the vector *Hyalesthes obsoletus*, herbaceous host plants and grapevine in South Tyrol (Northern Italy)

J. Berger, W. Schweigkofler, C. Kerschbamer, C. Roschatt, J. Dalla Via and S. Baric

Research Centre for Agriculture and Forestry Laimburg, Auer/Ora (BZ), Italy

Summary

Bois noir (BN) is a grapevine yellows disease caused by a phytoplasma of the Stolbur group (16SrXII-A). The planthopper *Hyalesthes obsoletus* is known to be the principal vector and can accidentally transmit the phytoplasma from its herbaceous host plants to grapevine (*Vitis vinifera*). Due to the increasing incidence of BN over the last decade, a monitoring study was conducted in South Tyrol (Northern Italy). Over a period of up to four years, 659 insect vector samples, 516 herbaceous plants of 41 potential host plant species as well as 56 grapevine samples from BN-affected vineyards were tested for the presence of the Stolbur phytoplasma using a nested PCR procedure. In addition, a recently developed TaqMan allelic discrimination assay was employed to determine different subtypes of BN in infected samples. The Stolbur phytoplasma could be detected in all three sample types analysed, and was shown to belong to two different subtypes, VK type I and VK type II. In most vineyards one subtype was found to be predominant. The average infection rate of *H. obsoletus* amounted to 24.1 %. Analysis of herbaceous plants revealed that 25.1 % of the *Convolvulus arvensis* samples tested positive for the BN phytoplasma, as well as 4.5 % of the *Urtica dioica* samples. Taken together, our results underline the role of these two species commonly found in the undergrowth vegetation of South Tyrolean vineyards as an important reservoir of the Stolbur phytoplasma.

K e y w o r d s : Bois noir, grapevine yellows, host plants, *Hyalesthes obsoletus*, Stolbur, phytoplasma, Italy.

Introduction

Grapevine yellows are diseases of *Vitis vinifera* caused by phytoplasmas, small cell wall-less bacteria that specifically inhabit the sieve tubes of plants. Two grapevine yellows, Bois noir (BN) and Flavescence dorée (FD), are responsible for causing great economic losses in European viticulture. Symptoms include chlorosis and downward rolling of leaves, stunted shoots and shrivelling of berries, making them unsuitable for wine production. BN or 'Vergilbungskrankheit' (VK) is caused by a phytoplasma of the Stolbur group (16SrXII-A, proposed name: '*Candidatus* Phytoplasma solani') (Lee *et al.* 1998, Seemüller *et al.* 1998). It is endemic to Europe and the Mediterranean area and has spread considerably over the last decades, causing increasing harm in affected vineyards (Daire *et al.* 1993, 1997). The Stolbur phytoplasma can be found in a wide range of host plants such as the bindweeds *Convolvulus arvensis* and *Calystegia sepium*, and the stinging nettle *Urtica dioica* (Maixner *et al.* 1995, Langer and Maixner 2004). It is known to be vectored by the polyphagous planthopper *Hyalesthes obsoletus* Signoret (Homoptera: Cixiidae) which is native in Central and Southern Europe as well as in the Middle East (Maixner 1994, Sforza *et al.* 1998). *V. vinifera* represents a dead-end host for the Stolbur phytoplasma, which is only incidentally transmitted by *H. obsoletus* from other host plants to the grapevine. In contrast, FD is transmitted vine-to-vine by the monophagous *Scaphoideus titanus* (Caudwell 1990).

A previous study distinguished three different subtypes of the Stolbur phytoplasma, VK type I, II and III, based on RFLP analysis of the elongation factor TU (*tuf*) gene (Langer and Maixner 2004). Furthermore, this study showed that the different phytoplasma subtypes are associated with a specific set of host plants: VK type I was detected only in *U. dioica* whereas VK type II was less specific and was found in *C. arvensis*, *C. sepium, Prunus spinosa*, and *Solanum nigrum*. So far, VK type III was detected only in *C. sepium* in the Mosel area in Germany. All three subtypes were also present in grapevine as well as in the insect vector *H. obsoletus*.

There are marked differences in the geographic distribution of Stolbur phytoplasma subtypes. In Germany and Austria, VK type II is the most prevalent subtype (Langer and Maixner 2004, Riedle-Bauer *et al.* 2008). However, in recent years an increased occurrence of VK type I was observed in Germany (Maixner *et al.* 2007). On the other hand, VK type I was found to be predominant in Central and Northern Italy (Bressan *et al.* 2007, Mori *et al.* 2007, Riolo *et al.* 2007). In South Tyrol in Northern Italy, the occurrence of both VK types I and II in infected grapevines was reported. However, a temporal shift of BN subtypes was observed in the past years: until 2003 only the *U. dioica*-associated VK type I was found, but a continuous spreading of VK type II was observed since its first detection in 2004 (Baric and Dalla Via 2007).

In this study, we sought to further characterise the occurrence and distribution of the two Stolbur phytoplasma subtypes in South Tyrol by analysing DNA isolates not only from symptomatic grapevines, but also from the insect vector *H. obsoletus* and various host plants collected in affected vineyards.

Correspondence to: Dr. S. Baric, Research Centre for Agriculture and Forestry Laimburg, Laimburg 6, 39040 Auer/Ora (BZ), Italy. Fax: +39-0471-969599. E-mail: sanja.baric@provinz.bz.it

Material and Methods

Sample collection: In order to reflect the diverse geographic and micro-climatic realities in the mountainous area of South Tyrol, 15 BN-affected commercial vineyards from different sub-appellations were monitored (Figure). The vineyards were planted with the white cultivars 'Chardonnay', 'Gewürztraminer', 'Kerner' and 'Müller-Thurgau', and the red cultivars 'Lagrein', 'Pinot Noir' and 'Zweigelt' (Tab. 1). The soil type of most vineyards consisted of silicate sands on slope debris, with the exception of two sites (FR and MI), which had a higher content of calcareous sands. Standard herbicide treatments were carried out on grapevine rows, whereas the plant cover between the rows was not treated chemically, but mulched repeatedly instead. No specific treatment was carried out to control *H. obsoletus*. Control of grapevine diseases was done according to the rules of Integrated Pest Management (IPM).

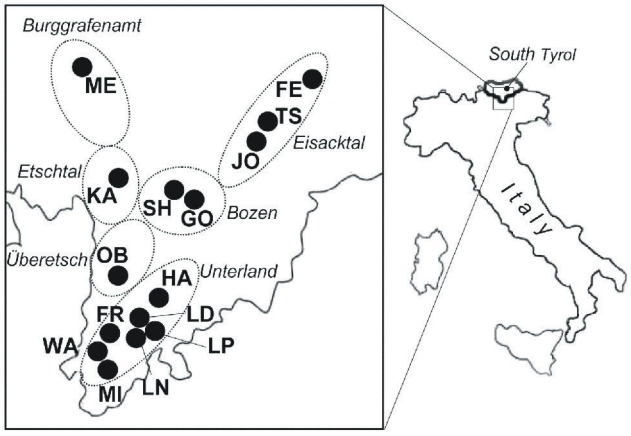

Figure: Map showing the location of the 15 vineyards (filled circles) within the main viticultural areas (dotted circles) in South Tyrol where samples were taken. The position of the enlarged area shown on the left and of South Tyrol (highlighted) within Italy is indicated.

Populations of *H. obsoletus* were monitored over a period of three years (2005-2007). A total of 659 adult insects were collected using sweep nets (100 strokes) at two-week intervals five to six times per year from June to August. Captured insects were stored at -20 ºC until DNA extraction. Additionally, 516 herbaceous plants of 41 different species belonging to 21 families were collected in the summers of 2006, 2007 and 2008 in the BN-diseased vineyards. After a visual survey of the undergrowth vegetation, representative specimens of the respective undergrowth flora were sampled in the immediate vicinity (< 2 m) of grapevines showing symptoms of BN. Only plants belonging to the class Dicotyledonae were collected due to their established role as host plants for the insect *H. obsoletus*. In the case of *C. arvensis*, specimens with symptoms like chlorosis and stunted growth likely induced by the Stolbur phytoplasma were sampled if present. After collection, plant samples were stored at 4 ºC for a maximum of five days until further processing. To confirm the presence of Stolbur phytoplasma and to determine the subtype, leaves from 56 symptomatic grapevines from five different cultivars ('Chardonnay', 'Lagrein', 'Müller-Thurgau', 'Pinot Noir' and 'Zweigelt') were collected in September and October 2007 in seven different vineyards, stored at 4 ºC and processed within two days of sampling.

DNA extraction: DNA was isolated from herbaceous plants and grapevine leaves following a modified version of the phytoplasma enrichment procedure as described previously by AHRENS and SEEMÜLLER (1992). Briefly, central leaf veins of a total of three leaves per plant were dissected and chopped into 2-mm-long pieces. 300 mg material was homogenised for 15 min in 1 ml chilled 'Mycoplasma-like Organism' (MLO) grinding buffer [95 mM dipotassium hydrogen phosphate (K_2HPO_4 x 3 H_2O), 30 mM potassium dihydrogen phosphate (KH_2PO_4), 10 % saccharose, 0.15 % bovine serum albumin (BSA) fraction V, 2 % polyvinyl pyrrolidone (pVp), 30 mM ascorbic acid, pH 7.6] in a vibration mill (Retsch GmbH, Haan, Germany) at maximum frequency (30/sec), using 5 mm stainless steel

Table 1

Characteristics of the vineyards investigated

Vineyard	Grape cultivar	Viticultural area	Elevation (m a.s.l.)	Pruning system	Vineyard slope
FE	Zweigelt	Eisacktal	650	Guyot	steep, south-facing
FR	Lagrein	Unterland	300	Guyot	gentle, east-facing
GO	Chardonnay	Bozen	350	Guyot	steep, north-facing
HA	Chardonnay	Unterland	230	Guyot	gentle, east-facing
JO	Müller-Thurgau	Eisacktal	700	Guyot	steep, east-facing
KA	Lagrein	Etschtal	280	Guyot	none
LD	Chardonnay	Unterland	230	Guyot	none
LN	Gewürztraminer	Unterland	230	Pergola	none
LP	Chardonnay	Unterland	230	Pergola	none
ME	Pinot Noir	Burggrafenamt	320	Guyot	steep, west-facing
MI	Chardonnay / Lagrein	Unterland	240	Guyot	gentle, east-facing
OB	Pinot Noir	Überetsch	500	Guyot	gentle, east-facing
SH	Chardonnay / Müller-Thurgau	Bozen	700	Pergola	steep, east-facing
TS	Zweigelt	Eisacktal	600	Guyot	steep, south-facing
WA	Chardonnay	Unterland	220	Guyot	none

beads (Qiagen, Hilden, Germany). After adding another 500 µl of chilled MLO grinding buffer and centrifuging for 5 min at 1000 x g and 4 ºC, the supernatant was transferred to a new tube and spun for 20 min at maximum speed (16,400 x g) and 4 ºC. The pellet was resuspended in 1 ml of CTAB buffer [3 % cetyltrimethyl ammonium bromide (CTAB), 0.1 M Tris-HCl, 1.4 M NaCl, 20 mM EDTA, 2 % PVP] containing 0.2 % beta-mercaptoethanol added just before use. Samples were incubated at 60 ºC for 1 h, chloroform:isoamylalcohol (24:1) extracted and precipitated with isopropanol. The pellet was washed with 70 % ethanol and dissolved in 100 µl TE buffer [10 mM Tris-HCl, 1 mM EDTA, pH 8.0]. DNA was extracted from *H. obsoletus* using the same protocol, with the exception that samples were homogenized directly in CTAB extraction buffer without PVP, containing 0.2 % beta-mercaptoethanol and 5 µl of a 10 mg·ml^{-1} proteinase K solution. DNA quality of plant extracts was checked by PCR-amplifying the chloroplast gene *mat*K as described by Matsumoto *et al.* (1998) using primers matK-AF and tranK-2R.

Detection of stolbur phytoplasma and determination of the BN subtype: The presence of Stolbur phytoplasma in *H. obsoletus* samples, herbaceous host plants and grapevine leaves was confirmed by nested PCR amplification using primers P1 and P7 for the first round of PCR (Schneider *et al.* 1995), followed by amplification of 1:50 diluted P1/P7 PCR products by a second round of PCR, using 16SrXII-A group-specific primers STOLF and STOLR (Maixner *et al.* 1995) or universal phytoplasma-specific primers R16F2 and R16R2 (Pasquini *et al.* 2001). All PCR reactions were carried out under the following conditions: initial denaturation for 10 min at 94 ºC, 35 cycles consisting of 30 s at 94 ºC, 30 s at 50 ºC, and 60 s at 72 ºC, and a final extension step of 2 min at 72 ºC.

The Stolbur phytoplasma subtype (VK type I or VK type II) was determined using a newly established TaqMan allelic discrimination assay, based on a single nucleotide polymorphism within the *tuf* gene (Berger *et al.* 2009). Since the typing assay consisted of a single-round real-time PCR, in contrast to the nested PCR carried out for the detection of the Stolbur phytoplasma, it was to be expected that some of the BN-positive specimens with low infection titres could not be typed.

Results

Stolbur phytoplasma infection rates of the vector *H. obsoletus:* A total of 659 *H. obsoletus* adults captured in BN-affected vineyards were analysed for the presence of Stolbur phytoplasma using nested PCR (Tab. 2). In 2005, 35 out of 210 (16.7 %) individuals tested positive for the Stolbur phytoplasma, compared to 81 out of 274 (29.6 %) in 2006 and 43 out of 175 (24.6 %) in 2007 (data not shown). The average infection rate of *H. obsoletus* in this three-year period was 24.1 %, but varied considerably between vineyards. In some vineyards, the infection rate reached up to 80 %, whereas in others no Stolbur phytoplasma-infected *H. obsoletus* individuals were found despite the presence of symptomatic grapevines. The Stolbur phytoplasma subtype could be determined in 138 out of 159 (86.8 %) BN-positive samples, with 73.9 % of vectors carrying VK type I and 26.1 % VK type II (Tab. 2). The prevalent BN subtype in most vineyards was the *U. dioica*-associated VK type I. In three out of 15 vineyards, both Stolbur phytoplasma subtypes could be detected in captured *H. obsoletus* specimens, albeit in different individuals. In two of these vineyards (HA and TS), planthoppers infected with different subtypes were captured in the same year. However, we never observed a co-infection with both subtypes in the same individual.

Table 2

Hyalesthes obsoletus individuals tested for the presence and subtype of Stolbur phytoplasma. Insects were captured in years 2005, 2006 and 2007 in 15 South Tyrolean vineyards. Values represent the sum of all three years

Vineyard	n tested	n positive	n VK type I	n VK type II
FE	28	4	3	0
FR	19	2	2	0
GO	79	33	32	0
HA	60	9	6	1
JO	35	21	0	19
KA	13	6	0	6
LD	21	4	3	1
LN	72	21	17	0
LP	160	32	29	0
ME	112	5	5	0
MI	15	2	0	0
OB	10	8	0	8
SH	17	5	3	0
TS	10	7	2	1
WA	8	0	-	-
Total	659	159	102	36

-: not determined as no positive samples were found.

Flight activity of *H. obsoletus* was observed from early June until the end of August. In general, most specimens were captured around mid July, but vector presence varied slightly from site to site and year to year, probably due to microclimatic conditions, soil type and annual variations of meteorological parameters. On two occasions, *H. obsoletus* specimens infected with different BN subtypes were captured on the same day in the same vineyard: In the vineyard HA, insects were collected at two different time points in 2005. At the second time point (July 11), four BN-positive *H. obsoletus* specimens were collected, of which three carried VK type I and one was infected with VK type II, whereas on the first sampling time point (June 29) only individuals infected with VK type I were captured. In the vineyard TS, six BN-positive *H. obsoletus* adults were collected on June 19 in 2007, of which three could be typed and were shown to be infected in two cases with VK type I and in one case with VK type II. One BN-positive specimen captured at the second time point (July 17) could not be typed.

Stolbur phytoplasma presence and subtype determination in herbaceous plant samples: To further elucidate the role of host

plants for *H. obsoletus* in the transmission of the Stolbur phytoplasma, 41 different herbaceous plant species belonging to 21 families collected in BN-afflicted vineyards were analysed for the presence of Stolbur phytoplasma (Tab. 3). The Stolbur phytoplasma could be detected in seven different species belonging to six families: *C. arvensis* (Family: Convolvulaceae), *Echium vulgare* (Family: Boraginaceae), *Polygonum aviculare* (Family: Polygonaceae), *Silene vulgaris* (Family: Caryophyllaceae), *Taraxacum officinale* (Family: Asteraceae), and the two Urticaceae species *U. dioica* and *U. urens*. 45 out of 179 (25.1 %) analysed *C. arvensis* samples tested positive for the BN phytoplasma, as well as five out of 111 (4.5 %) *U. dioica* samples. Only one sample of *U. urens* was analysed and proved to be positive.

Additionally, Tab. 3 summarises the results of the BN subtype determination in herbaceous plant samples, although informative results could be obtained in only 23 out of 57 (40.3 %) BN-positive samples. VK type II was detected in *C. arvensis, E. vulgare*, *P. aviculare*, *S. vulgaris*, and *T. officinale*. The three *U. dioica* samples that could be typed as well as the *U. urens* specimen were shown to be infected with VK type I.

Table 3

Detection and typing of Stolbur phytoplasma in various species of herbaceous plants, collected in BN-affected vineyards in years 2006, 2007 and 2008. Values represent the sum of all three years

Species	Family name	n tested	n positive	n VK type I	n VK type II
Achillea millefolium	Asteraceae	1	0	-	-
Artemisia vulgaris	Asteraceae	6	0	-	-
Atriplex papula	Chenopodiaceae	1	0	-	-
Calystegia sepium	Convolvulaceae	5	0	-	-
Chenopodium album	Chenopodiaceae	2	0	-	-
Chelidonium majus	Papaveraceae	7	0	-	-
Clematis vitalba	Ranunculaceae	1	0	-	-
Convolvulus arvensis	Convolvulaceae	179	45	0	15
Crepis biennis	Asteraceae	1	0	-	-
Echium vulgare	Boraginaceae	2	1	0	1
Erigeron annuus	Asteraceae	2	0	-	-
Fallopia convolvulus	Polygonaceae	2	0	-	-
Fragaria vesca	Rosaceae	3	0	-	-
Fumaria officinalis	Fumariaceae	2	0	-	-
Galinsoga ciliata	Asteraceae	2	0	-	-
Lamium album	Lamiaceae	9	0	-	-
Lamium purpureum	Lamiaceae	1	0	-	-
Malva neglecta	Malvaceae	1	0	-	-
Malva sylvestris	Malvaceae	1	0	-	-
Oxalis stricta	Oxalidaceae	3	0	-	-
Parietaria officinalis	Urticaceae	11	0	-	-
Peucedanum ostruthium	Apiaceae	1	0	-	-
Plantago lanceolata	Plantaginaceae	9	0	-	-
Plantago major	Plantaginaceae	3	0	-	-
Plantago media	Plantaginaceae	1	0	-	-
Polygonum aviculare	Polygonaceae	14	1	0	1
Rumex spp.	Polygonaceae	1	0	-	-
Salvia glutinosa	Lamiaceae	1	0	-	-
Saponaria officinalis	Caryophyllaceae	1	0	-	-
Silene dioica	Caryophyllaceae	1	0	-	-
Silene latifolia ssp. alba	Caryophyllaceae	4	0	-	-
Silene vulgaris	Caryophyllaceae	1	1	0	1
Solanum nigrum	Solanaceae	5	0	-	-
Sonchus arvensis	Asteraceae	2	0	-	-
Taraxacum officinale	Asteraceae	114	3	0	1
Trifolium pratense	Fabaceae	1	0	-	-
Urtica dioica	Urticaceae	111	5	3	0
Urtica urens	Urticaceae	1	1	1	0
Viola arvensis	Violaceae	1	0	-	-
Verbascum phlomoides	Scrophulariaceae	1	0	-	-
Vincetoxicum hirundinaria	Apocynaceae	1	0	-	-
Total (41 species)	21 families	516	57	4	19

-: not determined as no positive samples were found.

BN subtype distribution in *H. obsoletus*, herbaceous plants and grapevines in selected vineyards: Seven vineyards with high BN phytoplasma infection rates in vector and herbaceous plant samples were selected to study the concordance of Stolbur phytoplasma subtypes detected in *H. obsoletus*, herbaceous host plants and infected grapevines in greater detail. Cultivars grown in these vineyards were 'Chardonnay', 'Lagrein', 'Müller-Thurgau', 'Pinot Noir', and 'Zweigelt'. In addition to insect vectors and two herbaceous plant species (*C. arvensis* and *U. dioica*), leaves of symptomatic grapevines were collected in the same vineyards and tested for the presence and subtype of the Stolbur phytoplasma (Tab. 4). For instance, symptomatic grapevines in five out of seven vineyards were infected with only one subtype, whereas in the two remaining vineyards, KA and OB, both Stolbur phytoplasma subtypes could be detected. There was a relatively good correspondence between the BN subtypes found in different sample types collected in the same vineyard, especially when comparing grapevines and insect vectors. However, *C. arvensis* samples that tested positive for Stolbur phytoplasma were found in all vineyards, but in seven out of 19 samples the subtype could not be determined. In two vineyards (LP and FE), both *C. arvensis* and *U. dioica* specimens were found to be BN-positive.

Two vineyards (JO and LP) with high numbers of BN-positive samples producing informative typing results illustrate the prevalence of one BN subtype especially well: In the vineyard JO, 21 positive *H. obsoletus* specimens were captured, of which all 19 samples that could be typed were identified as being VK type II. The same subtype was detected in all eight grapevine leaves analysed as well as in six BN-positive *C. arvensis* samples. In contrast, vineyard LP was mainly infested with VK type I phytoplasma: all 29 BN-positive *H. obsoletus* samples that could be typed as well as all grapevine leaves analysed were shown to be infected with this subtype. Additionally, in one of the two BN-positive *U. dioica* specimens VK type I phytoplasma could be detected. However, also four *C. arvensis* samples in this vineyard tested positive for Stolbur phytoplasma, and in one of these samples the subtype could be determined to be VK type II.

Examination of the undergrowth flora revealed that vineyard JO was vegetated with *C. arvensis*, *T. officinale* and other weeds, whereas vineyard LP was clearly dominated by *U. dioica*, with few additional dicotyledonous species found (data not shown).

Table 4

Summary of VK type determination in the vector *Hyalesthes obsoletus*, symptomatic grapevines leaves (*Vitis vinifera*) and the two herbaceous host plants *Convolvulus arvensis* and *Urtica dioica* in selected BN-affected vineyards in South Tyrol

Vineyard	Sample	n tested	n positive	VK type I	VK type II
JO	*H. obsoletus*	35	21	0	19
	V. vinifera	8	8	0	8
	C. arvensis	11	6	0	6
	U. dioica	0	-	-	-
LP	*H. obsoletus*	160	32	29	0
	V. vinifera	8	8	8	0
	C. arvensis	8	4	0	1
	U. dioica	15	2	1	0
KA	*H. obsoletus*	13	6	0	6
	V. vinifera	10	5	4	1
	C. arvensis	8	2	0	2
	U. dioica	0	-	-	-
HA	*H. obsoletus*	60	9	6	1
	V. vinifera	8	8	8	0
	C. arvensis	38	2	0	1
	U. dioica	36	0	-	-
OB	*H. obsoletus*	10	8	0	8
	V. vinifera	8	6	2	4
	C. arvensis	4	1	0	1
	U. dioica	1	0	-	-
FE	*H. obsoletus*	28	4	3	0
	V. vinifera	6	6	6	0
	C. arvensis	17	3	0	1
	U. dioica	18	3	2	0
ME	*H. obsoletus*	112	5	5	0
	V. vinifera	8	8	8	0
	C. arvensis	12	1	0	0
	U. dioica	16	0	-	-

-: not determined.

Discussion

To shed some light on the epidemiology of the Stolbur phytoplasma, samples of the insect vector *H. obsoletus*, various herbaceous host plants and symptomatic grapevine leaves were collected over a period of up to four years in BN-affected vineyards in South Tyrol and tested for the presence of the phytoplasma.

H. obsoletus was detected and sampled in all 15 vineyards. Insect captures were lower than those reported for some wine growing areas in Germany (Darimont and Maixner 2001), and higher than in some areas in eastern Austria (Tiefenbrunner *et al.* 2007), but standardised population densities could not be estimated due to the clustered distribution of the insect. Our study revealed that infection rates of the vector *H. obsoletus* reached an average of 24.1 % over a three-year period of monitoring (2005-2007). A case study conducted over a similar period (2003-2005) in the Northern Italian region of Verona reported comparable infection rates of 20-30 % in *H. obsoletus* populations (Bressan *et al.* 2007).

Determination of the BN subtype revealed that both subtypes, VK type I and II, were present in the BN-positive *H. obsoletus* population in South Tyrolean vineyards; however, VK type I was detected three times more frequently. This agrees with our previous results showing only a recent appearance of VK type II phytoplasma in BN-infected grapevines in South Tyrol (Baric and Dalla Via 2007). In correspondence with our findings, other studies conducted in Northern Italy also related the predominance of VK type I in BN-infected *H. obsoletus* specimens (Bressan *et al.* 2007, Lessio *et al.* 2007, Mori *et al.* 2007). Our study shows that in a given vineyard most *H. obsoletus* specimens were infected with the same BN subtype. However, two vineyards contained vector populations infected with both subtypes in the same year, but we never observed a co-infection of VK type I and II in the same individual. In many European regions, including other parts of Italy, one Stolbur phytoplasma subtype seems to prevail in the BN-infected *H. obsoletus* population. In Germany however, where the *C. arvensis*-associated VK-type II was predominant until recently (Langer and Maixner 2004), a sudden increment of the population density of planthoppers infected with VK type I phytoplasma was observed in the past ten years, together with an increased exploitation of nettle as a host plant (Maixner *et al.* 2007). It has been suggested that the two *H. obsoletus* populations infected with VK type I or VK type II differ with respect to flight periods when found in the same area (Maixner *et al.* 2007, Angelini *et al.* 2008). The flight phase in South Tyrol lasts from early June to the end of August with a maximum around mid July (Schweigkofler *et al.* 2006). Seasonal emergence of the first individuals in South Tyrol varies slightly based on spring temperatures and micro-climates. A tendency to earlier start of the flight in the sun-exposed steep slopes with light soils (e.g. in the Eisacktal) compared to the valley grounds with heavy loamy soils (e.g. some vineyards in the Unterland) was observed. Due to the limited number of sampling dates and of infected *H. obsoletus* specimens that could be typed, we were not able to observe clear differences in the flight activity of *H. obsoletus* infected with either of the two BN subtypes. On two occasions, insect vectors infected with different BN subtypes were captured on the same day and in the same vineyard. Both of these time points (June 19 and July 11) can be allocated to a period where the flight curves of *H. obsoletus* individuals carrying VK type I as well as VK type II phytoplasmas overlap in Northern Italy (Angelini *et al.* 2008).

Since the planthopper *H. obsoletus* is known to be polyphagous, a visual plant survey of the undergrowth vegetation in each vineyard was carried out. 12 to 24 dicotyledonous herbaceous species were identified per vineyard, in addition to a number of monocotyledonous species, which were not examined further because they are not known to be host plants for the insect vector *H. obsoletus* (Nickel 2003). A representative cross section of 41 herbaceous plant species commonly occurring in South Tyrolean vineyards was then tested for the presence of Stolbur phytoplasma, with the main emphasis on the known host plants *C. arvensis* and *U. dioica* and the potential host *T. officinale*. In the case of *C. arvensis*, specimens with symptoms like chlorosis and stunted growth, likely induced by the Stolbur phytoplasma, were sampled if present, whereas no symptoms are described for infected *U. dioica*. Only seven species tested positive, with *C. arvensis* showing the highest infection rate. The infection rates determined in *C. arvensis* and *U. dioica* were comparable to the ones reported by previous studies (Langer and Maixner 2004, Riolo *et al.* 2007). None of the other 34 plant species analysed were positive for Stolbur phytoplasma, although some, such as *Solanum nigrum*, have been described as host plants before (Langer and Maixner 2004). It should be noted however, that the sample number per species was in many cases very low; for 19 species only one individual was tested. In most of the species that we found to be BN-positive, the Stolbur phytoplasma was detected before (Arzone *et al.* 1995, Skoric *et al.* 1998, Batlle *et al.* 2000, Riedle-Bauer *et al.* 2006). To our knowledge, *U. urens* has not yet been described as a host plant for Stolbur phytoplasma. However, the importance of this finding needs to be viewed critically in light of *U. urens* being an annual plant, in contrast to other known Stolbur phytoplasma host plants, including *U. dioica*, which are perennial. It seems plausible that infection of *H. obsoletus* occurs predominantly during feeding of the nymphs at the roots of host plants during winter (Darimont and Maixner 2001). For this reason it is thought that annual plants probably do not act as very efficient Stolbur phytoplasma reservoirs, especially as phytoplasmas are not thought to be transmitted through seeds (Christensen *et al.* 2005).

As two out of 18 *T. officinale* specimens were found to be BN-positive in the first year of sampling, it was assumed that this species could act as a reservoir for the phytoplasma in South Tyrol. For this reason, 96 additional specimens were collected in the following years. However, only one more *T. officinale* specimen appeared to be infected with BN phytoplasma. *T. officinale* has been shown to test positive for the Stolbur phytoplasma in previous studies (Credi *et al.* 2006, Riedle-Bauer *et al.* 2006), but it still needs to be confirmed that this species, which is very

abundant in South Tyrolean vineyards, can act as a source of inoculum.

Analysis of the BN subtype in herbaceous plants revealed the presence of VK type II not only in *C. arvensis* but in four additional species, thereby confirming earlier reports that this subtype is less specific regarding its host plants (Langer and Maixner 2004). VK type I phytoplasma was detected in *U. dioica* and also in the dwarf nettle *U. urens*. Our data show that in South Tyrol *U. dioica* and *C. arvensis* represent the two major host plants and can therefore act as inoculum sources for the Stolbur phytoplasma, as is the case in most European wine-growing areas. In all cases where typing was possible these host plants were infected with their associated BN subtypes.

With respect to the relative homogeneity of the BN subtype distribution in all samples in a given vineyard, it is tempting to speculate that the host plant species dominating the weed flora of the vineyard (*U. dioica* or *C. arvensis*) will determine the phytoplasma subtype. As more than 10 % of the herbaceous plant specimens investigated in the present study were found to be BN-positive, our data support the hypothesis that undergrowth vegetation represents an important source of the Stolbur phytoplasma in vineyards. Therefore, wine growers affected by the BN disease should be aware of this problem. Mowing, mulching, well-directed herbicide treatments and the use of non-host-plant seed mixes in newly planted vineyards are measures that can be taken to eliminate weeds from vineyards and thereby minimise the risk of a BN infection.

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