Survey and molecular detection of Bois noir in vineyards of the Czech Republic – Short communication

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Abstract

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Stolbur phytoplasma is an important pathogen associated with the Bois noir (BN) disease of grapevines. In the Moravia wine region, plants exhibiting BN-symptoms were observed in 14 localities, covering all four of the wine sub-regions. On other hand in the Bohemia wine region, symptomatic plants occurrence was only sporadic. All of the stolbur phytoplasma isolates were ascribable to the 16SrXII-A subgroup and the tuf-b type. The loss of grape clusters reached 50%, comparing the fully affected grapevine plants with the healthy ones.

Keywords: grapevine; stolbur phytoplasma; tuf-b; nested PCR; grape clusters

Bois noir (BN) is a serious grapevine disease, associated with an infection by the stolbur phytoplasma (subgroup 16SrXII-A). The disease is endemic to the European and Mediterranean areas, and over the last decades it has spread extensively (Maixner 2011). In the Czech Republic, probably the first reference on the occurrence of stolbur on tomatoes in South Moravia was published by Baudyš (1933). The first observations of typical BN-symptoms on grapevines (cvs Chardonnay and Blaufränkisch) came from South Moravia (Polešovice and Velké Pavlovice locations) in 2003, and the BN occurrence was officially confirmed by the Plant Protection Service in 2006.

In this work, the occurrence of BN disease in the Bohemian and Moravian wine regions is presented, the phytoplasmas affecting grapevines are molecularly identified and preliminary data on the negative effects upon yields are published.

MATERIAL AND METHODS

The study was conducted between 2005 and 2011 in Bohemian and Moravian vineyards (Table 1). At least 1,000 grapevines per intensive vineyard were visually inspected for typical BN-symptoms such as leaf reddening or yellowing, incomplete lignification of the canes, and shrivelling of the berries. The frequency of symptomatic vines was evaluated with the scale: sporadic (≤ 1%), frequent (≤ 10%), and massive (> 10%) occurrence. A minimum of 20 symptomatic and non-symptomatic vines were randomly sampled in the August–September and subjected to molecular identification of phytoplasma (Table 1).

Total DNA was extracted using the phytoplasma enrichment procedure by Ahrens and Seemüller (1992) from petioles and leaf midribs. For the detection and identification of phytoplasma,

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Table 1. The highest observed incidence of Bois noir disease at different localities of the Czech wine regions during period 2005–2011

	Sub-region	Vineyeard	BN-symptoms	PCR/RFLP	Incidence	Cultivar
Moravian wine region	Mikulov	Březí	+	stolbur	•	MT, Sv
	Mikulov	Iváň	+	nt	••	
	Mikulov	Perná	+	stolbur	••	Fr
	Slovácko	Blatnice pod Sv. Antonínkem	+	nt	•	
	Slovácko	Polešovice	+	stolbur	•	Zw
	Velké Pavlovice	Čejkovice	+	stolbur	•	Zw
	Velké Pavlovice	Hustopeče	+	stolbur	••	
	Velké Pavlovice	Nosislav	+	stolbur	•••	Zw
	Velké Pavlovice	Velké Němčice	+	nt	••	Fr, Zw
	Velké Pavlovice	Žabčice	+	stolbur	•	Ch, CM
	Znojmo	Božice	+	stolbur	•••	An, Sv, Zw
	Znojmo	Dolní Kounice	+	stolbur	••	MP, Zw
	Znojmo	Hnánice	+	nt	•	
	Znojmo	Trboušany	+	stolbur	••	
Bohemian wine region	Litoměřice	Litoměřice	+	stolbur	•	Dr, Sv, Zw
	Mělník	Karlštejn	+	stolbur	•	
	Mělník	Kutná hora	–	nt	–	
	Mělník	Louny	–	nt	–	
	Mělník	Mělník	–	nt	–	
	Mělník	Most	–	nt	–	
	Mělník	Praha	+	nt	•	
	Mělník	Slaný	–	nt	–	

incidence: ≤ 1% (•), ≤ 10% (••), > 10% (•••); symptoms present (+), without symptoms (–); (An) André, (MP) Blauer Portugieser, (MT) Müller Thurgau, (Fr) Blaufränkisch, (CM) Cabernet Moravia, (Ch) Chardonnay, (Dr) Dornfelder, (Sv) Saint Laurent, (Zw) Zweigeltrebe; nt – not tested

nested PCR with universal primers P1/P7 followed by R16F2/R2, and subsequent RFLP analysis with *Alu*I, *Mse*I, *Rsa*I and *Taq*I restriction enzymes were employed (Lee et al. 1998; Quaglino et al. 2009). All obtained stolbur phytoplasma isolates were subjected to genotyping on *tuf* gene as described by Langer and Maixner (2004).

The number of grape clusters was evaluated during 2009 and 2010 harvests in the Perná vineyard. Twenty BN-symptomatic, PCR-positive vines and twenty asymptomatic, PCR-negative vines of cv. Blaufränkisch were analyzed. The statistical analyses of yield were carried out using one-way ANOVA, Multifactorial ANOVA, and Kruskal-Wallis statistical test, $P = 0.05$ (Statistica Cz 10, StatSoft CR, Prague, Czech Republic).

RESULTS AND DISCUSSION

In the Bohemian wine region (ca. 550 ha area) only sporadic occurrence of symptomatic plants was noted. The presence of BN was only marked in three out of eight inspected localities (Table 1, Fig. 1). A different situation was noted in the Moravian wine region (ca. 17,450 ha area), where plants showing BN-symptoms were observed in all of the 14 localities to varying extents. The most extreme incidence was noted in the Božice vineyard, with 68% of the vines showing BN-symptoms in 2005. The same situation was found in 2006, and the vineyard was eliminated in 2007. The second highest occurrence was noted in the Nosislav vineyard, with the BN incidence about 11.5% observed during the years 2006–2008 (13.98% in 2006, 11.15% in 2007, 9.31% in 2008). That was similar to BN incidence on Zweigelt and Rheinriesling reported from Austria (Riedle-Bauer et al. 2006). Surprisingly, during the next two years, the frequency of symptomatic vines decreased to 2.06% (3.10% in 2009 and 2.06% in 2010). This variation in the incidence of the disease is commonly connected with the phenomenon of recovery (Maixner 2011). However, recovery does not lead to disease disappearance and the return to full yields; other authors have noted a 25–30% decrease in yields or a 25% decrease in the number of fruit clusters, compared to healthy plants (Morone et al. 2007; Zahavi et al. 2009). Yet a different situation was noted in the Perná locality, where a BN incidence of about 5% was constant during the period 2006–2010 (evaluated from 1,380 vines, cv. Blaufränkisch); with the affected vineyard eliminated in 2011.

The first symptoms of leaf reddening or yellowing and the shrivelling of berries were observed at the end of July in the stage of fruit development (BBCH-77). The symptoms included incomplete lignification of the canes being fully developed in the second half of August and September (BBCH-81). The most affected blue grapes cultivars were Zweigeltrebe, and Saint Laurent; plus Chardonnay of the white grape cultivars (Table 1).

A total of 271 plants were collected and analysed for the presence of stolbur phytoplasma afterwards. Nested PCR provided positive results in 91% (106 out of 116) of the BN-symptomatic grapevines tested. Only stolbur phytoplasma (16SrXII-A) was identified by following the RFLP analyses of the R16F2/R2 amplicons. Asymptomatic plants and plants manifesting typical symptoms of damage caused by *Stictocephala bisonia* feeding, symptoms of ESCA syndrome, and grapevine leaf-roll disease were phytoplasma-negative (Fig. 2).

All of the stolbur phytoplasma isolates were successfully amplified in the nested PCR with Tuf1f/r followed by TufAyf/r primers. All the *Hpa*II RFLP profiles obtained were attributable to the stolbur tuf-b type. The results obtained confirm the tuf-b

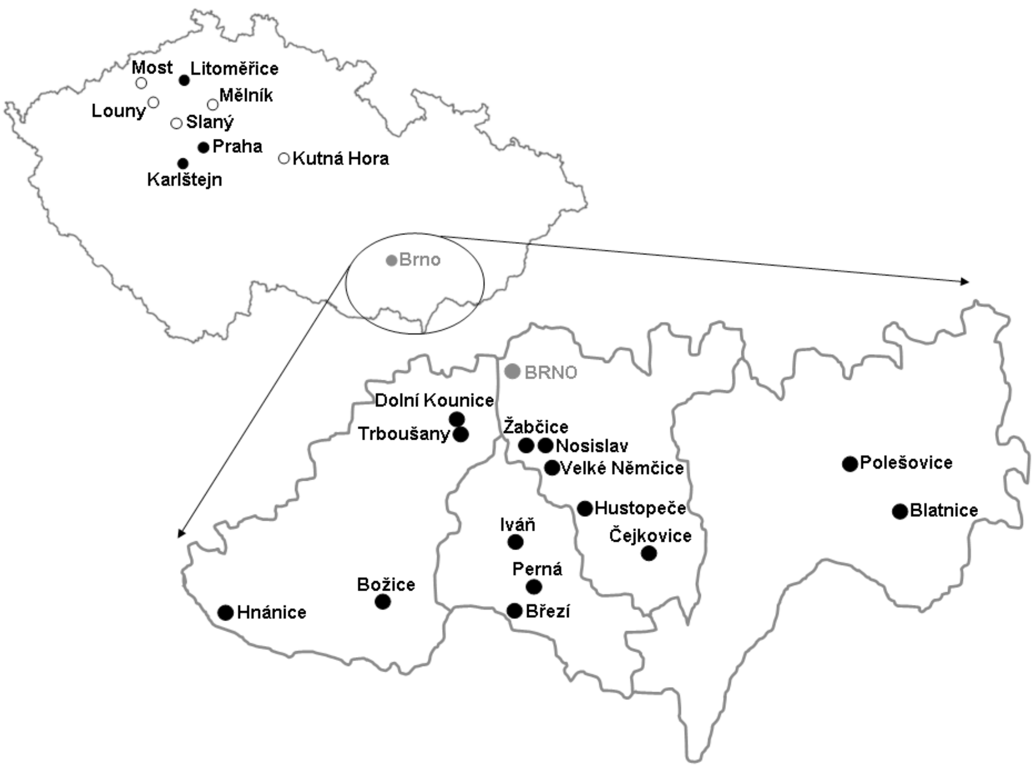

Fig. 1. Distribution of BN disease caused by stolbur phytoplasma in Bohemian and Moravian grapevine regions
Stolbur phytoplasma absence (○), stolbur phytoplasma presence (●)

Fig. 2. Bois noir and other non–bois noir symptoms on grapevine: (A) leaf reddening and rolling of stolbur phytoplasma infected grapevine cv. Blaufränkisch with shrivelling of berries; (B) leaf reddening caused by *Stictocephala bisonia* sucking (indicated by arrow); (C) ESCA syndrome symptoms (red brown spots) on leaves of grapevine cv. Blaufränkisch; (D) reddish purple coloration of leaf with typical green veins caused by complex grapevine viruses

type as a predominant type in the eastern and southern wine regions (Fialová et al. 2009; Ember et al. 2011).

In 2009–2010, development of clusters of symptomless grapes (PCR stolbur negative) and fully BN-symptomatic (PCR stolbur positive) vines at the Perná vineyard (cv. Blaufränkisch) were evaluated. The average number of clusters per vine was 6.45 (2009) and 5.0 clusters (2010) on symptomatic vines and 11.8 (2009) and 10.7 clusters (2010) on healthy vines. This almost 50% difference was significant ($P = 0.05$) in both years. A similar situation was described by Morone et al. (2007) in northwestern Italian vineyards infected with Flavescence dorée. Yield losses in our work were estimated at 70% (data not shown). Zahavi et al. (2009) noted even 85% yield losses in stolbur-affected grapevines.

In summary, the results of this study indicate that the stolbur phytoplasma is the important pathogen associated with the BN of grapevines in the Czech Republic. The affected vines had lower yields than non-affected vines. The results obtained from this and previous studies (Fialová et al. 2009) could be well useful for the development and application of both better and more specific control strategies of stolbur phytoplasma and its vectors.

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