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Incidence of "bois noir" phytoplasma in different wine-growing regions of Spain and its relation to *Hyalesthes obsoletus*

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Abstract

"Bois noir" (BN) disease was first identified in Spain in 1995, but in recent years there seems to be a recrudescence in different wine regions. During 2006 and 2007, the areas where this increase was observed were surveyed for *Hyalesthes obsoletus* and other potential vectors. Not very high populations of *H. obsoletus* have been detected, but a positive relation between *H. obsoletus* presence and disease incidence was highlighted. The vineyards with the highest BN incidence (55-70%) are the ones where the majority of *H. obsoletus* were captured. The maximum total number of individuals caught in seven samplings of four aspirations each was fifteen. In low BN incidence vineyards (around 5%) the total number of individuals caught was 3-5. Only in one high BN-incidence vineyard (70%) in Catalonia, *H. obsoletus* couldn't be captured in 2006 and only a few were captured in 2007. In this case, the *Euscelidius variegatus* population was very high. The percentage of stolbur-bearing *H. obsoletus* individuals ranged between 20 and 100%.

Key words: "Bois noir", stolbur phytoplasma, *Hyalesthes obsoletus, Euscelidius variegatus*.

Introduction

"Bois noir" (BN) disease is caused by a phytoplasma which belongs to the stolbur group, also referred to as the 16SrXII-A group (Seemüller *et al*., 1998). The disease is widespread in different vine growing regions in Spain, such as La Rioja, Alava, Navarra, Catalonia and Aragón. Studies previously done in Catalonia, in the wine areas of the Penedès and the Conca de Barbera showed that *Hyalesthes obsoletus* Signoret was present in very low populations; nevertheless surveys in La Rioja showed its presence. During 2006 and 2007 the search of *H. obsoletus*, the main vector of the stolbur in vineyards, and of other potential vectors was extended to five geographical areas of Spain. Likewise, the percentage of BN infection has been related, in each of the plots, with the *H. obsoletus* presence and with their percentage of infection.

Materials and methods

During 2006 and 2007, weekly from June to September, the insects were captured with a D-vac aspirator, with four aspirations, of 2 minutes each, over weeds within and at the edges of grapevine plots. The PCR technique was used for phytoplasma detection in plants and insects. DNA was extracted following the methods used in previous studies (Batlle *et al*., 2000). The universal primers for phytoplasma detection, P1/P7, were used in the first amplification step. The second step was performed with the fstol/rstol specific primers for the stolbur group (Maixner *et al*., 1995). Negative samples with the specific primers were analyzed in the second step using the universal primer pair fU5/rU3 to determine the presence of phytoplasmas diverse from stolbur group. Ten µl of the mixture containing the amplified DNA in the second step were digested with T*ru*I enzyme. The non ribosomal specific primers stol4f/r (Daire *et al*., 1997) were also used.

Results

In most of the cases a correlation was obtained between the disease incidence and the importance of the *H. obsoletus* population. This is the case of Viñas del Vero-1 (Aragon) with an incidence of the disease of 55% and a total of 15 individuals of *H. obsoletus* captured, followed by Montitura plot (Navarre) with an incidence of 70% and 10 individuals of *H. obsoletus* captured and Aranzana (La Rioja) with an incidence of 70% and 8 individuals captured (table 1). In both plots in Alava and in the plot of Navarre (Saso) with minor incidence of the disease (1-10%), the populations of *H. obsoletus* were lower. Individuals of *H. obsoletus* couldn't be detected in the plots of "La Rioja baja" (Autol and Tudelilla), where the incidence of the disease is practically nil. Nevertheless, a correlation was not observed between BN incidence and *H. obsoletus* presence in the plot of the area of the Penedès (Catalonia) where the percentage of the disease was high (70%), *H. obsoletus* was not found in 2006, and only 2 individuals were captured in 2007. In this case, however, there was an abundant presence of other potential vectors as *Euscelidius variegatus* (Kirschbaum) (Sabaté *et al.,* 2006*)*. On the other hand, in occasional samplings in other zones of Catalonia, such as Mediona (Alt Penedès), Avinyo (Bages) and Corbera (Terra Alta) *H. obsoletus* individuals were captured (figure 1); in all the zones where the cixiide was captured individuals positive to

Table 1. BN incidence in different wine-growing regions and most relevant species of insects captured in 2006.

Location	BN incidence	no. insects captured (no. positives insects/total analyzed)						
		H. obsolethus	*E. variegatus*	*A. laevis*	*M. qadripunctulatus*	*P. striatus*	*L. striatellus*	*N. fenestratus*
Alava								
Morrolavieja	1%	4(1+/3)	13(2+/13)	12/0+/3)	2(1+/2)	45(0+/10)	1	2
Carrabañas	3-4%	5(2+/5)	3(0+/2)	14(0+/2)	3(1+/3)	59(1+/7)	2(0+/1)	5(0+/3)
Aragón								
Vero1	55%	15(6+/8)	41(0+/41)	7(0+/2)	0	92(0+/13)	5(0+/4)	1(1+/1)
Vero2	53%	4(1+/2)	32(1+/32)	60(0+/11)	0	343(0+/19)	34(0+/17)	5(2+/4)
La Rioja								
Manjarres	70%	1(1+/1)	7(0+/7)	5(2+/3)	0	57(1+/25)	14(0+/1)	1(1+/1)
Aranzana	70%	8(1+/5)	13(0+/13)	26(0+/9)	1 (0+/1)	1(0+/1)	5(0+/5)	1(0+/1)
Tudelilla	2%	0	1(0+/1)	3(0+/3)	0	6(0+/3)	8	0
Autol	2%	0	4(0+/4)	4(0+/2)	0	1(0+/1)	0	0
Navarra								
Montitura	70%	10 (5+/10)	174(4+/140)	100(1+/2)	0	480(0+/15)	44(3+/15)	0
Saso	10%	3(1+/3)	47(0+/39)	30(1+/10)	0	53(0+/12)	13(0+/6)	1(1+/1)
Cataluña								
Pla de Penede	70%	0	138 (2+/130)	7		13	41(1+/40)	0
Avinyo	5%	5(2+/2)	1	6		7	0	0

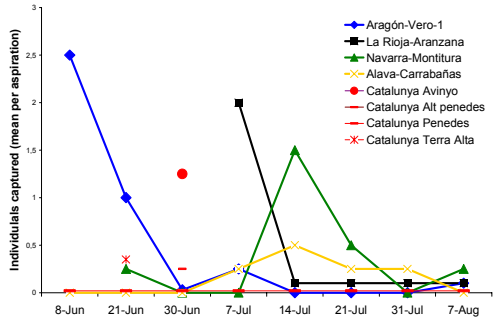

Figure 1. Population evolution of *H. obsoletus* in different wine plots of Spain.
(In colour at www.bulletinofinsectology.org).

stolbur were identified, the percentage of infected individuals ranging between 20 and 100% (table 1). The peaks of population of *H. obsoletus* were attained in 2006 at different dates, depending on the sampled zone, between June 6th and July 14th. Like in previous studies, in the plots with a major incidence of the disease, like Montitura (Navarra) and Manjarrés (La Rioja), other species of insects have been identified as carriers of stolbur phytoplasma: *E. variegatus*, *Agallia laevis* Ribaut, *Psammotettix striatus* (L.), *Laodelphax striatellus* (Fallén), *Neoaliturus fenestratus* (Herrich-Schäffer) and *Macrosteles quadripunctulatus* (Kirschbaum) (table 1).

Discussion

Results showed that population of *H. obsoletus* was higher in plots of Aragón, Navarra and La Rioja that in the plots of Alava and Catalunya. This indicates a correlation between the presence of this species and the disease incidence in most of the cases. Other insect species as *E. variegatus* could be ivolved in disease transmission in those areas where *H. obsoletus* is not common.

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