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PRELIMINARY RESULTS ON PUTATIVE VECTORS OF '*CANDIDATUS* PHYTOPLASMA SOLANI' IN BOIS NOIR-AFFECTED VINEYARDS IN FRANCIACORTA (LOMBARDY REGION, NORTH ITALY)

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Bois noir phytoplasma (BNp) strains are transmitted by the planthopper *Hyalesthes obsoletus* Signoret (Homoptera: Cixiidae), a polyphagous insect living preferentially on weeds. In vine-growing areas where *H. obsoletus* is absent, the presence of BNp implies the existence of alternative vectors. Recently, *Reptalus panzeri* has been reported as a natural vector of BNp in Serbian vineyards. In the present study, field surveys and molecular analyses were carried out in a BN-affected vineyard in Franciacorta (Lombardy region, North Italy) in 2013 to identify putative insect vectors. Insects (1100 specimens) were captured by entomological net and sticky traps from May to October. Phenotypic analysis by stereomicroscope allowed the identification of 42 taxonomic groups at different levels (26 species and 16 genera), grouped in 624 pools for molecular detection. Specific PCR-based amplification of *stamp* gene revealed the presence of BNp in 64 analysed pools (10%) belonging to 20 taxonomic groups (15 species and 5 genera). Further analyses will be carried out to (i) characterize the BNp strains identified in insect specimens by *stamp* gene sequence and compare with strains identified in grapevines and weeds, (ii) determine the transmission capability by trials performed in controlled conditions.

INTRODUCTION

Grapevine yellows (GY) are a phytoplasma-associated disease complex that induces severe crop losses in almost all varieties used for wine production. Among GY, Bois noir (BN), associated with *'Candidatus* Phytoplasma solani', is responsible for serious crop losses in the Euro-Mediterranean area and in other continents (QUAGLINO et al., 2013). BN phytoplasma (BNp) strains are transmitted to grapevine by *Hyalesthes obsoletus* Signoret (Homoptera: Cixiide), a polyphagous vector living preferentially on nettle (*Urtica dioica L.*), bindweed (*Convolvulus arvensis L.*), and chaste tree (*Vitex agnus-castus* L.) inside and/or around vineyards (LANGER und MAIXNER, 2004; KOSOVAC et al., 2015). Furthermore, in vine-growing areas where *H. obsoletus* is absent, the presence of BNp implies the existence of alternative vectors. *Reptalus panzeri* has been reported as a natural vector of BNp in Serbian vineyards (CVRKOVIĆ et al., 2014). Additionally, *Anaceratagallia ribauti* and *Reptalus quinquecostatus* were experimentally confirmed as vectors of 'Ca. P. solani' but not to grapevine (PINZAUTI et al., 2008; RIEDLE-BAUER et al., 2008); therefore, currently, such insects are not considered to be involved in BNp transmission to grapevine. Other studies reported that other Cixiidae and Cicadellidae have been captured within or near BN-diseased vineyards and found to contain BNp. Based on such information, it appears that, even though the role of these nume-

rous hosts in BNp transmission has not been proven, it is probable that other host plants are involved in the epidemiology of BN disease, harboring additional insect species capable of spreading the disease (MORI et al., 2015; OLIVERI et al., 2015). In the present study, field surveys and molecular analyses were carried out in a BN-affected vineyard in Franciacorta (Lombardy region, North Italy) in 2013 to identify putative insect vectors.

MATERIAL AND METHODS

SAMPLING OF INSECT SPECIMENS. A field survey was carried out in a vineyard (variety Chardonnay), located in Gussago (BS), Franciacorta (Lombardy, North Italy), selected for the high incidence of BN disease (>70% affected grapevines). During 2013, insects were monitored and captured every week, from May to September, by yellow sticky traps (placed within, around the borders and in the neighborhood of the vineyard) and entomological nets.

INSECT IDENTIFICATION. All the insect specimens, captured in the examined vineyard, were maintained in ethanol 90% and identified by stereomicroscope based on phenotypic characters. Specimens of the same taxonomic group were pooled (1-3 specimens per pool) for further molecular analyses.

BNP-SPECIFIC DETECTION. Total genomic DNA was extracted from 624 insect pools. Briefly, the ethanol-preserved adults were dried on filter paper and homogenized in CTAB-based buffer plus ascorbic acid 0.5%. After incubation at 60°C for 30min, DNA was extracted with one volume of chloroform:isoamylalcohol 24:1v/v solution and then precipitated with the addition of one volume of isopropanol. The DNA pellet was then washed with 70% ethanol, vacuum dried and resuspended in 100µL TE pH8.0.

Specific detection of BNp ('*Ca*. P. solani', subgroup 16SrXII-A) was carried out by nested-PCR based amplification of *stamp* gene as previously described (FABRE et al., 2011). Total nucleic acids from periwinkle plants infected by phytoplasma strains EY1 ('*Ca*. P. ulmi'), STOL ('*Ca*. P. solani'), and AY1 ('*Ca*. P. asteris') were used as reference controls. Total nucleic acids from healthy periwinkle and PCR mixture devoid of nucleic acids were used as negative controls.

RESULTS AND DISCUSSION

INSECT DIVERSITY IN EXAMINED VINEYARD. Methods employed to capture insects (entomological net and sticky traps) allowed an accurate monitoring of the entomofauna present in the examined vineyard. In detail, during field activities, 1100 insect specimens were collected (717 by net and 383 by sticky traps). Stereomicroscope analysis, based on observation of morphological characters, allowed the identification of 42 distinct taxonomic groups. Based on such evidence, it was possible to classify the majority of captured specimens at species level (28); however, other taxonomic groups were defined only at genus level (16) (Table 1). Further studies will be carried out to describe such groups at species level by integrating the morphological approach with the utilization of the DNA-barcoding techniques.

PHYTOPLASMA IDENTIFICATION. PCR-based amplification of *stamp* gene revealed the presence of BNp in 64 out of 624 analyzed insect pools, belonging to 20 taxonomic groups (15 species and 5 genera) (Table 1).

Interestingly, some of the insects harboring BNp in the present work have been found in previous studies as vectors of other phytoplasmas (i.e. *Fieberiella florii for* '*Ca*. P. mali' and *Scaphoideus titanus* for Flavescence dorée phytoplasma) (WEINTRAUB und BEANLAND, 2006) and/or other bacterial plant pathogens (i.e. *Philaenus spumarius* for *Xylella fastidiosa*) (SAPONARI et al., 2014). Moreover, molecular detection analysis confirmed the presence of BNp in other insects, such as *Euscelis* spp., recently found infected by BNp in vineyards in South Italy (OLIVERI et al., 2015). In the last few years, multiple gene analysis was proposed and employed to describe phytoplasma species distinguished by evident molecular diversity and representing ecologically separated populations. Moreover, this approach was also applied to the investigation of the genetic diversity among phytoplasmas associated with several diseases in order to identify strain-specific molecular markers useful for improving the understanding of complex phytoplasma ecologies (QUAGLINO et al. 2013). To determine the possible vectoring activity of insects found BNp-infected in Franciacorta, further analyses will be carried out to

Table 1. Detection of '*Ca*. P. solani' in insects captured in BN-affected vineyard in Gussago (BS)

Insect (species)	No. of tested pools		Infection %
	collected	BNp-infected	
Allygidius furcatus	24	2	8
Aconurella prolixa	1	-	-
Anoplotettix fuscovenosus	12	1	8
Aphrodes makarovi	21	5	24
Asiraca clavicornis	6	2	33
Caliscelis bonellii	7	-	-
Centrotus corutus	1	-	-
Cercopis vulnerata	120	2	2
Cicadella viridis	35	6	17
Dictyophara europaea	47	-	-
Eupteryx vittata	2	1	50
Evacanthus acuminatus	3	2	67
Fieberiella florii	5	1	20
Goniagnathus brevis	5	-	-
Haematoloma dorsatum	7	-	-
Hephathus nanus	25	-	-
Hishimonus hamatus	4	-	-
Hyalesthes obsoletus	44	13	30
Hyalesthes scotti	6	-	-
Japananus hyalinus	2	-	-
Laodelphax striatella	56	5	9
Metcalfa pruinosa	35	9	26
Mocydia crocea	2	-	-
Neoaliturus fenestratus	2	1	50
Philaneus spumarius	28	2	7
Scaphoideus titanus	1	1	100
Stictocephala bisonia	1	-	-
Toya propinqua	9	-	-

Insect (genus)	No. of tested pools		Infection %
	collected	BNp-infected	
Balcanocerus spp.	5	-	-
Cixus spp.	2	-	-
Dicraneura spp.	1	-	-
Dicranotropis spp.	15	2	13
Empoasca spp.	1	1	100
Euscelis spp.	46	5	11
Kelisia spp.	2	-	-
Macrolestes spp.	4	-	-
Macropsis spp.	6	-	-
Megophthalmus spp.	4	2	50
Mocydiopsis spp.	2	-	-
Psammotettix spp.	12	-	-
Reptalus spp.	2	-	-
Thamnotettix spp.	5	1	20
Typhlocyba spp.	5	-	-
Verdanus spp.	1	-	-

(i) characterize the BNp strains identified in such insects by nucleotide sequence analysis of stamp gene and other genes (*tuf, vmp*1) and comparison with sequences of strains identified in grapevines and weeds, (ii) determine the transmission capability of insects harboring BNp strains genetically identical to those present in grapevine by trials performed in controlled conditions.

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