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'Bois noir' phytoplasma can be transmitted to healthy *Vitis vinifera* L. plants by rootstocks

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Abstract

The role of the rootstocks Kober 5BB, 420A and SO4 in 'bois noir' (BN) phytoplasma transmission was investigated in cuttings, named 'trionti', created by grafting a BN-infected grapevine cane (inoculum source) and a healthy grapevine bud grafted onto a healthy rootstock. Typical grapevine yellows symptoms were observed on five originally healthy grapevine sprouts, and specific real-time PCR analyses confirmed the presence of BN phytoplasmas. These findings evidenced that BN phytoplasma translocated from the infected cane to the healthy bud through the rootstock, highlighting the possible rootstock role in spreading BN phytoplasma in the nurseries.

Key words: Stolbur, *trionte*, nursery, transmission.

Introduction

'Bois noir' (BN), a disease of the grapevine yellows (GY) complex associated with stolbur phytoplasmas of group 16SrXII, was firstly reported in Veneto region in 1983 (Egger and Borgo, 1983). Nowadays, BN infects vineyards of all north-eastern Italy (Belli *et al*., 2010), including the mother-plant vineyards, where its epidemics heavily impact on viticulture. Almost all *Vitis vinifera* L. varieties are susceptible to GY infection and show typical disease symptoms. On the other hand, rootstock varieties do not show any symptoms and are considered tolerant. Recently, GY symptoms were reported also in rootstocks, where phytoplasmas associated with 'flavescence dorée' were identified (Borgo *et al*., 2009). As phytoplasmas infecting rootstocks could be transmitted by grafting in nurseries, a three-year research project entitled "Prevention and control of grapevine 'bois noir' in the Veneto region" studied the role of different rootstocks in the transmission of BN phytoplasma to the scion. In this work, we reported the results of the first two years.

Materials and methods

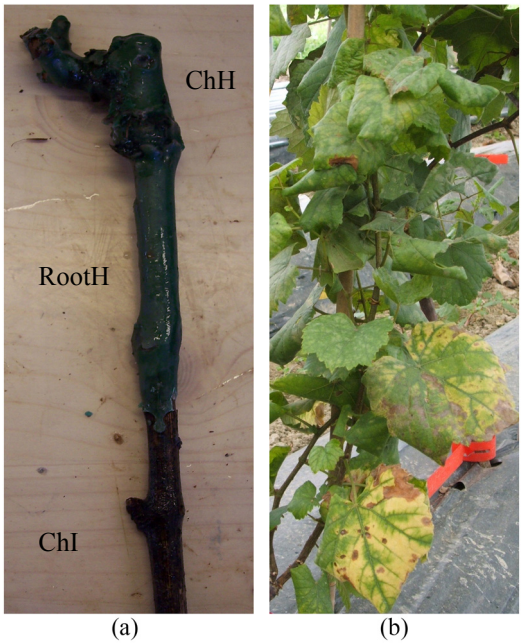
Double grafted cuttings, named 'trionti', were created by grafting a healthy Chardonnay bud grafted on a healthy rootstock (top) onto a BN-infected Chardonnay cane, used as inoculum source (bottom) (figure 1a). BN-infected or healthy Chardonnay plants and rootstocks (Kober 5BB, 420A, and SO4) used for creating the 'trionti' were selected by analyses through a real-time PCR TaqMan allelic discrimination assay for the specific detection of BN phytoplasma. Primers and probes were designed on ribosomal protein gene nucleotide sequences (data under publication). In total, 1,175 'trionti' have been grafted: 385 in 2009 and 790 in 2010 (table 1). Double grafts have been carried out at nurseries in Veneto region with a bench omega-type grafting machine. The grafted cuttings have been forced, planted, and maintained in a screen-house protected against phytoplasma vectors. GY symptoms were visually observed on sprouted cuttings in September 2009 and 2010. TaqMan real-time PCR assays were performed on DNAs extracted from leaves of sprouted cuttings for validating the visual observation and for revealing the possible presence of BN phytoplasma even in absence of symptoms.

Results

The sprouting percentages recorded have been very low: in the first year, 59 out of 385 rooted cuttings (15.3%); in the second year, 120 out of 790 (15.2%) (table 1). Leaf yellowing and rolling were observed in one 'trionte' sprouted in 2009 (rootstock SO4) and in two 'trionti' in 2010 (rootstock 420A) (table 1 and figure 1b). Molecular analyses by TaqMan real-time PCR confirmed the presence of BN phytoplasma in symptomatic 'trionti'. Further, these analyses allowed to identify BN phytoplasma also in two symptomless plants, one grafted on SO4 in 2009 and one on 420A in 2010 (table 1).

Table 1. 'Trionte' sprouting, GY-symptom observation and molecular detection of BN phytoplasma in the years 2009 and 2010.

Year	Rootstock	Number of 'trionti'			
		Grafted	Sprouted	GY-symptomatic	BN-infected
2009	Kober 5BB	189	34	0	1
	SO4	105	14	1	1
	420A	91	11	0	0
	Total	385	59	1	2
2010	Kober 5BB	205	27	0	0
	SO4	300	31	0	0
	420A	285	62	2	3
	Total	790	120	2	3
Overall Total		1175	179	3	5



(a) (b)

Figure 1. Double grafted cutting ('trionte') (a) and symptomatic plant on rootstock 420A (b). ChH: bud from healthy Chardonnay; RootH: healthy rootstock; ChI: cane from BN-infected Chardonnay.
(In colour at www.bulletinofinsectology.org)

Discussion and conclusions

Obtained results suggested that the rootstocks Kober 5BB, 420A and SO4 can transmit BN phytoplasma to healthy *Vitis vinifera* L. plants. In 2011, observation and molecular analyses will be repeated on all sprouted 'trionti' maintained in screen-house in order to verify the possible presence of further BN phytoplasma transmission events. Interestingly, a recent BN survey in Friuli Venezia Giulia region reported the highest number of BN infections on vines grafted on rootstocks 420A and SO4 (Ermacora *et al*., 2011). Our results highlighted that not only FD phytoplasma (Borgo *et al*., 2009), but also BN phytoplasma can be transmitted through different rootstocks. This evidence opens new avenues for nursery management against the spreading of economically important phytoplasma diseases.

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