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Epidemiological observations on Bois Noir in Austrian vineyards

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Bois Noir is widespread in several vine growing regions in Austria. Therefore Auchenorrhyncha species, which are considered to be potential vectors of this disease, and possible weedy host plants of Bois Noir were studied in six vineyards distributed all over the Austrian viticultural area. Bois Noir phytoplasmas were ascertained in Hyalesthes obsoletus, Reptalus panzeri, Psammotettix spp. *(mainly* P. alienus*),* Macrosteles spp. *(*M. laevis, M. cristatus, M. sexnotatus*),* Balclutha *sp.*, Neoaliturus fenestratus, Anoplotettix fuscovenosus, Mocuellus collinus, Errastunus ocellaris, Dryodurgades reticulatus, Emelyanoviana mollicula *and in* Eupteryx atropunctata. Hyalesthes obsoletus*, the only confirmed vector species in grapevine was not captured in 2004, in 2005 only a few individuals were caught. Disease incidence increased in the same period. Thus other vectors besides* H. obsoletus *are suggested to transmit Bois Noir disease in grapevine. Based on our results the most probable species are* Psammotettix alienus*,* Macrosteles spp. *(*M. laevis, M. cristatus, M. sexnotatus*),* Anoplotettix fuscovenosus, Dryodurgades reticulatus, and Reptalus panzeri.
Key words: *Vitis vinifera* L., Bois Noir, *Auchenorrhyncha*

Beobachtungen zur Epidemiologie von Schwarzholzkrankheit an Rebe in Österreich. *Die Schwarzholzkrankheit (Bois Noir) ist in einigen Weinbauregionen Österreichs weit verbreitet. Deshalb wurden einige Auchenorrhyncha-Spezies, die als potenzielle Vektoren für die Krankheit gelten, und einige mögliche Wirtspflanzen für die Schwarzholzkrankheit in sechs Weingärten aus ganz Österreich untersucht. Bois Noir Phytoplasmen wurden gefunden in:* Hyalesthes obsoletus, Reptalus panzeri, Psammotettix spp. *(hauptsächlich* P. alienus*),* Macrosteles spp. *(*M. laevis, M. cristatus, M. sexnotatus*),* Balclutha *sp.*, Neoaliturus fenestratus, Anoplotettix fuscovenosus, Mocuellus collinus, Errastunus ocellaris, Dryodurgades reticulatus, Emelyanoviana mollicula *und in* Eupteryx atropunctata*. Im Jahr 2004 wurde* Hyalesthes obsoletus*, die einzige bestätigte Vektorspezies bei Rebe, nicht nachgewiesen und im Jahr 2005 wurden nur einige Individuen gefangen. Das Auftreten der Krankheit nahm im gleichen Zeitraum zu. Deshalb wird angenommen, dass andere Vektoren als* H. obsoletus *die Schwarzholzkrankheit bei Rebe übertragen. Auf Grund der vorliegenden Resultate sind die wahrscheinlichsten Spezies* Psammotettix alienus*,* Macrosteles spp. *(*M. laevis, M. cristatus, M. sexnotatus*),* Anoplotettix fuscovenosus, Dryodurgades reticulatus und Reptalus panzeri.
Schlagwörter: *Vitis vinifera* L., Schwarzholzkrankheit, Stolbur-Phytoplasmen, *Auchenorrhyncha*

Observations sur l'épidémiologie de la maladie du Bois noir des vignes en Autriche. *Le Bois noir est largement répandu dans quelques régions viticoles autrichiennes. De ce fait, des recherches ont été réalisées sur certaines espèces d'Auchenorrhyncha, qui sont considérées comme vecteurs de la maladie, et sur quelques plantes hôtes du Bois noir dans six vignobles dans toute l'Autriche. Du phytoplasme du stolbur a été trouvé dans :* Hyalesthes obsoletus, Reptalus panzeri, Psammotettix spp. *(notamment* P. alienus*),* Macrosteles spp. *(*M. laevis, M. cristatus, M. sexnotatus*),* Balclutha *sp.*, Neoaliturus fenestratus, Anoplotettix fuscovenosus, Mocuellus collinus, Errastunus ocellaris, Dryo-

durgades reticulatus, Emelyanoviana mollicula *et dans* Eupteryx atropunctata*. En 2004,* Hyalesthes obsoletus*, la seule espèce vectrice confirmée de la vigne, n'a pas été constatée du tout, et en 2005 elle n'a été constatée que chez quelques individus. Pourtant, la maladie s'est manifestée plus souvent au cours de cette période. On suppose de ce fait que d'autres vecteurs que* H. obsoletus *transmettent la maladie du stolbur de la vigne. Sur la base des résultats obtenus, les espèces les plus probables sont* Psammotettix alienus, Macrosteles spp. *(*M. laevis, M. cristatus, M. sexnotatus*),* Anoplotettix fuscovenosus, Dryodurgades reticulatus *et* Reptalus panzeri.
Mots clés : *Vitis vinifera* L., maladie du Bois noir, phytoplasmes du stolbur, *Auchenorrhyncha*

During the last years inspections revealed a significant increase of Bois Noir in Austrian vineyards. Symptoms are yellowing or reddening of the leaves, curling of the leaves, shrivelling of flowers and berries and the absence of lignification in fall. Sometimes infected vines die off during winter. Laboratory analyses revealed the presence of phytoplasmas belonging to the Stolbur subgroup in the infected vines.

Phytoplasmas belonging to this subgroup attack a wide range of host plants such as vegetables, e.g. *Solanum nigrum, Lycopersicon esculentum*, *Daucus carota*, field crops like *Beta vulgaris* and several weeds (VALENTA et al., 1961; GATINEAU et al., 2001). In the vineyard pest plants like C*onvolvulus arvensis* and *Urtica dioica* are frequently infected and considered as sources of the pathogen (MAIXNER et al., 1995; LANGER et al., 2003).

Studies on epidemiology of Bois Noir in grapevine proved that the pathogen can be transmitted by the planthopper species *Hyalesthes obsoletus* (MAIXNER, 1995; SFORZA et al., 1998). The leafhoppers *Euscelis obsoletus, Aphrodes bicinctus, Issus* sp. and *Euscelidius variegatus* can infect *in vitro*-grapevines (LAVIÑA et al., 2006). Several other leafhopper and planthopper species are also suspected to be involved in transmission (BOSCO et al., 1997; BATLLE et al., 2000; KLEIN et al., 2001; BERTACCINI et al., 2003; PALERMO et al., 2004; LA ROSA et al., 2006 and others).

Our study focused on Auchenorrhyncha species as possible vectors for Bois Noir and on potential host plants of the pathogen in Austrian vineyards.

Material and Methods

Sampling

From April 2004 to November 2005 Auchenorrhyncha populations were surveyed in six Bois Noir infected vineyards situated in Klosterneuburg, Langenzersdorf, Zemendorf, Illmitz, Deutsch Schützen and Lutzmannsburg. Yellow sticky traps were placed at cover crop level or in vineyards without cover crop at canopy level. Emergence traps were positioned above *Convolvulus arvensis* plants. Traps were controlled weekly. Additionally in Klosterneuburg and Langenzersdorf insects were collected by spraying *Convolvulus arvensis* plants and grapevine leaves with an adhesive (Insekten-Fangleim, Schacht, Germany). Identification of insect species was performed as described (RIBAUT, 1952; OSSIANNILSSON, 1978; OSSIANNILSSON, 1981; OSSIANNILSSON, 1983; HOLZINGER et al., 2003; BIEDERMANN and NIEDRINGHAUS, 2004). All Cixiidae and all other frequent (exceeding single individuals) hopper species were stored at -18 °C and subjected to PCR analysis (with the exception of *Empoasca vitis*).

Disease incidence in the vineyards was recorded in September 2004 and in September 2005. All vines were checked visually and infected plants were registered.

Surveys on potential host plants were made in four stolbur infected vineyards located in Klosterneuburg, Langenzersdorf, Deutsch Schützen and Klöch. In all selected vineyards soil is covered by naturally growing weed flora. Plants with disease symptoms, perennial weeds and plant species frequently occurring in and around the vineyards were sampled for further analysis.

PCR-analysis

DNA was extracted from stems, petioles and leaves as described by MAIXNER et al. (1995). 60 mg of plant tissue were ground in 750 µl of extraction buffer (2% cetyltrimethylammoniumbromide, 1.4 M NaCl, 0.2% mercaptoethanole, 20 mM EDTA, 100 mM Tris, pH 8,0). After addition of 250 µl of extraction buffer samples were incubated at 60 °C for 15 min and centrifuged for 1 min at 3.200 g. The supernatant was mixed with an equal volume of chloroform/isoamylalcohol (24:1) and centrifuged for 5 min at 3.200 g. The supernatant was mixed with an equal volume of isopropanol. After centrifugation the precipitated pellet was washed with 70% ethanol, dried and resuspended in 100 µl of TE buffer.

Insect samples consisted of one to five individuals of the same species. Insects were ground 1:15 (w/v) in extraction buffer and processed as described for plant samples. Phytoplasmas were detected by a nested PCR procedure. The primer pairs were fU5 (LORENZ et al., 1995) /P7 (SMART et al., 1996) for the first PCR and STOLF/STOLR (MAIXNER et al., 1995) for the second amplification. The primerpair STOL11f2/STOL11r1 followed by primerpair STOL11f3/STOL11r2 (CLAIR et al., 2003) was also used. Amplification products were separated on 1.5% agarose gels, stained with ethidium bromide and examined under UV light.

Results

PCR analysis of 31 Auchenorrhyncha species showed the presence of stolbur phytoplasmas in a number of leafhoppers and planthoppers: *Hyalesthes obsoletus, Reptalus panzeri, Macrosteles* spp., *Macrosteles sardus*, *Balclutha* sp. *Psammotettix* spp., *Neoaliturus fenestratus, Anoplotettix fuscovenosus, Errastunus ocellaris, Mocuellus collinus, Dryodurgades reticulatus, Emelyanoviana mollicula* and *Eupteryx atropunctata* (Table 1).
Catches on yellow sticky traps revealed that in Klosterneuburg, Langenzersdorf and Deutsch Schützen *Macrosteles* spp. (*M. laevis, M. cristatus* and *M. sexnotatus*), *Psammotettix spp*. (*P. alienus*, *P. confinis* and low numbers of *P. kolosvarensis* and *P. cephalotes*), *Macrosteles sardus*, *Neoaliturus fenestratus, Ophiola decumana, Anaceratagallia ribauti, Empoasca vitis, Emelyanoviana mollicula, Eupteryx atropunctata, Eupteryx curtisii*., *Zyginidia pullula, Arboridia erecta* and *Laodelphax striatella* are common species. *Anoplotettix fuscovenosus* and *Dryodurgades reticulatus* were frequenly trapped in Klosterneuburg and Langenzersdorf. The prevalent species in Zemendorf, Illmitz and Lutzmannsburg were *Empoasca vitis, Emelyanoviana mollicula, Eupteryx atropunctata, Eupteryx curtisii, Zyginidia pullula, Arboridia erecta* and *Laodelphax striatella*. In 2004 no *Hyalesthes obsoletus* was captured, in 2005 it was only found sporadically on sticky traps and in emergence traps in all studied vineyards.
On sticky leaves of *Convolvulus arvensis* the following leafhopper and planthopper species were captured: *Macrosteles* spp. (*M. laevis, M. cristatus* and *M. sexnotatus*), *Macrosteles sardus, Psammotettix alienus, Ophiola decumana, Mocuellus collinus, Emelyanoviana mollicula, Eupteryx atropunctata, Eupteryx curtisii, Zyginidia pullula*, *Laodelphax striatella.* On sticky vine leaves apart from *Empoasca vitis* only low numbers of leafhoppers were present. *Macrosteles* spp. (*M. laevis, M. cristatus* and *M. sexnotatus*), *Macrosteles sardus, Psammotettix alienus, Mocuellus collinus, Errastunus ocellaris, Neoaliturus fenestratus, Cicadella viridis, Emelyanoviana mollicula, Eupteryx atropunctata* and *Eupteryx curtisii* were trapped.
32 plant species were tested for the presence of stolbur phytoplasmas by PCR. PCR-positive results are illustrated in Table 2. Infected Convolvulus plants showed yellowing and stunting of leaves and shoots, infected *Taraxacum officinale* and *Polygonum aviculare* plants turned red. No disease symptoms were observed on *Urtica dioica.*

Table 1: Presence of stolbur phytoplasmas in insect samples

Auchenorrhyncha families/species	Number of PCR samples	Number of infected samples
Aphrophoridae		
Neophilaenus campestris (F., 1805)	1	0
Philaenus spumarius (L., 1758)	1	0
Cicadellidae		
Typhlocybinae		
Arboridia erecta (RIB.1931*)*	6	0
Emelyanoviana mollicula (BOH.,1845)	18	2
Eupteryx atropunctata (GOEZE, 1778)	18	2
Zyginidia pullula (BOH.,1845)	3	0
Cicadellinae		
Cicadella viridis (L., 1758)	1	0
Agalliinae		
Anaceratagallia ribauti (OSS., 1938)	5	0
Dryodurgades reticulatus (H.-S., 1834)	18	2
Deltocephalinae		
Allygus modestus SCOTT, 1876	1	0
Anoplotettix fuscovenosus (FERR., 1882)	5	1
Allygidius atomarius (FABR., 1794)	1	0
Arthaldeus striifrons (KBM., 1868)	1	0
Balclutha sp.	6	2
Errastunus ocellaris (F., 1806)	6	1
Macrosteles sardus RIB., 1948	13	2
Mocuellus collinus (BOH., 1850)	3	1
Macrosteles spp. (M. laevis (RIB., 1927), *M. sexnotatus* (F., 1806), *M. sexnotatus* (F., 1806)*)*	49	1
Mocydia crocea (H.-S., 1834)	1	0
Neoaliturus fenestratus (H.-S., 1834)	14	1
Ophiola decumana (KONTK., 1949)	21	0
Psammotettix spp. (P. alienus (DHLB., 1850), *P. confinis* (DHLB., 1850), *P. cephalotes* (H.-S., 1834), *P. kolosvarensis* (MATS., 1908)*)*	25	5
Cixiidae		
Hyalesthes obsoletus SIGN., 1965	6	1
Pentastiridus sp.	1	0
Reptalus panzeri (P. LÖW, 1883*)*	3	1
Delphacidae		
Laodelphax striatella (FALL., 1826)	8	0

Table 2: List of plant species infected with stolbur phytoplasmas

Plant species	Klosterneuburg	Langenzersdorf	Deutsch Schützen	Klöch
Convolvulus arvensis L.	+	+	+	+
Taraxacum officinale WEB.	o	o	+	o
Urtica dioica L.	o	o	-	+
Polygonum aviculare L.	+	+	-	-

\+ plant species positive in PCR detection
o plant species present in the studied region but no infected plants detected
\- plant species not observed at the studied site

Disease incidence increased during the observation period at all experimental sites. As an example data of the vineyard in Klosterneuburg are given in Table 3.

Discussion

The increase of infected vines in the studied vineyards indicates the presence of efficient vectors for Bois Noir. The pathogen was ascertained in a range of planthopper and leafhopper species. The presence of the phytoplasma in an insect alone, however, is no positive proof for the vectoring ability of a species (VEGA et al., 1993). Up to now only transmission tests with *Hyalesthes obsoletus* resulted in infections of grapevines (MAIXNER, 1995; SFORZA et al., 1998). In the present study *H. obsoletus* was either not captured (2004) or only in low numbers (2005). Possibly due to its low incidence the species was never found on sticky Convolvulus plants or grapevine leaves. Based on an observation period of two years it cannot be excluded that the number of *H. obsoletus* in the investigated vineyards declined from 2003 to 2004 as a consequence of e.g. unsuitable wheather conditions. Possibly the species was more frequent in 2002 and 2003 and thus responsible for the outbreak of Bois Noir disease at the studied sites. On the other hand *H. obsoletus* was never observed frequently in Austria (HOLZINGER, pers. comm.) and disease incidence increased during the observation period. Transmission of stolbur phytoplasma without the presence of *H. obsoletus* is also reported from vineyards in Spain and Italy (BATLLE et al., 2000; LA ROSA et al., 2006). These facts suggest that besides *H.obsoletus* other efficient vectors exist, possibly some of the species found stolbur-infected in our study. We found stolbur phytoplasma in *Reptalus panzeri,* which coincides with Hungarian findings (PALERMO et al., 2004). Like *Hyalesthes obsoletus* it belongs to the family Cixiidae. Therefore a transmission of stolbur phytoplasma seems possible although up to now no transmission tests have been carried out. In our study, however, the species has been trapped rarely.

In *Psammotettix alienus* (Deltocephalinae) the presence of stolbur phytoplasma was detected several times. The species was also frequently trapped on yellow boards and on Convolvulus and grapevine leaves. Although it is well-established that *P. alienus* generally colonizes grasses, it seems possible that it also feeds on other plant species (HOLZINGER, pers.comm.). *P. alienus* has never been confirmed as vector of phytoplasmas, but Batlle et al. (2000) have demonstrated that another member of the genus *Psammotettix, P. striatus* carried stolbur phytoplasmas in Spain. It was also shown that *P. striatus* is able to transmit stolbur phytoplasma to artificial feeding medium (SABATÉ et al., 2003).

Macrosteles (Deltocephalinae) individuals were abundantly caught on yellow traps and they also occurred frequently both on vines and on *Convolvulus arvensis*. Several leafhopper species of the genus Macrosteles are known as stolbur vectors in other crops (ALTABELLA et al., 2002).

The Deltocephalinae *Anoplotettix fuscovenosus* and the Agalliinae *Dryodurgades reticulatus* are frequent species in vineyards and should therefore be observed although they have never been mentioned in literature as vectors of stolbur phytoplasma.

Table 3: Progress of Bois Noir in the studied vineyard in Klosterneuburg

Variety	Total number of plants	Number of infected plants 2004	Number of newly infected plants 2005
Rheinriesling	961	59	46
Zweigelt	587	48	13
Chardonnay	560	21	11
Grüner Veltliner	560	5	4

Visual inspection and molecular analysis of potential host plants revealed that at all experimental sites stolbur phytoplasmas were present in *Convolvulus arvensis.* It can be presumed that this species plays a key role in disease spread in Austria. Contrarily to other European countries, where *Urtica dioica* is a very important weed host, in our study infected *Urtica dioica* plants were only found at one investigated site (Klöch). The relevance of *Taraxacum officinale* has to be further investigated. Occasionally samples of *Polygonum aviculare* were also infected by stolbur phytoplasmas. To our knowledge stolbur infections of *P. aviculare* have never been reported before.

The gained information on host plants and possible vectors of Bois Noir in Austria could be a basis for development of control strategies. Further work is required to determine the vectoring abilities of the suspicious leafhopper species.

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