New findings on phytoplasmas-affected Auchenorrhyncha populations in Sardinian vineyards

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Abstract – Epidemiological research was carried out in two vineyards affected by "Bois noir" (BN). *Auchenorrhyncha* potential vectors of BN, were monitored periodically between May to November 2004 in a vineyard and between May to June 2005 in the other. *Auchenorrhyncha* samples were tested to assess phytoplasmas presence using PCR and RFLP. *Euscelis lineolatus* was positive to 16SrI-C ("Clover phyllody" reference strain) in 2004 while, in 2005, at the preimaginal age, at the 16SrXII-A ("Stolbur" reference strain) phytoplasmas. *Exitianus taeniaticeps* acquired 16SrI-B ("Maryland aster yellow" reference strain), 16SrV-A ("Elm yellow" reference strain) and 16SrX-C ("Pear decline" reference strain) phytoplasmas in 2004. Resulted news host of phytoplasmas: *Psammotettix alienus* was positive to 16SrI-B, 16SrV-A, 16SrX-A phytoplasmas ("Apple proliferation" reference strain) in 2004 and to 16SrXII-A in 2005. *E. lineolatus* and *P. alienus* for 16SrXII-A. *E. taeniaticeps* for 16SrV-A 16SrX-C and *P. alienus* for 16SrV-A and 16SrX-A. Researches on the effective epidemiological role of *E. lineolatus* and *P. alienus* in BN are in progress.

Keywords: *Auchenorrhyncha*, Bois noir, phytoplasmas, potential vectors.

INTRODUCTION

Phytoplasma infections known as "yellows" are widespread in European vineyards. In Sardinia typical symptoms were observed on different varieties of *Vitis vinifera*, and attributed to phytoplasmas belonging to the 16SrXII-A taxonomic group. These phytoplasmas are known to be the etiological agents of the grapevine "Bois noir" (BN) and *Hyalesthes obsoletus* Signoret is their natural vector.

The high occurrence of the disease, in areas where this vector is absent, i.e. in Sardinia, suggests that the presence of other possible vectors for 16SrXII-A phytoplasmas would be actual.

This study aimed to identify new potential vectors of BN phytoplasmas.

MATERIALS AND METHODS

Epidemiological investigations carried out in 2003 on a vineyard affected with BN revealed that different insects species could acquire phytoplasmas of the 16SrI-C, 16SrI-B, 16SrX-A and 16SrXII-A taxonomic groups [3] .

Subsequent studies were carried out in 2004 in symptomatic Vernaccia plants in a vineyard of the Centre-Western Sardinia. In May and June 2005 further researches were carried out in a mixed Chardonnay and Vermentino vineyard in North-Western Sardinia.

In the first case the survey was carried out from May to November at regular intervals between the plant rows. Insects were collected from the herbaceous plants with entomological net, in four rows (150 meters length). The same system was used to monitor the second vineyard in 2005.

Graminaceae, Chenopodiaceae, Convolvulaceae, Portulacaceae, Urticaceae, Amarantaceae and *Solanaceae* were present at sites investigated.

The *Auchenorrhyncha* collected from the two sites were divided, according to their taxonomy, into different groups. The number of insects grouped in each batch was 5 to 30 according to their size.

The insects were tested to verify the Stolbur or other phytoplasmas presence. Samples were tested by PCR and RFLP. Total DNA extraction was carried out using the protocol suggested by Doyle and Doyle [2]. Amplification assays were carried out using direct PCR with universal primers P1/P7 [1] followed by two nested PCR using R16F1 [6] /B6 [7] and then R16F2n/R2 [4] primers.

Polymorphic analysis of the length of the restriction fragments of the ribosomal amplified DNA was carried out using the *Tru*I enzyme and then *Rsa*I, *Ssp*I and *Hha*I. The digested products were analyzed in 5% polyacrylamide gel and visualized on an UV transilluminator.

RESULTS

The results from the surveys carried out during the 2004 are shown in Tab.1. The most present *Auchenorrhyncha* species were: *Laodelphax striatellus* (Fallén), *Anaceratagallia ribauti* (Ossialnisson), *Euscelis lineolatus* (Brullè), *Exitianus taeniaticeps* (Kirschbaum), *Goniagnathus guttulinervis* (Kirschbaum), *Psammotettix alienus* (Dahlbom), *Thamnotettix zelleri* (Kirschbaum) and *Zyginidia scutellaris* (Herrich-Shäffer). *Zyginia rhamni* (Ferrari) and *Jacobiasca lybica* (Bergenin & Zanon) were also sporadically present (not shown in the table).

The results of molecular assays are shown in Tab. 2. *E. lineolatus* had the ability to acquire 16SrI-C, Aster yellows group phytoplasmas ("Clover phyllody" reference strain), at the immaginal stage in July and August 2004.

In May 2005, nymphs from the same species collected in the vineyard located in the North-Western Sardinia, was found positive for 16SrI-B phytoplasmas ("Maryland aster yellows" reference strain) and, for the first time, 16SrV-A phytoplasmas ("Elm yellows" reference strain), 16SrX-A ("Apple proliferation" reference strain) during July 2004 and found positive for the 16SrXII-A phytoplasmas presence. A sample of *P. alienus* from Centre-Western Sardinia was 16SrXII-A in June 2005. Remarkably *E. taeniaticeps* was found as a new host for the 16SrI-B phytoplasmas (November 2004), and for the 16SrV-A and 16SrX-C ("Pear decline" reference strain) in July 2004.

Tab. 1. Species captured with entomological net from May to November 2004, in vineyards of Central-Western Sardinia.

Auchenorrhyncha										
Family	*Delphacidae*		*Cicadellidae*							
Subfam.			*Deltocephalinae*							*Tiflocibinae*
Captured 2004	*Laodelphax striatellus*		*Anaceratagalla ribauti*	*Euscelis lineolatus*		*Exitianus taeniaticeps*	*Goniagnathus guttulinervis*	*Psammotettix alienus*	*Thamnotettix zelleri*	*Zyginidia scutellaris*
	A	N	A	A	N	A	A	A	A	A
15 /05	-	-	-	2	5	-	-	-	57	-
07 /06	55	-	-	2	-	1	-	3	14	2
05 /07	17	-	2	7	-	9	-	57	-	66
25 /07	8	3	-	6	6	19	-	48	-	4
10 /08	1	-	3	5	-	12	5	20	-	1
30 /08	2	-	-	1	-	-	2	-	-	-
20 /09	-	-	-	-	-	-	-	-	-	-
10 /10	1	-	-	-	1	3	6	6	-	-
15 /11	1	-	-	-	4	6	-		-	-

A:adulti; N: nymphs

Tab. 2. Proportion of positive samples within *Auchenorrhyncha* captured in 2004 and 2005 in two vineyards in North and Central Sardinia.

Species		N° insects per sample	No. of positive samples from those taken and the phytoplasmas found							% of positive samples
			May	June	July	August	Sept	Oct	Nov	
Euscelis lineolatus	A	5	-	-	1/1 16SrI-C	1/1 16SrI-C	-	-	-	100
Euscelis lineolatus	N*	5	2/2 16SrXII-A 16SrI-C		-	-	-	-	-	100
Exitianus taeniaticeps	A	5			2/5 16SrV-A 16SrX-C	0/1		0/3	1/3 16SrI-B	25.0
Goniagnathus guttulinervis	A	5	-		-	0/1	-	0/4	-	0
Psammotettix alienus	A	10	-		3/9 16SrI-B 16SrX-A 16SrV-A	1/2 16SrX-A	-	0/2	-	30.7
	A*	3	-	1/5 16SrXII-A	-	-	-	-	-	20.0

* indicates captured in 2005; A: adults; N: nymphs

CONCLUSIONS

We have checked the presence, in the two sites, of new natural phytoplasmas hosts among the *Auchenorrhyncha*. It remains to clarify, in further studies, the role of these insects in the "vineyard system", with the different botanic groups present and the probable ecological cycle of the prokaryotes identified. It is worth emphasizing that more than one species of insects may be BN vectors and substitute the *Hyalesthes obsoletus* action in disease spreading. However this has not yet been proved. In addition, the presence of *Auchenorryncha* infected with 16SrI-C and/or 16SrI-B phytoplasmas confirms that these two groups of prokaryotes ar e widespread in the vineyards [5]. Their role however is not clear. In our experience *E lineolatus* and *P. alienus* are new hosts of 16SrXII-A phytoplasmas, *E. taeniaticeps* with respect to 16SrV-A, 16SrX-C and *P. alienus* for 16SrV-A and 16SrX-A phytoplasmas.

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