Biodiversity of planthoppers (Auchenorrhyncha) in vineyards infected by the Bois noir phytoplasma

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Abstract

The presence and density of plant- and leafhoppers was investigated in eleven vineyards infected with *Ca.* Phytoplasma solani, causal agent of Bois noir (BN), in South Tyrol (Northern Italy) using insect nets for sampling the understory vegetation. The confirmed vector *Hyalesthes obsoletus* Signoret was sampled from early June to mid August of 2006; its abundance was positively correlated to the presence of BN symptoms on grapevines. An additional 56 Auchenorrhyncha species were sampled; the most numerous being *Psammotettix confinis* (Dahlbom), *Laodelphax striatella* (Fallén), *Dicranotropis hamata* (Boheman), *Psammotettix alienus* (Dahlbom), *Falcotoya minuscula* (Horváth), *Macrosteles cristatus* (Ribaut), *Dictyophara europaea* (L.), *Philaenus spumarius* (L.), *Anaceratagallia ribauti* (Ossiannilson) and *Neoaliturus fenestratus* (Herrich-Schäffer). Several invasive species, such as *Stictocephala bisona* Kopp & Yonke and *Metcalfa pruinosa* (Say) were sampled in the investigated vineyards, whereas *Scaphoideus titanus* Ball, the vector of the Grapevine yellow Flavescence dorée, was not found. *Recilia horvathi* (Then) (Cicadellidae) was found for the first time in South Tyrol.

Keywords: *Vitis vinifera*, planthoppers, Bois noir, biodiversity, invasive species, Auchenorrhyncha, South Tyrol, Italy

Zusammenfassung

Biodiversität von Zikaden (Auchenorrhyncha) in Schwarzholz-infizierten Weinbergen

Das Vorkommen und die Dichte von Zikadenpopulationen im Unterwuchs von elf Weingärten in Südtirol, welche von *Ca.* Phytoplasma solani, dem Erreger der Schwarzholzkrankheit (Bois noir; BN) befallen sind, wurde mittels Käscherung erhoben. Der bekannte Vektor *Hyalesthes obsoletus* Signoret wurde von Anfang Juni bis Mitte August 2006 gefangen, seine Dichte war positiv korreliert zur Präsenz von BN Symptomen an Weinreben. Zusätzlich wurden weitere 56 Auchenorrhyncha-Arten gefangen: am häufigsten *Psammotettix confinis* (Dahlbom), *Laodelphax striatella* (Fallén), *Dicranotropis hamata* (Boheman), *Psammotettix alienus* (Dahlbom), *Falcotoya minuscula* (Horváth), *Macrosteles cristatus* (Ribaut), *Dictyophara europaea* (L.), *Philaenus spumarius* (L.), *Anaceratagallia ribauti* (Ossiannilson) und *Neoaliturus fenestratus* (Herrich-Schäffer). Verschiedene invasive Arten, wie die Büffelzikade *Stictocephala bisona* Kopp & Yonke und *Metcalfa pruinosa* (Say) wurden in den untersuchten Weingärten gefunden, nicht jedoch *Scaphoideus titanus* Ball, der Überträger der Vergilbungskrankheit Flavescence dorée. Die Präsenz von *Recilia horvathi* (Then) (Cicadellidae) wird zum ersten Mal für Südtirol berichtet.

1. Introduction

A considerable number of planthopper species (Auchenorrhyncha; Hemiptera) are known vectors of plant pathogenic microbes (esp. viruses and phytoplasmas) and play a crucial role for the epidemiology of important plant diseases (Weintraub &Beanland 2006, Weintraub 2007). A disease complex named Grapevine yellows recently has received considerable attention because of the severe economic impact. Grapevine yellows are characterized by chlorosis and downward rolling of leaves combined with imbalanced nutrient content (Schweigkofler et al. 2008), stunted shoots and shriveling of berries, which leads to significant losses in the crop quality and yield (Schweigkofler & Roschatt 2008). The infected grapevine (*Vitis vinifera* L.) often remains weakened, which in combination with severe frost damage, might lead to an increased death rate.

Although Grapevine yellows are always caused by a phytoplasma, a cell wall less pleomorphic bacterium, and transmitted by a planthopper, different diseases can be defined based on the pathogen and vector involved. Bois noir (BN), widespread in several countries in Southern and Central Europe, is caused by a phytoplasma of the Stolbur group (16SrXII-A, proposed name: '*Candidatus* Phytoplasma solani') (Seemüller et al. 1998). The only confirmed vector of BN so far is *Hyalesthes obsoletus* (Cixiidae), a monovoltine (two generations per year were reported in Israel; Sharon et al. 2005), polyphagous species distributed from Asia minor, Kazakhstan, southern Russia, and the Mediterranean region to southern parts of central Europe (Holzinger et al. 2003). Flavenscence dorée (FD), another Grapevine yellow disease with very similar symptoms, is caused by the phytoplasma '*Candidatus* Phytoplasma vitis' (Group 16SrV, Elm yellows), which is vectored by *Scaphoideus titanus*, an invasive species originating from North America. *H. obsoletus* is a polyphagous species which prefers herbaceous, perennial host plants, such as the bindweeds *Convolvulus arvensis* L. and *Calystegia sepium* (L.) R. Br. (Convolvulacaeae), *Ranunculus* spp. (Ranunculaceae), *Senecio* spp. and *Artemisia* spp. (Asteraceae) and the stinging nettle *Urtica dioica* L. (Urticaceae) (Maixner 1994, Holzinger et al. 2003, Langer & Maixner 2004). *V. vinifera* acts only as a secondary host plant of *Ca.* Phytoplasma solani. The grapevine is a dead end host, because grape-to-grape transmission doesn't occur, which interrupts the infection cycle. The epidemiological significance of other confirmed host plants (among them *Taraxacum officinale* G. H. Weber ex Wiggers and *Polygonum aviculare* L. s.l (Berger et al. 2009)) has still to be studied in more detail. Although the first report of *H. obsoletus* in South Tyrol dates back to the nineteenth century (Hellrigl 1996), severe outbreak of BN was reported only in the 1990s. The possibility that planthoppers other than *H. obsoletus* might vector *Ca.* Phytoplasma solani in vineyards, especially in winegrowing areas with very low densities of *H. obsoletus* (Riedle-Bauer et al. 2006), cannot be excluded. *Ca.* Phytoplasma solani infecting corn (*Zea mays* L.) was reported to be vectored by the ciixid *Reptalus panzeri* (Löw) (Jović et al. 2007), and the insect can infect *in vitro*-grapevines (Laviña et al. 2006). In addition, several other Auchenorrhyncha species were reported recently to contain detectable amounts of the phytoplasma (Riedle-Bauer et al. 2006, 2008).

Our study focused on the biodiversity of Auchenorrhyncha species in selected vineyards in South Tyrol to identify possible additional vectors of *Ca.* Phytoplasma solani.

2. Material and Methods

Eleven commercial vineyards planted with different white and red cultivars from different sub-appellations within the diverse mountainous area of South Tyrol were sampled (see Table 1). Based on the differing vineyard sizes, two to ten plots were sampled on each site. The time period was chosen based on earlier experiences of the flight cycle of *H. obsoletus* in South Tyrol. 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. The understory vegetation in the vineyards was surveyed visually. No specific treatment was carried out to control *H. obsoletus*. Control of grapevine diseases (including chemical control of *Empoasca vitis* (Goethe)) was done according to the rules of Integrated Pest Management (IPM), with the exception of the organically managed vineyard FE. The BN infection rate of the grapevines was surveyed based on the presence of clear visual symptoms in October 2006. Planthoppers were sampled from the understory vegetation (height above ground approx. 0-50 cm) at two-week intervals from June 7 to August 17, 2006, using insect nets (open diameter: 33 cm; 100 strokes / plot), transferred into plastic bags (6 l), and transported to the laboratory, where they were treated with CO_2 the same day and stored at –20°C until morphological identification.
For each site, the biodiversity of Auchenorrhyncha species was calculated using the Shannon-Wiener-Index, $H = - S\ p_i\ ln\ (pi)$, with p_i = the relative abundance of species *i*. The species evenness E was calculated as $E = H/H_{max.}$ Maximum diversity possible: $H_{max} = ln\ (1/s)$ (s = total number of species).

Table 1: Characteristics of the vineyard sites used in this study.

Site	Grapevine cultivar	Geographic area	Elevation (m.a.s.l.)	Pruning system	Slope	BN infection rate 2006	Plots/ vineyard
FE	diverse; organic	Eisacktal	600	Guyot	gentle, east-facing	3.8	6
FR	Lagrein	Unterland	300	Guyot	gentle, east-facing	5.2	4
HA	Chardonnay	Unterland	230	Guyot	gentle, east-facing	2.0	10
KA	Lagrein	Etschtal	280	Guyot	none	3.7	6
LN	Gewürztraminer / Lagrein	Unterland	230	Pergola	none	0	2
LO	Chardonnay	Unterland	230	Guyot / Pergola	none	28.4	8
ME	Pinot Noir	Burggrafen-amt	320	Guyot	steep, west-facing	25.5	6
MI	Chardonnay / Lagrein	Unterland	240	Guyot	gentle, east-facing	3.1	4
OB	Pinot Noir	Überetsch	500	Guyot	gentle, east-facing	1.1	5
PE	Lagrein	Unterland	260	Guyot	gentle, west-facing	1.1	3
WA	Chardonnay	Unterland	220	Guyot	none	1.3	4

3. Results

3.1 Meteorological data

The sampling period between June 7 and August 17, 2006, was characterized by relatively cool temperatures until June 10, which was followed by a very warm and dry period until July 25 and a cool and wet period until August 17. Median air temperatures as measured at the Meteorological Station Laimburg (222 m m.a.s.l.; geographic coordinates: 11°17'18" east, 46°22'59" north) were 21.5° C in June (Tmin: 3.5° C, Tmax: 34.0° C); in July 24.6° C (Tmin: 13.8° C, Tmax: 37.3° C) and in August 18.7° C (Tmin: 5.8° C, Tmax: 28.8° C). Monthly precipitation rates were: June, 83.6 mm (6 rainy days); July, 92.6 mm (13 rainy days) and August, 133.6 mm (15 rainy days).
Occurrence and density of Auchenorrhyncha species with soil dwelling larval stages like *H. obsoletus* are also influenced by soil temperatures during larval development from depositing of eggs in late July / August until hatching of the adults in early June of the following year. Fifth instar larvae were found on the roots of *Urtica dioica* in soil layers between approximately 3 and 20 cm deep (data not shown). The soil temperature at 20 cm depth fluctuated between 22.0° C (August 2005) and 0.3° C (January 2006), with an annual mean of 12.5° C. Microclimatic conditions (temperature, precipitation, wind strength) varied slightly between the eleven sites used for sampling, but the general weather trend was similar.

3.2 Characterization and treatment of the understory vegetation

The understory vegetation was characterized visually during the summer months. In general, in most vineyards (with the exception of the organically managed site FE) grapevine rows (approximately 20 cm) were treated with herbicides and therefore free of vegetation or containing only a very sketchy vegetation composed of pioneer plants like *C. arvensis.* The area between the rows (approx. 1.8 to 2.1 m) were not treated chemically, but mulched repeatedly, resulting in a high degree of plant cover (>80 % of the surface). Between 12 and 24 herbaceous dicotyledonous plant species and a few grasses dominated the understory vegetation of the vineyards (Table 2). The presence of *Ca.* Phytoplasma solani in selected plants from these vineyards was reported recently (Berger et al. 2009). The sites FE, HA, ME, MI were adjoining typical mixed forests characterized by the presence of *Castanea sativa* Mill., *Fraxinus ornus* L., *Larix decidua* Mill., *Picea abies* (L.) Karst., *Pinus nigra* Arnold and *Quercus pubescens* Willd., among others, whereas the other sites were entirely surrounded by vineyards.

3.3 Sampling and identification of Auchenorrhyncha species

Planthoppers were sampled in eleven vineyards during the summer of 2006. Because the flight period of *H. obsoletus* is restricted to a few weeks, this period was analyzed in further detail. A total of 217 *H. obsoletus* specimens were sampled. The flight cycle of *H. obsoletus* is shown in Figure 1a. Close to 90 % of all *H. obsoletus* specimens were caught

Table 2: Plant species growing in the understory of South Tyrolean vineyards. Selected plants were tested for the presence of *Ca.* Phytoplasma solani using PCR (for details see Berger et al. 2009).

Species	**Family**	**PCR test**
Achillea millefolium agg. L.	Asteraceaea	-
Aeogopodium podagraria L.	Apiaceae	n.d.
Amaranthus sp.	Amaranthaceae	n.d.
Arrhenatherum elatius (L.) P. Beauv. ex J. Presl & C. Presl	Poaceae	n.d.
Artemisia verlotiorum Lamotte	Asteraceae	n.d.
Artemisia vulgaris L.	Asteraceae	-
Campanula rapunculus L.	Campanulaceae	n.d.
Chenopodium album agg. L.	Chenopodiaceae	-
Convolvulus arvensis L.	Convolvulaceae	+
Cynodon dactylon (L.) Pers.	Poaceae	n.d.
Digitaria sanguinali (L.) Scop.	Poaceae	n.d.
Diplotaxis tenuifolia (L.) DC	Brassicaceae	n.d.
Echinochloa crus-galli (L). Beauv.	Poaceae	n.d.
Elymus repens (L.) Gould.	Poaceae	n.d.
Equisetum arvense L.	Equisetaceae	n.d.
Erigeron annuus (L.) Pers.	Asteraceaea	n.d.
Fragaria vesca L.	Rosaceae	-
Galinsoga ciliata (Raf.) S.F.Blake	Asteraceae	-
Galium mollugo agg. L.	Rubiaceae	n.d.
Geranium pusillum Burm. f. ex L.	Geraniaceae	n.d.
Glechoma hederacea L.	Lamiaceae	n.d.
Lactuca serriola L.	Asteraceae	n.d.
Lamium album L.	Lamiaceae	-
Lolium perenne L.	Poaceae	n.d.
Medicago sativa L.	Fabaceae	n.d.
Oxalis stricta L.	Oxalidaceae	-
Parietaria officinalis L.	Urticaceae	-
Plantago lanceolata L.	Plantaginaceae	-
Polygonum aviculare agg. L. s.l.	Polygonaceae	+
Portulaca oleracea L.	Portulacaceae	n.d.
Senecio inaequidens DC.	Asteraceae	n.d.
Setaria viridis L. (Beauv.)	Poaceae	n.d.
Silene latifolia (alba) Poiret	Caryophyllaceae	-
Solanum nigrum L.	Solanaceae	n.d.
Sonchus oleraceus L.	Asteraceae	-
Taraxacum officinale agg. G.H. Weber ex Wiggers	Asteraceae	+
Trifolium pratense L.	Fabaceae	-
Trifolium repens L.	Fabaceae	n.d.
Urtica dioica L.	Urticaceae	+
Viola arvensis Murr.	Violaceae	-

Table 3: Auchenorrhyncha species sampled from vineyards. Number of sampled specimens, relative abundance of each species and the site where the species was sampled is shown. In addition, key biological features which might determine if a species is a possible vector of phytoplasmas are listed. A: abundance of the species, F: feeding pattern (mono- or polyphage; host plants listed when known), T: plant tissue on which the planthopper feeds (phloem, xylem, mesenchym), G: generations/year, O: over-wintering stage (A: adult; E: egg; N: nymph); V: vector (microbes associated with the planthopper are listed).

Species	No.	A [%]	Site	F	T	G	O	V*
CIXIIDAE								
Cixiinae								
Hyalesthes luteipes Fieber	2	0.05	FR, LO	P (mainly *Ulmus* and *Celtis*)	P	1	N	
Hyalesthes obsoletus Signoret	217	5.67	FE, FR, HA, KA, LN, LO, ME, MI, OB, WA	P	P	1	N	Phyto (Stolbur)
Reptalus cuspidatus Fieber	19	0.50	FR, MI, OB, PE	P (woody plants)	P	1	N	
Cixius sp.	2	0.05	ME	-	P	-	-	
Cixiidae unknown	2	0.05	PE	-	P	-	-	
DELPHACIDAE								
Asiracinae								
Asiraca clavicornis (Fabricius)	14	0.37	FR, HA, LN, LO, PN	P	P	1	A	
Kelisiinae								
Kelisia praecox Haupt	1	0.03	HA	P (*Carex* spp.)	P	1	A	
Delphacinae								
Dicranotropis hamata (Boheman)	376	9.83	FR, HA, KA, LN, LO, ME, OB, PE, WA	P (grasses)	P	2	N	MDRV, OSDV, PGSV
Falcotoya minuscula (Horváth)	243	6.35	FR, HA, KA, OB	P (grasses)	P	1	E	
Javesella pellucida (Fabricius)	2	0.05	HA, KA	P (grasses)	P	2	N	OSDV, MRDV, EWSMV
Laodelphax striatella (Fallén)	424	11.09	FE, FR, HA, KA, LN, LO, ME, MI, OB, PE, WA	P (grasses)	P	2	N	BYSMV, MRDV, NCMV, RSV, RBSDV, WCSV, WSTV
Megadelphax sordidula (Stål)	21	0.55	OB	P (grasses)	P	2	N	PGSV
Ribautodelphax albostriata (Fieber)	1	0.03	FE	M (*Poa pratense*)	P	2	N	
Ribautodelphax imitans (Ribaut)	38	0.99	FR, HA, OB, PE	M (*Festuca arundinaceae*)	P	2	N	
Ribautodelphax sp.	4	0.10	HA	P (grasses)	P	2	N	
Delphacidae unknown	117	3.06	FR, HA, KA, LN, LO, OB, ME, WA	-	P	-	-	
DICTYOPHARIDAE								
Dictyopharinae								
Dictyophara europaea (L.)	175	4.58	FE, FR, HA, KA, LN, LO, ME, MI, OB, PE, WA	P	P	1	E	
ISSIDAE								
Issinae								
Issus coleoptratus (Fabricius)	1	0.03	FE	P (woody plants)	P	1	N	
FLATIDAE								
Metcalfa pruinosa (Say)	2	0.05	FR, PE	P (woody plants et al.)	P	1	E	Phyto (16SrI)
Fulgoromorpha unknown	3	0.08	MI	-	P	-	-	
CERCOPIDAE								
Cercopis vulnerata Rossi	3	0.08	PE	P	X	1	N	
APHROPHORIDAE								
Philaenus spumarius (L.)	103	2.69	FE, FR, HA, LN, LO, ME, MI, PE	P	X	1	E	

Species	No.	A [%]	Site	F	T	G	O	V*
MEMBRACIDAE								
Smiliinae								
Stictocephala bisonia Kopp & Yonke	7	0.18	FE, ME, OB	P (woody plants)	P	1	E	
CICADELLIDAE								
Agalliinae								
Anaceratagallia ribauti (Ossiannilson)	76	1.99	FE, FR, HA, KA, LN, LO, ME, MI, OB	P	P	1	A	Phyto (Stolbur)
Dryodurgases reticulatus (Herrich-Schäffer)	1	0.03	FE	M (*Viccia tenuifolia*)	P	1	A	Phyto (Stolbur)
Aphrodinae								
Aphrodes makarovi Zachvatkin	20	0.52	FE, FR, HA, LN, LO, ME, OB, WA	P	P	1	E	
Aphrodes sp.	8	0.21	FE, FR, LO	-	P	-	-	
Aphrodinae unknown	1	0.03	FR					
Cicadellinae								
Cicadella viridis (L.)	13	0.34	FE, HA, ME	P (grasses)	X	1-2	E	
Evacanthus acuminatus (Fabricius)	1	0.03	FE	P	X	1	E	
Deltocephalinae								
Aconurella prolixa (Lethierry)	5	0.13	FR, WA	P? (grasses)	P	?	E	
Allygidius abbreviatus (Lethierry)	1	0.03	PE	?	P	1	E	
Allygidius atomarius (Fabricius)	3	0.08	HA, ME, MI	P (woody plants et al.)	P	1	E	
Anoplotettix fuscovenosus (Ferrari)	46	1.20	FR, HA, ME, MI, PE	P (woody plants)	P	1	E	Phyto (Stolbur)
Arocephalus longiceps (Kirschbaum)	13	0.34	FE, FR, ME	P (grasses?)	P	2	E	
Balclutha punctata (Fabricius) sensu Wagner	12	0.31	FE, HA, LN, ME, PE, WA	P (woody plants et al.)	P	1	A	
Balclutha saltuella (Kirschbaum)	3	0.08	FR, HA, WA	P (grasses)	P	1 (?)	A (?)	
Balclutha sp.	4	0.10	FE	-	P	-	-	
Deltocephalus pulicaris (Fallén)	1	0.03	LO	P (grasses)	P	2	E	
Euscelis incisus (Kirschbaum)	39	1.02	FE, FR, HA, KA, LN, LO, ME, OB, PE	P	P	1-2	E	Phyto (16SrXII, 16SrVI, 16SrI)
Fieberiella septentrionalis Wagner	1	0.03	FR	P	P	1	E	
Fiebriella sp.	1	0.03	ME	P	P	1	E	
Idiodonus cruentatus (Panzer)	1	0.03	FE	P (woody plants et al.)	P	1	E	
Jassargus flori (Fieber)	53	1.39	FE, HA, ME	P (grasses)	P	2	E	
Jassargus obtusivalvis (Kirschbaum)	4	0.10	ME	P (grasses)	P	2	E	
Macrosteles cristatus (Ribaut)	233	6.09	HA, KA, LN, LO, PE, OB, WA	P (grasses)	P	2	E	Phyto (16SrI, 16SrIII)
Macrosteles quadripunctulatus (Kirschbaum)	2	0.05	KA, LO	P	P	2	E	Phyto (16SrIX, 16SrI)
Macrosteles variatus (Fallén)	1	0.03	LO	M (*Urtica dioica*)	P	2	E	
Macrosteles sp.	6	0.16	HA, KA	-	P	-	-	
Mocydia crocea (Herrich-Schäffer)	2	0.05	ME	P (grasses)	P	1	A	
Neoaliturus fenestratus (Herrich-Schäffer)	63	1.65	FE, FR, HA, KA, LN, LO, MI, OB, PE, WA	P	P	2	A	Phyto (Stolbur)
Ophiola decumana (Kontkanen)	18	0.47	FR, HA, KA, LO	P	P	2	E	

Species	No.	A [%]	Site	F	T	G	O	V*
Phlogotettix cyclops Mulsant & Rey	1	0.03	MI	P (grasses?)	P	1	E	
Psammotettix alienus (Dahlbom)	259	6.77	FE, FR, HA, KA, LN, LO, ME, MI, OB, PE, WA	P (grasses)	P	2	E	BMWR, WDV
Psammotettix confinis (Dahlbom)	728	19.03	FE, FR, HA, KA, LN, LO, ME, MI, OB, PE, WA	P (grasses)	P	2	E	
Psammotettix sp.	243	6.35	FE, HA, KA, LO, PE, ME, OB, WA	-	P	-	-	
Recilia horvathi (Then)	2	0.05	FR		P	1 (?)	E (?)	
Recilia schmidtgeni (Wagner)	48	1.26	FR, HA, ME, OB, WA		P	1 (?)	E (?)	
Recilia sp.	1	0.03	HA					
Rhopalopyx elongata Wagner	1	0.03	ME	P (grasses)	P	2 (?)	E	
Rhopalopyx sp.	2	0.05	ME					
Thamnotettix confinis Zetterstedt	6	0.16	FE, FR	P (woody plants et al.)	P	1	N	
Thamnotettix exemtus Melichar	1	0.03	FE		P			
Deltocephalinae unknown	70	1.83	FE, FR, HA, KA, LN, LO, ME, MI, OB, PE, WA					
Macropsinae								
Macropsis cf *scotti* Edwards	2	0.05	LO, MI	M (*Rubus fruticosus*)		1	E	Phyto (16SrV)
Macropsis cf *scutellata* (Boheman)	1	0.03	LO	M (*Urtica dioica*)		1	E	
Typhlocybinae								
Emelyanoviana mollicula (Boheman)	25	0.64	HA, KA, LO, ME, OB, WA	P	M	2	E (A)	Phyto (Stolbur)
Empoasca decipiens Paoli	3	0.08	HA	P (woody plants et al.)	M, P	2 (?)	A	
Empoasca sp.	5	0.13	HA, OB, PE, WA,					
Eupteryx cf *calcarata* Ossiannilsson	1	0.03	FE	M (*Urtica dioica*)	M	2	E	
Eupteryx decemnotata Rey	1	0.03	WA	P	M	2	E	
Zyginidia pullula (Boheman)	7	0.18	FE	P (grasses)	M	4	A	
Zyginidia sp.	1	0.03	KA					
Typhlocybinae unknown	2	0.05	MI					
Cicadellidae unknown	7	0.18	HA, KA, LO, MI, WA	-	-	-	-	

*BMWR: Band Mosaic of Wheat and Rye
WDV: Wheat Dwarf Virus
OSDV: Oat Sterile Dwarf Virus,
MRDV: Maize Rough Dwarf Virus,
EWSMV: European Wheat Striate Mosaic Virus,
PGSV: Phleum Green Stripe Virus
NCMV: Northern Cereal Mosaic Virus
RSV: Rice Stripe Virus
RBSDV: Rice Black-Streaked Dwarf Virus
WCSV: Wheat Chlorotic Streak Virus
WSTV: Wheat Rosette Stunt Virus

on only two successive collection dates (July 5 and July 19). *H. obsoletus* was found in all surveyed vineyards with the exception of Site PE. No clear geographic effect of the sampling site on *H. obsoletus* flight pattern or density was observed.
In addition to *H. obsoletus*, 3607 other Auchenorrhyncha specimens were sampled; and 3347 (87.5%) of them identified, representing 9 families and 58 species (Table 3). Larval stages comprised of 226 specimens (5.91%), which were identified to species level in most cases. Insect collecting continued until September 27, 2006, and an additional number of approximately 2000 specimens caught, but not identified to species level (data not shown).
The Cicadellidae was the most species-rich family with 39 species sampled, whereas six families (the Dictyopharidae, Issidae, Flatidae, Cercopidae, Aphrophoridae and Membracidae) were represented by one single species each. Among the Cixiidae, *H. obsoletus* and two other species were identified (*H. luteipes* Fieber and *R. cuspidatus* Fieber) and four additional unknown ciixid specimens sampled.
The vast majority of sampled specimens belonged to only a few species: the three most common species *P. confinis*, *L. striatella* and *D. hamata* comprised 40.0% of all specimens, whereas sixteen species (27.6%) were represented by one single specimen. The flight curves of the ten most common Auchenorrhyncha species (*P. confinis, L. striatella, D. hamata, P. alienus, F. minuscula, M. cristatus, D. europeae, P. spumarius, A. ribauti, and N. fenestratus*) are shown in Figure 1b. Seasonal variations of the densities of several species were found, esp. *D. hamata, F. minuscula, L. striatella* and *P. confinis*, but were never as pronounced as that of *H. obsoletus*.

3.4 Site-specific differences

The total number of sampled specimens per site varied from 76 to 1379 (Table 4). In order to allow for the different site size, the average number of sampled specimens was calculated for the plots per site, which varied between 16.3 at Site LO and 149.5 at Site FR (ratio 1:9.2). Species richness per site varied from 14 (Site LN) to 27 (Site HA). Only four species were sampled at all eleven sites (*L. striatella, D. europaea, P. alienus* and *P. confinis*). A relatively high number of species (8) were sampled exclusively from the organically managed Site FE. The Shannon Wiener-Index of species diversity varied from $H = 1.7681$ (Site MI) to $H = 2.5922$ (Site PE), and the Evenness factor from $E = 0.6117$ (Site MI) to $E = 0.8386$ (Site PE). The abundance of *H. obsoletus* (expressed as average number specimens / plot) showed a positive correlation to the presence of BN symptoms in ten vineyards planted with *V. vinifera* cultivars known to be moderately to very susceptible to Grapevine yellows (Figure 2a). On the other hand, elevated numbers of *H. obsoletus* were sampled in the single vineyard LN planted with the less susceptible variety Gewürztraminer, which showed no BN symptoms.
Fife Auchenorrhyncha species (*A. fuscovenosus, A. ribauti, D. reticulates, E. mollicula, N. fenestratus*), shown recently to be associated with the Stolbur phytoplasma in Austria (Riedle-Bauer et al. 2006, 2008), and the ciixid species *R. cuspidatus* were sampled in low numbers on several sites (Table 3). The density of only one species, *A. fuscovenosus,* was positively correlated to the presence of BN symptoms in ten vineyards planted with *V. vinifera* cultivars known to be moderately to very susceptible to Grapevine yellows (Figure 2b).

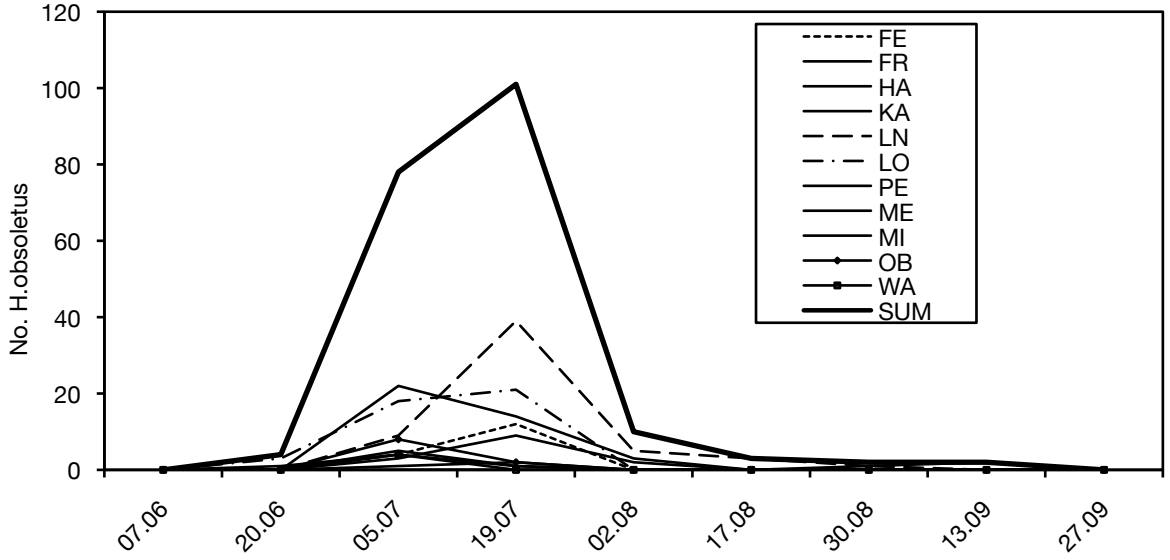

Figure 1a: Flight cycle of *H. obsoletus* in vineyards in South Tyrol measured in bi-weekly intervals from early June to end of September 2006. Shown are the numbers of sampled specimens from each individual site and the sum off all samples for each sampling date.

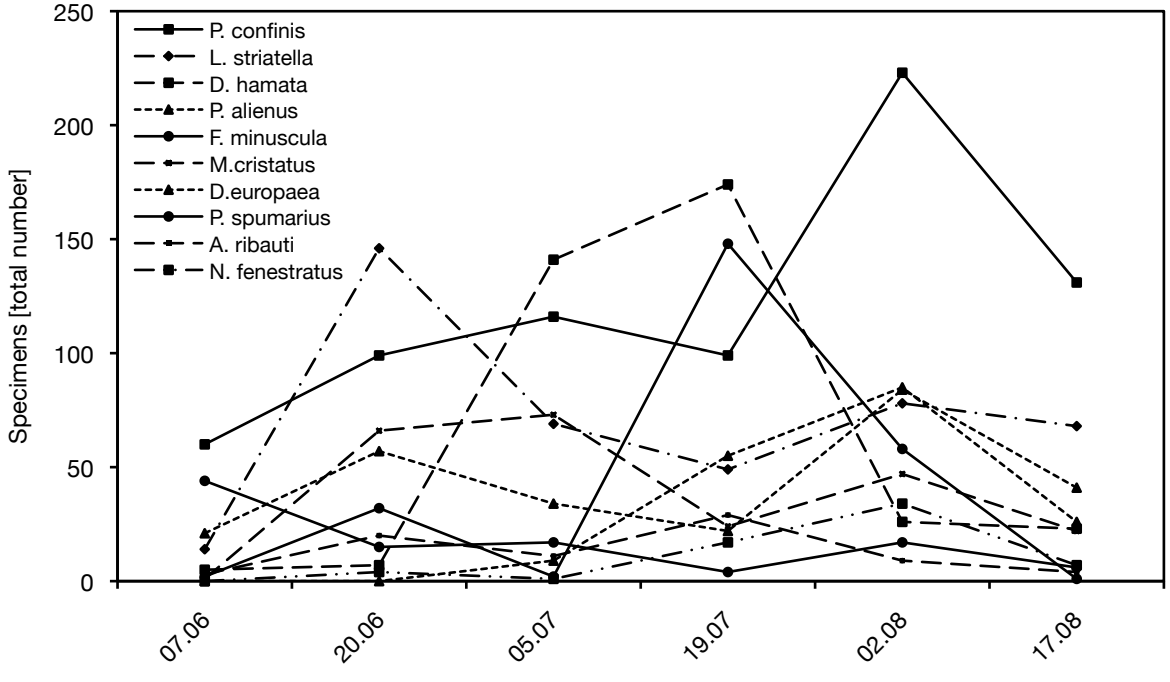

Figure 1b: Flight cycle of the ten most common Auchenorrhyncha species in vineyards in South Tyrol. Co-specific specimens from the eleven sites were summed up for each sampling date from early June to Mid August of 2006.

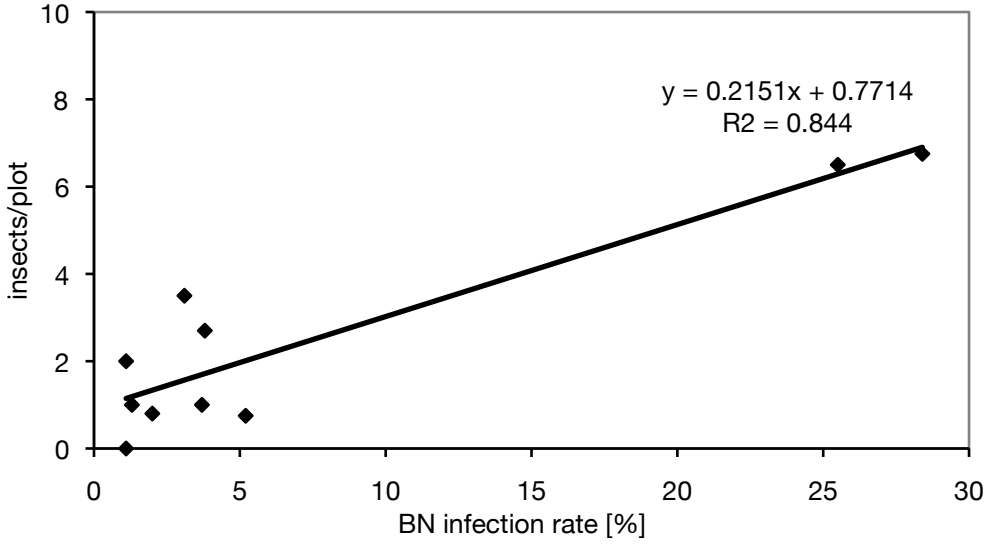

Figure 2a: Correlation between abundance of the vector *H. obsoletus* (number specimens/plot) and Bois noir infection rate in the vineyard.

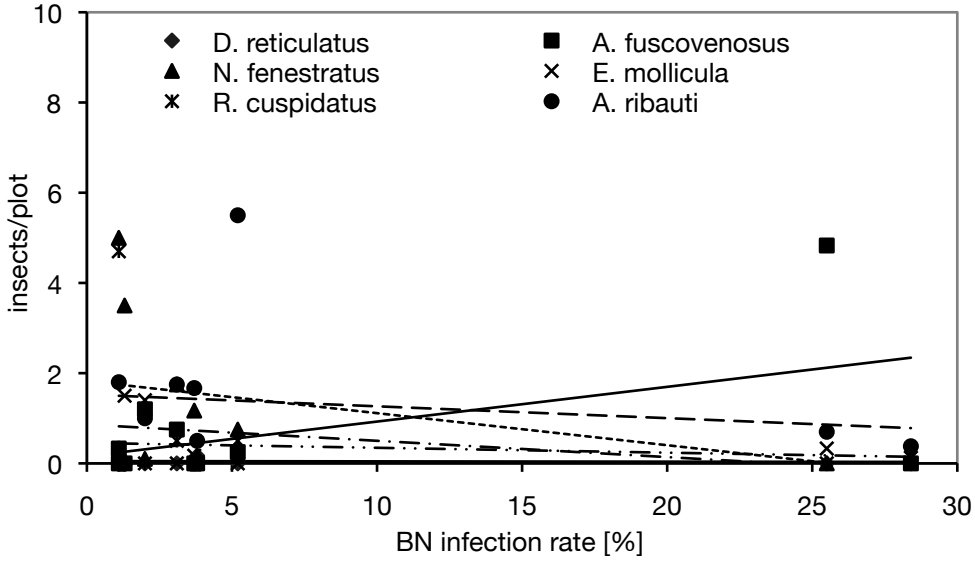

Figure 2b: Correlation between abundance of *A. fuscovenosus, A. ribauti, E. mollicula, N. fenestratus* and *R. cuspidatus* (number specimens/plot) and Bois noir infection rate in the vineyard.

Table 4: Site-specific effects on sampling of Auchenorrhyncha species (*H. obsoletus* listed separately from the other species). *H* = Shannon-Wiener-Index of species diversity; *E* = evenness of species.

Site	species	Hobs	Hobs/ plot	No. other species	other specimens	specimens/ plot	H	E
FE	*Hobs, Lstr, Ralb, Deur, Icol, Pspu, Sbis, Arib, Dret, Amak, Cvir, Eacu, Alon, Bpun, Einc, Icru, Jflo, Nfen, Odec, Pali, Pcon, Tcon, Texe, Ecal, Zpul*	16	2.7	25	131	21.8	2.5169	0.7329
FR	*Hlut,Hobs, Rcu, Acla, Dham, Fmin, Lstr, Rimi, Deur, Mpru, Pspu, Arib, Amak, Apro, Afus, Alon, Bsal, Einc, Fsep, Nfen, Pali, Pcon, Rhor, Rsch, Tcon*	3	0.75	25	598	149.5	2.2979	0.6516
HA	*Hobs, Acla, Kpra, Dham, Fmin, Jpel, Lstr, Rimi, Deur, Pspu, Arib, Amak, Cvir, Aato, Afus, Bpun, Bsal, Einc, Jflo, Mcri, Nfen, Odec, Pali, Pcon, Rsch, Emol, Edec*	8	0.8	27	1379	137.9	2.2969	0.6460
KA	*Hobs, Dham, Fmin, Jpel, Lstr, Deur, Arib, Einc, Mcri, Mqua, Nfen, Odec, Pali, Pcon, Emol*	6	1.0	15	309	51.5	2.1452	0.7046
LO	*Hlut, Hobs, Acla, Dham, Lstr, Deur, Pspu, Arib, Amak, Dpul, Einc, Mcri, Nfen, Mqua, Mvar, Odec, Pali, Pcon, Msco, Mscu, Emol*	54	6.75	21	129	16.2	2.4898	0.7554
LN	*Hobs, Acla, Dham, Lstr, Deur, Pspu, Arib, Amak, Bpun, Einc, Mcri, Nfen, Pali, Pcon*	59	29.5	14	107	50.4	1.9695	0.7103
ME	*Hobs, Dham, Lstr, Deur, Pspu, Sbis, Arib, Amak, Aato, Afus, Alon, Bpun, Einc, Jflo, Jobt, Mcro, Pali, Pcon, Rsch, Relo, Emol*	39	6.5	21	241	40.2	2.5179	0.7478
MI	*Hobs, Rcu, Lstr, Deur, Pspu, Arib, Cvir, Aato, Afus, Nfen, Pcyc, Pali, Pcon, Msco*	14	3.5	14	179	44.8	1.7681	0.6117
OB	*Hobs, Rcu, Dham, Fmin, Lstr, Msor, Rimi, Deur, Sbis, Arib, Amak, Einc, Mcri, Nfen, Pali, Pcon, Rsch, Emol*	10	2	18	374	74.8	2.3262	0.7419
PE	*Rcu, Acla, Dham, Lstr, Rimi, Deur, Mpru, Cvul, Pspu, Aabb, Afus, Bpun, Mcri, Einc, Nfen, Pali, Pcon*	0	0	17	76	25.3	2.5922	0.8386
WA	*Hobs, Dham, Lstr, Deur, Amak, Apro, Bpun, Bsal, Mcri, Nfen, Pali, Pcon, Rsch, Emol, Edec*	4	1	15	84	21.0	2.4834	0.8290

4. Discussion

Bois noir is a grapevine disease causing considerable economic damages in several European countries. In order to study the epidemiology of the disease in South Tyrol (Northern Italy) and develop sustainable control strategies, the Research Centre Laimburg started a long-term program in 2005 to survey the density and biology of the only established vector *H. obsoletus* (Schweigkofler et al.2006, Schweigkofler & Roschatt 2008, Berger et al. 2009). *H. obsoletus* was sampled consistently in most vineyards showing BN symptoms; insect captures were lower than those reported previously in other wine growing areas with a recent BN outbreak in Germany (Darimont & Maixner 2001), but higher than in Austria (Tiefenbrunner et al. 2007). Calculations of *H. obsoletus* densities in a given area are difficult because of the highly clustered distribution on the host plants. The main flight activity of *H. obsoletus* in South Tyrol takes place consistently from early June to late August, with only small variances at the different sites. A slightly earlier flight was observed in the very warm and dry year of 2007 (Schweigkofler & Roschatt 2008), but so far, we did not observe the two-peaked flight curve which in Germany was reported to occur based on the host plant (with *H. obsoletus* feeding on bindweed flying approximately three weeks before *H. obsoletus* feeding on stinging nettle; Maixner & Langer 2006).
In this study we widened our approach and analyzed the complete Auchenorrhyncha fauna of eleven model vineyards representing the different winegrowing areas in South Tyrol during the flight period of *H. obsoletus* (June 7 through August 17, 2006) in order to gain first information on the presence and density of potential additional vectors.
Similar studies were carried out previously in BN infected vineyards in several European countries (Bosco et al. 1997, Riedle-Bauer et al. 2006, Mazzoni et al. 2001, Seljak et al. 2003). Bosco et al. (1997) identified 32 species in the Piedmont region (Northwestern Italy), most of them belonging to the Deltocephalinae, whereas the overwhelming majority of specimens belonged to the Typhlocybinae species *Empoasca vitis* (Goethe) and *Zygina rhamni* Ferrari. Similar species composition was reported from vineyards in Austria (Riedle-Bauer et al. 2006; Tiefenbrunner & Tiefenbrunner 2007) and Slovenia (Seljak et al. 2003). In most of these studies, sampling was done using yellow sticky traps in the canopy zone (approx. 1.5 m above ground), which favors sampling of species which are either regularly feeding on the grapevine (like *E. vitis* and *S. titanus*) or good fliers. The color of the sticky traps might also have an influence on sampling results with yellow probably being the most attractive for all Auchenorrhyncha species, whereas the results using green, red or blue traps vary depending on insect species (Tiefenbrunner & Tiefenbrunner 2007).
In South Tyrol, studies on planthoppers focused mainly on a limited number of species causing problems on important crops, such as apples and grapevine (Berger et al. 2009, Günthart 1989, Pernter 2009). The Auchenorrhyncha fauna in natural habitats was studied recently by Carl (2007, 2008) at three ecological diverse sites: the Schlern mountain (the westernmost peak of the Dolomites range; different sampling sites: 1020-2560 m.a.s.l.), the Ritten plateau (forest area: 1770 m.a.s.l.) and the Montiggl forest (550 m.a.s.l.). The distances of these three sites to the vineyard sites range from approximately 5 to 30 km. A total of 116 leafhopper species was collected by Carl (2007, 2008), but only two species (*Forcipata obtusa* Vidano, *Speudotettix subfusculus* (Fallén) were found on all three sites.
In our study we sampled a total of 57 species from vineyards (selected examples are shown on Figure 3); eleven of them were reported also from the Schlern mountain (*R. albostriata, C. vulnerata, P. spumarius, C. viridis, E. acuminatus, B. punctata, O. decumana, R. elongata, T. confinis* and *E. mollicula*), seven from Ritten (*Anaceratagallia ribauti, Deltocephalus pulicaris, Jassargus flori, Megadelphax sordidula, Philaenus spumarius, Ribautodelphax*

albstriatus, Thamnotettix confinis) and five from Montiggl (*Balclutha punctata, Cercopis vulnerata, Dicranotropis hamata, Empoasca vitis, Issus coleoptratus*) (Carl, 2007, 2008). The rather low overlap in species composition from the different sites most likely reflects the differences in host plant availability and microclimatic conditions.

In this study, *Recilia horvathi* (Cicadellidae) was sampled for the first time in South Tyrol. Two male specimens were sampled on August 17 from Site FR. This species has a wide range extending from Mongolia to Central Europe and Northern Italy, where it occurs in isolated populations at xerothermic sites (Nickel 2003). We have no further information on the presence and possible ecological role of this species in vineyards.

Adjacent sites with similar microclimate and understory vegetation might contain similar Auchenorrhyncha species. In this study, the only two sites neighboring each other were LN and LO. Only one plot was used at Site LN, a very small vineyard, whereas 8 plots were sampled at the bigger vineyard presenting Site LO, which most likely explains the lower number of Auchenorrhyncha species sampled at Site LN (14) compared to Site LO (21). However, 13 out of the 14 species sampled at Site LN were also sampled at Site LO, the only exception being *B. punctata* (1 specimen). The understory of both sites was characterized by the abundant presence of *U. dioica*, which might also explain the relatively high density of *H. obsoletus* at both sites.

Biological parameters of the sampled Auchenorrhyncha species that might play an important role for the acquisition and spread of phytoplasmas, like known host plants and feeding patterns, are listed in Table 3. The Deltocephalinae were the prevailing group both in number of species and specimens. The ten most common Auchenorrhyncha species (*P. confinis, L. striatella, D. hamata, P. alienus, F. minuscula, M. cristatus, D. europeae, P. spumarius, A. ribauti, N. fenestratus*) are all polyphagous, with five species (*P. confinis, L. striatella, P. alienus, F. minuscula, M. cristatus*) restricted to grasses. Woody plants are known host plants for only a limited number of the sampled species. *Macropsis scutellata* and *Eupteryx calcarata*, two species described to be monophagous on *Urtica dioica* (Nickel 2003), were both sampled only once during this study, despite *U. dioica* being common at several sites. The two species, which are not known to transmit phytoplasmas, most probably play no major role in the epidemiology of *Ca.* Phytoplasma solani in the sampled area.

Most species sampled in this study are feeding on the phloem; whereas *P. spumarius* is the only xylem-feeding species sampled consistently. Because phytoplasmas were never detected from the xylem so far, xylem-feeding planthoppers are not regarded to be possible vectors.

Due to a similar life cycle and feeding pattern as *H. obsoletus,* members of the genus *Reptalus* might show the prerequisites for being a vector of phytoplasmas. Indeed, recent studies in Serbia showed that *R. panzeri* is a vector of the Stolbur phytoplasma in corn (Jović et al. 2007). In this study, we only sampled few specimens of *R. cuspidatus* from four sites (FR, MI, OB, PE), but the species was absent at the two sites showing the heaviest BN infection (LO and ME). Although *Reptalus* sp. seems to have in general a more scattered presence in vineyards in South Tyrol than the closely related *H. obsoletus*, high local densities were found recently on dry grassland adjacent to a vineyard (data not shown).

Phytoplasmas of the Stolbur type were detected recently from a number of Auchenorrhyncha species using species-specific PCR (Table 3; Riedle-Bauer et al. 2006). Whereas phloem-sucking insects might ingest phytoplasmas rather frequently from infected host plants in quantities above the detection limit, several other prerequisites have to be fulfilled to become an efficient vector, like crossing of the phytoplasma from the intestinal tract into the hemocoel and eventually the salivary gland where mass multiplying takes place. In addition, host preference and population density also play an important role. *A. ribauti*

was shown recently to transmit the Stolbur phytoplasma in greenhouse tests (Riedle-Bauer et al. 2008). In the study presented here, *A. ribauti* was sampled in nine out of eleven sites in South Tyrol, although at low densities. *A. fuscovenosus, D. reticulates, E. mollicula* and *N. fenestratus*, other species found to be associated with the Stolbur phytoplasma in Austria (Riedle-Bauer et al. 2006), were also present at several sites. *D. reticulates* was sampled only once at the organically managed Site FE. The density of only one of this possible phytoplasma vectors (*A. fuscovenosus*) showed a positive, however weak, correlation with the presence of the BN symptoms in the vineyards. Further field observations, analysis of phytoplasma presence and transmission tests are required to shed light on the actual role of these planthoppers for BN epidemiology.

Members of the Typhlocybinae are predominantely feeding on mesenchym, with the exception of *Empoasca* spp., which feed on mesenchym and phloem. The polyphagous green leafhopper *Empoasca vitis* is a well-known pathogen of grapevine, causing leaf necrosis and eventually reduced sugar content in grapes. *E. vitis* is common in vineyards in South Tyrol, and chemical control is often necessary to protect crop yield (data not shown). *E. vitis* is rarely found on the understory vegetation, but can be trapped easily using yellow sticky traps in the leaf zone (approx. 1.5 m above the ground) (Bosco et al. 1997). *Scaphoideus titanus* (Cicadellidae), the vector of *Ca.* Phytoplasma vitis which causes Flavescence doree, was not sampled in this study. The presence of *S. titanus* is confirmed for the Province of Trento, South Tyrol's southern neighbor. *Metcalfa pruinosa,* another invasive species originating from North America, first found in Italy (Veneto region) in 1979 was sampled in some areas on the southern border of the observed range, mainly on grapevines (less on the understory) damaging grape berries by the abundant production of honey dew. Another invasive species from North America, *Stictocephala bisonia*, seems to extend its area continuously in South Tyrol. Damage caused by this species on grapevines is easily detectable by the puncture spot and the discoloration of the apical leafs. These symptoms have been noticed increasingly over the last few years and are sometimes confused with those caused by Grapevine yellows by inexperienced observers.

An analysis of the plants comprising the understory vegetation in vineyards is essential to understand the dynamics of the spread of BN. The grapevine itself acts as a 'dead end' for the *Ca.* Phytoplasma Stolbur, from which no new infection cycle can start, in contrast to *Ca.* Phytoplasma vitis, which is transmitted grape-to-grape by the monophagous leafhopper *S. titanus*. *H. obsoletus* over winters as a nymph feeding on roots of herbaceous plants, from which phytoplasmas can be ingested. Therefore, it is tempting to speculate, that mainly perennial plants serve as a long-term phytoplasma reservoir, whereas annual plants without an over wintering root system have a more transient effect, if any. In many woody plants (like the apple tree *Malus domestica* (Borkh.)) the phloem system above ground degrades every winter, and phytoplasmas can survive only in the root system. The phloem of *V. vinifera,* on the other hand, survives for two to three years, but data on the survival rate of phytoplasmas above ground during winter are still missing.

The dense plant cover of the sample sites is typical for South Tyrol, where due to ecological reasons and to prevent erosion the use of herbicides is limited to a narrow band along the grapevine row. Still, conventional agricultural practices like mowing, mineral fertilizing and application of pesticides were shown to have a negative effect on Auchenorrhyncha species richness (Nickel & Achtziger, 2005). In addition to the direct effects of insecticides, impoverishment of the vegetation diversity and eventually loss of habitats for oligotopic and stenotopic species might occur. The species composition varies considerably from one site to another, but in general 12 to 24 herbaceous species prevail in each site together

with a limited number of grass species. The well established host plants of *H. obsoletus* (*C. arvensis* and *U. dioica*) were present at all sites, and the number of sampled *H. obsoletus* showed a positive correlation with the abundance of the host plants (esp. *U. dioica*; data not shown). Several other plants, esp. *Taraxacum officinale* and *Polygonum aviculare*, were tested positive for the presence of *Ca.* Phytoplasma solani using PCR (Berger et al. 2009 Riedle-Bauer et al. 2006), but their role for the epidemiology of BN has still to be clarified. Species richness and biodiversity index *H* of the leafhopper communities were slightly higher at the organically managed Site FE than on most other sites, but because only one organically managed site was included in this study no general conclusions on the effect of management strategies on planthopper abundance and diversity can be drawn yet. The evenness factor *E* represents the numerical ratio of species abundance at a given site, with values ranging from 0 (one species very dominant) to 1 (all species present with the same abundance). On the eleven vineyard sites, *E* values from 0.6117 (Site MI) to 0.8386 (Site PE) were measured. Sites with lower *E* values were in general characterized by the presence of one or a few dominant species (in the case of Site MI *D. europaea* and *P. spumarius*). With an *E* value of 0.7329 the organically managed Site FE did not differ significantly from the other sites.

5. Conclusion

The biodiversity and abundance of planthoppers in South Tyrol's vineyards is similar to the situation reported for other wine growing areas in Europe. Density and infection rates of *H. obsoletus*, the only well-established vector of the Bois noir phytoplasma, seem to be high enough to explain the epidemiology of Bois noir in the area. However, several other Auchenorrhyncha species occur in the vineyards which might have the potential to act as vectors of viral or bacterial plant diseases, including phytoplasmas. As long as some key factors for the understanding of Grapevine yellows are still not known in detail, such as the role of different host plants, new or additional vectors, virulence of different phytoplasma subtypes and the interaction between these factors, further environmental field studies are needed to establish sustainable control strategies.

Hyalesthes obsoletus (Winden-Glasflügelzikade)

Reptalus cuspidatus (Östliche Glasflügelzikade)

Reptalus panzeri (Rosen-Glasflügelzikade)

Reptalus panzeri (Rosen-Glasflügelzikade)

Empoasa vitis (Reben-Blattzikade)

Scaphoideus titanus (Amerikanische Rebenzikade)

Laodelphax striatella (Wander-Spornzikade)

Philaenus spumarius (Wiesen-Schaumzikade).

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