Bulletin of Insectology **70** (2): 221-229, 2017
ISSN 1721-8861

Influence of foraging strip crops on the presence of leafhoppers and planthoppers associated to grapevines' phytoplasmas

Federico LESSIO, Rosemarie TEDESCHI, Alberto ALMA
DISAFA, University of Torino, Italy

Abstract

The possible colonization of vineyards by leafhoppers, planthoppers and their allies, known or suspected vectors of phytoplasmas to vines, living on adjacent foraging strip crops seeded for pollinators was evaluated in Piedmont, NW Italy. Strip crops consisted in a seed mixture of Fabaceae and in rape (*Brassica napus* var. *oleifera* L.). Insects were collected with a sweep net on the strips, in the vineyard inter-row (close to and far from the strip), and in the surrounding habitat. DNA was extracted from single specimens, and PCR was performed to identify phytoplasmas of different groups, with a particular emphasis on groups 16SrV and 16SrXII. A one-way ANOVA was made for detecting differences in catches between strips and vineyards'inter-rows of the most abundant species. Among vectors, *Neoaliturus fenestratus* (Herrich-Schaffer), *Euscelis incisus* (Kirschbaum) and *Dictyophara europaea* (L.) were locally abundant as a consequence of the presence of weeds serving as host plants. Phytoplasmas of groups 16SrV and 16SrIX were identified in three specimens of *D. europaea* and one of *N. fenestratus*, respectively. *D. europaea* was more abundant on the strips, whereas the patterns of *N. fenestratus* changed from site to site. Concerning other species (not associated to 16SrV and 16SrXII phytoplasmas), high levels of *Psammotettix alienus* (Dalbholm) and *Philaenus spumarius* (L.) were observed. The presence of a flowering strip close to a vineyard does not seem to be a threat, if a good ground cover is achieved by the seed mixture. Otherwise, the development of certain weeds may cause an increase of leafhopper species known or suspected vectors of phytoplasmas.

Key words: agroecology, leafhoppers, phytoplasmas, PCR.

Introduction

Agroecology provides the basic ecological principles to study, design and manage agroecosystems that are both productive and natural resource conserving (Altieri, 2002). The functional biodiversity in agroecosystems may be increased by means of ecological compensation areas (ECAs) for the multiplication of both natural enemies (Burgio *et al*., 2004; 2006; Ponti *et al*., 2005) and pollinators (Nicholls and Altieri, 2013). In particular, pollinators are crucial in many ecosystems, including crops, as they permit plant reproduction, and many species may also act as bio-indicators (Kevan, 1999). Simplified ecosystems such as monocultural crops may not provide sufficient pathways for pollinator guilds, and have a heavy chemical input of pesticides that depresses pollinator communities. The input of adjacent non-food, foraging crops at field margins may overcome this problem at different levels of scale. Moreover, some cover crops may have other beneficial effects by increasing also natural enemies' populations (Burgio *et al*., 2016). In viticulture, one of the main problems is represented by phytoplasmas transmitted by leafhoppers and planthoppers (Hemiptera: Cicadomorpha and Fulgoromorpha) (Alma *et al*., 2015). In Europe, two grapevine yellows are associated to phytoplasmas: Flavescence dorée (FD), is associated to phytoplasmas of the 16SrV-C and 16SrV-D subgroups transmitted by *Scaphoideus titanus* Ball (Alma *et al*., 2015) and, to a lesser extent, possibly, also by *Dictyophara europaea* (L.) (Filippin *et al*., 2009) and *Orientus ishidae* (Matsumura) (Lessio *et al*., 2016), whereas Bois noir (BN) is associated to '*Candidatus* Phytoplasma solani' (16SrXII-A) transmitted by *Hyalesthes obsoletus* Signoret (Lessio *et al*., 2007), *Reptalus quinquecostatus* (Dufour) (Chuche *et al*., 2016), and *Reptalus panzeri* (Low) (Cvrković *et al*., 2014). Moreover, recently, a phytoplasma of the group 16SrIX has been identified in vines in Turkey (Bianco, 2013). From an Integrated Pest management (IPM) point of view, habitat manipulations with replacement of host plants for vector species with suitable cover crops may be a further mean of control. For instance, *S. titanus* lives strictly on *Vitis* spp., both wild and cultivated (Chuche *et al*., 2014), and it is a threat particularly in presence of many uncultivated areas colonized by wild grapevines (Pavan *et al*., 2012; Lessio *et al*., 2014). Therefore, the removal of wild grapevines has been proposed as a pest management measure (Alma *et al*., 2015). On the other hand, *H. obsoletus* is polyphagous and lives mainly on stinging nettle, *Urtica dioica* L. (Alma *et al*., 1988), and the removal of nettle from edges has a long-term beneficial effect in decreasing vector's populations (Alma *et al*., 2015). The use of side non-food crops may therefore be implemented in this direction.

However, a side-effect of these (partially) unmanaged ecosystems on adjacent crops may be expected also in terms of pests harmful to crops and which may take advantage of these new habitats. Many other species of leafhoppers and planthoppers are suspected to be vectors of phytoplasmas to grapevine, and many of them are actually vectors under laboratory conditions. The presence of a non-food crop close to vineyards must therefore be evaluated also concerning this aspect. The aim of this research was to investigate into communities of leafhoppers and planthoppers living on foraging strips and that may switch onto grapevines and cause phytoplasma transmission.

Materials and methods

Study area and strip composition

The research was conducted over a three-year period (2012-2014) in three sites placed in vine-growing areas of Piedmont (NW Italy), and belonging to the Operation Pollinator® Network (www.operationpollinator.com).

The first site was in the district of Canelli (AT), and was surrounded by a corn/wheat rotation crop, a hazelnut orchard, a meadow, and a vineyard of cv Moscato: the strip was seeded perpendicularly to the vineyard and parallel to the corn crop (figure 1A). The second was in La Morra (CN), and was settled between two vineyards of cv Dolcetto, whereas no other crops were present in the surroundings: the strip was settled parallel to the first and before the second vineyard (figure 1B). The third was in Serralunga d'Alba (CN) and was close to a vineyard of cv Chardonnay: in this case there were two strips, settled N and W of the vineyard, respectively (figure 1C). In all of the sites, both 16SrV and 16SrXII phytoplasmas were identified in grapevines, with an approximate proportion of 3:1. The main features of the experimental sites are listed in table 1.

Flowering strips were up to 70 m long, and 5-6 m wide. Two-thirds of the strip length was seeded with a mix of the following species: *Medicago sativa* L. (alfalfa), *Lotus corniculatus* L., *Hedysadrum coronarium* L., *Onobrychis viciifolia* Scopoli, *Trifolium pratense* L. (red clover), and *Trifolium repens* L. (white clover). The remaining part was seeded with rape, *Brassica napus* L. Legumes were seeded in 2012 during spring (March-April), and mowed twice a year, generally at the end of June and at the end of September-beginning of October; rape was seeded in autumn (September-October) and mowed in June.

The adjacent vineyards were subject to standard pest management, and in particular to mandatory insecticidal sprays against *S. titanus*, within the frame of FD control, with two sprays: the first with Thiamethoxam (end of June) and the second with organophosphates (end of July).

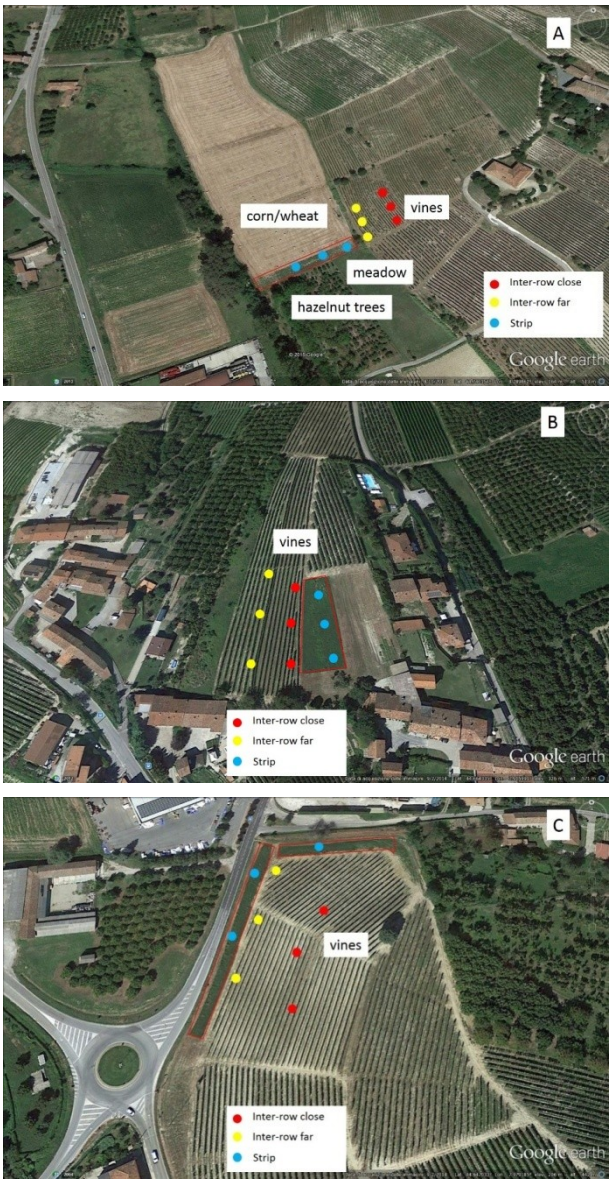

Figure 1. Map of the experimental sites: Canelli (A), La Morra (B) and Serralunga d'Alba (C).

Field collections and surveys

Insects were collected using a sweep net with a diameter of 40 cm. The sweep-net method was chosen because it allows an unbiased collection of specimens, regardless of flight activity, morphs (e.g. brachypterous morphs), gender, etc. of the different species inhabiting the strip and the surrounding environments (Alma *et al*., 2015). The samples were taken from the strip, in the vineyard inter-rows, and to some extent in adjacent plots. Inside the vineyard, two positions were sampled: close to (distance 3 m circa) and far from (minimum distance 30 m) the strip crop. Within different positions,

Table 1. Main features of experimental sites. BS: bare soil; DC: dicothyledons; MC: monocothyledons.

Exp. site	Canelli	La Morra	Serralunga d'Alba
Year sampled	2012-2013	2012-2014	2012-2014
Grapevine cv	Moscato	Dolcetto	Chardonnay
Soil texture	Medium/silty	Medium/silty/loam	Medium
Inter-row composition	BS, DC	DC, MC	BS, DC
Surrounding crops	Corn/wheat Hazelnut trees Meadow	Meadow	None
Grapevine yellows' symptoms	5%	2%	9%

three blocks of 9 m^2 (3 × 3 m) each were identified. At each sampling date, each block was swept following a scheme with 4 replicates of 20 sweepings each, consisting in a strike and a counter-strike on vegetation. Captured insects were collected with a mouth aspirator, placed into glass tubes (8 × 20 mm), and killed by freezing them. The sampling started at the middle of May and was repeated at 20-30 days intervals until the beginning of October. Only one sampling per season was made on rape, which was mowed in June; since no (or very negligible numbers of) specimens were captured, these data were excluded from analyses.

The ground cover of the strips was evaluated visually at the end of June, before grass cutting, by observing three replicates of a square (100 × 100 cm). Three categories of soil coverage were considered: OP seed mixture, weeds, and bare soil. The different species of dicothyledon weeds that developed on the strip were also reported; we omitted to identify Gramineae as they are quite difficult to determine. Plant species were determined according to Pignatti (1982).

Laboratory analyses

Insect specimens were identified in the laboratory using morphological features, up to the genus level. Identification of adults to species (or species complex) level was made either through morphological features or following the extraction of male genitalia, according to Holzinger *et al.* (2003) and Biedermann and Niedringhaus (2004). When doubtful, the identification of females was left at genus level.

Molecular analyses were conducted on specimens belonging to acknowledged or suspected vectors of group 16SrV and 16SrXII phytoplasmas. For each species, at least 20% of the total specimens captured was tested. Total DNA from single insects was extracted following a CTAB method as described by Bertin and Bosco (2013) and resuspended in 100 µl TE buffer. Phytoplasma detection was carried out through nested PCR with the universal phytoplasma primer pairs P1/P7 and R16F2n/R16R2 (Gundersen and Lee, 1996). Then, species already known to be vectors or suspected vectors of phytoplasmas to grapevine were analyzed also with specific primers. In particular, *Agallia consobrina* Curtis, *Anaceratagallia* spp., *D. europaea*, *Euscelis incisus* (Kirschbaum), *H. obsoletus* and *Reptalus* spp. were tested in SYBER Green RealTime PCR with the primers StolFw/StolRev (Galetto *et al*., 2005) for '*Ca.* Phytoplasma solani', *D. europaea*, *Euscelidius variegatus* (Kirschbaum) and *E. incisus* were tested in SYBER Green RealTime PCR with the 16SrV-group specific primers fAY/rEY (Marcone *et al*., 1996); while *Neoaliturus fenestratus* (Heirrich-Schaeffer) was tested in direct PCR with 16SrIX-group semi-specific primers ALWF2/ALWR2 (Abou-Jawdah *et al*., 2003).

Statistical analyses

A one-way ANOVA followed by a Tukey test was performed on the data of single vector species captures in different positions (within the strip, inter-row close to the strip, inter-row far from the strip, and adjacent plots) in different sites. The analysis was run pooling captures from different years of sampling and keeping sites separated. Data were square root transformed to meet the assumption of normality and variance homogeneity. ANOVA was calculated with the SPSS 21.0® statistical software.

Results

Ground cover of flowering strips

The rate of ground cover due to seed mixture and weeds changed in different years and sites (figure 2). Generally, weeds increased over time due to a decrease in seeded species. The best rate of cover with the seed

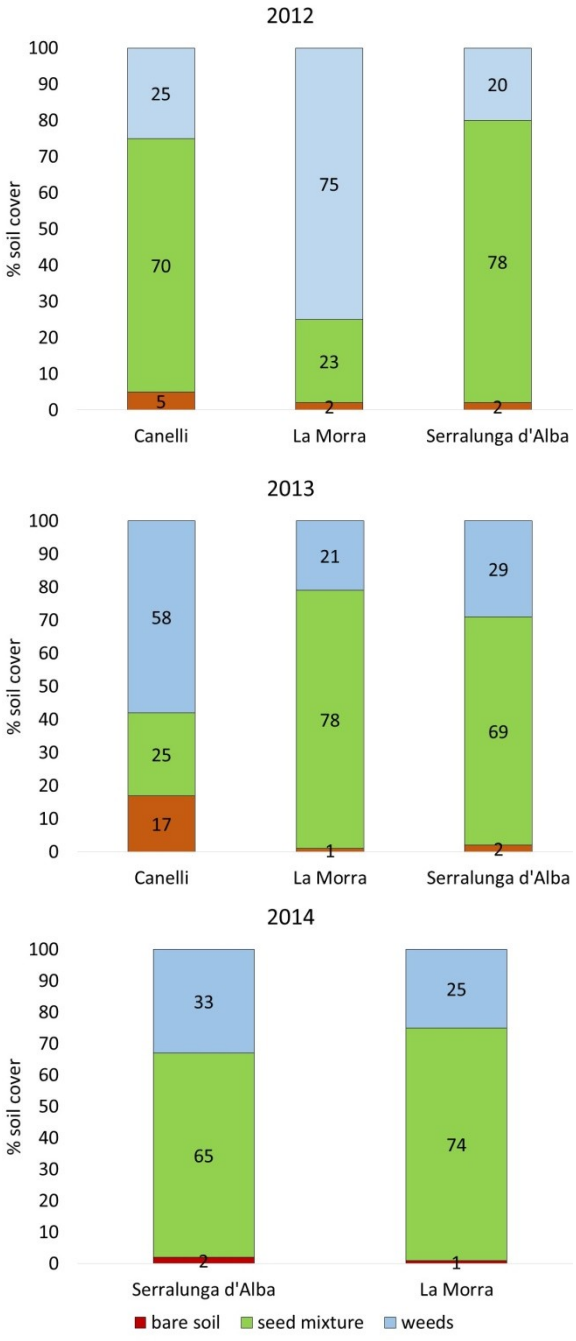

Figure 2. Percentage of ground cover of strip crops in different sites and years.

Table 2. Weeds observed within the strip in the different sites.

Species	Canelli	La Morra	Serralunga d'Alba
Achillea millefolium L.	+		
Amaranthus retroflexus L.	+		+
Brassica nigra (L.)	+		+
Brassica oleracea L.		+	
Centaurea spp.	+		+
Chenopodium album L.	+		+
Cichorium intybus L.		+	+
Cirsium arvense (L.)	+	+	+
Convolvulus arvensis L.		+	
Crepis spp.		+	
grasses	+	+	+
Hieracium spp.			+
Knautia arvensis (L.)	+		
Leontodon spp.	+		
Leucanthemum vulgare (Vaill.)	+		
Lythrum salicaria L.	+		
Melilotus officinalis (L.)	+		+
Mentha piperita L.	+		+
Papaver rhoeas L.	+	+	
Picris hieracioides L.	+	+	+
Plantago lanceolata L.	+		
Polygonum persicaria L.	+		
Potentilla reptans L.	+		
Pulicaria spp.	+		
Rumex spp.	+		
Salvia pratensis L.		+	
Scabiosa spp.	+		
Taraxacum officinale Weber			+
Trifolium repens L.	+	+	
Urtica dioica L.	+		

mixture was noted in Serralunga d'Alba (65-78%). La Morra was badly covered during the first year of seeding, probably because of little rain during spring, and was seeded again in 2013 achieving a better result. Canelli had a good performance during the first year, but went down badly in the second and last year, and had the highest rate in bare soil.

Among the species seeded, alfalfa (*M. sativa*) and *O. vicifolia* were the most performant, whereas the others did not emerge in a considerable number. Concerning weeds, some important species such as *Convolvolus arvensis* L., *U. dioica*, *Amaranthus retroflexus* L., and *Picris hieracioides* L. (table 2), which are host plants for vectors and in some cases a source of phytoplasma inoculum, were identified.

Leafhopper captures and molecular analyses

On the whole, 1099 adult specimens were collected and 62 species were identified. The most abundant were *Psammotettix alienus* (Dalbholm) (288 specimens, 26% of the total captured) and *Philaenus spumarius* (L.) (168, 15%). Other numerous species, representing 4-5% of the total captured each, were *Laodelphax striatellus* (Fallen) (48), *N. fenestratus* (50), *Empoasca vitis* (Goethe) (48), and *D. europaea* (48). Among the species captured, 8 are acknowledged as vectors of 16SrV and/or 16SrXII phytoplasmas, the most abundant being *D. europaea* (48), *E. incisus* (44) and *E. variegatus* (36), whereas 9 are considered potential vectors, the most abundant being *N. fenestratus* (50). Data are shown in table 3.

On the whole, 157 specimens, belonging to 17 different species, were subject to DNA extraction and PCR for phytoplasma identification. Phytoplasmas of the 16SrV group were detected in three specimens of *D. europaea*, caught on the strips in the sites of La Morra (1 specimen) and Serralunga d'Alba (2 specimens) during 2014. Group 16SrIX phytoplasmas were detected in one specimen of *N. fenestratus* captured on the strip in Canelli during 2013. None of the other specimens showed positive signals (table 3).

Differences between strips and vineyard

Differences in abundance on different positions were calculated for the following: *D. europaea*, *E. variegatus*, *E. incisus*, *N. fenestratus*, *A. venosa*. In the site of Canelli, both *D. europaea* and *N. fenestratus* were more abundant on the strips, whereas *E. variegatus* showed no differences at all and *E. incisus* was more abundant in the surroundings. On the other hand, in the site of Serralunga d'Alba *N. fenestratus* was found only in the vineyard inter-row whereas *A. venosa* showed no differences. Data are illustrated in table 4.

Table 3. Captures and molecular analyses of species known or suspected vectors of phytoplasmas of groups 16SrV and XII (according to Alma *et al*., 2015, unless differently specified). CAN: Canelli; MOR: La Morra; SRL: Serralunga d'Alba.

	Species	16Sr	Captured				PCR				
			CAN	MOR	SRL	Total	Tested	+F2n/R2	16SrV	16SrIX	16Sr XII-A
Acknowledged vectors	*Euscelidius variegatus* (Kirschbaum)	V	28	5	3	36	19	0	0	-	-
	Euscelis incisus (Kirschbaum)	XII	42	2	0	44	9	0	-	-	0
	Anaceratagallia ribauti (Ossiannlison)	XII	3	4	12	19	17	0	-	-	0
	Aphrodes bicincta (Schrank)	XII	1	0	6	7	6	0	-	-	0
	Hyalesthes obsoletus Signoret	XII	0	1	0	1	1	0	-	-	0
	Reptalus quinquecostatus (Dufour)	XII	0	1	0	1	1	0	-	-	0
	Reptalus spp.[(1)]	XII	2	3	4	9	2	0	-	-	0
	Dictyophara europaea (L.)	V; (XII)[(2)]	32	3	13	48	10	0	3	-	0
Suspected vectors	*Anoplotettix fuscovenosus* (Ferrari)	V	7	4	16	27	6	0	0	-	0
	Agallia consobrina Curtis	V	1	2	4	7	4	0	-	-	0
	Anaceratagallia laevis (Ribaut)	XII	7	4	11	22	8	0	-	-	0
	Anaceratagallia venosa (Fourcroy)	XII[(3)]	4	6	23	33	16	0	-	-	0
	Anaceratagallia spp.	XII[(4)]	3	6	5	14	12	0	-	-	0
	Aphrodes makarovi Zachvaktin	V[(5)]; XII[(6)]	2	0	4	6	3	0	-	-	0
	Hyalesthes scotti Ferrari	XII[(7)]	14	0	0	14	3	0	-	-	0
	Reptalus cuspidatus (Fieber)	XII	0	0	3	3	1	0	-	-	0
	Neoaliturus fenestratus (Heirrich-Schaffer)	XII	24	6	20	50	39	0	-	1	0

(1) Females, either *R. quinquecostatus* or *R. panzeri*. *R. melanochaetus* in Piedmont is quite rare (Luca Picciau, personal communication).
(2) Suspected vector of 16SrXII.
(3) Drobnjaković *et al*., 2010.
(4) Either *A. venosa* or *A. ribauti*.
(5) Positive after microinjection (Bressan *et al*., 2006) but no transmission.
(6) Positive after field collection (Sanna *et al*., 2016).
(7) Skorić, 2013.

Table 4. Abundance of leafhoppers (mean ± s.d. per replication) on different positions in different experimental sites. Different letters indicate significant differences between positions (ANOVA + Tukey test, P<0.05).

Total species	Position	Canelli	Serralunga d'Alba
Dictyopara europaea (L.)	inter-row (far)	1 ± 1 a	-
	inter-row (close)	1.7 ± 1.5 a	-
	strip	7.3 ± 3.5 bc	-
	surrounding	0.7 ± 0.6 a	-
Euscelidius variegatus (Kirschbaum)	inter-row (far)	1.7 ± 0.6 a	-
	inter-row (close)	0.3 ± 0.6 a	-
	strip	4.3 ± 4 a	-
	surrounding	3 ± 1 a	-
Euscelis incisus (Kirschbaum)	inter-row (far)	0 ± 0 a	-
	inter-row (close)	0 ± 0 a	-
	strip	3.3 ± 1.2 a	-
	surrounding	10.7 ± 2.3 b	-
Neoaliturus fenestratus (Heirrich-Schaeffer)	inter-row (far)	0 ± 0 a	2.3 ± 1.2 a
	inter-row (close)	0 ± 0 a	4.3 ± 0.6 a
	strip	8 ± 1 b	0.0 ± 0.0 b
	surrounding	0 ± 0 a	-
Anaceratagallia venosa (Fourcroy)	inter-row (far)	-	2.0 ± 1.0 a
	inter-row (close)	-	3.6 ± 6.4 a
	strip	-	2.0 ± 3.5 a

Discussion

In the present research, 17 species of leafhoppers or planthoppers known to be vectors (7) or potential vectors (10) of grapevine phytoplasmas were captured either on the flowering strip or in the vineyard inter-row. The presence and the abundance of a certain species depended mainly on the botanical composition of the crop considered, including weeds. In many cases, no significant differences between captures in the vineyard inter-row were noted with respect to the distance from the flowering strip: it is likely therefore that no massive movement from the strip to the vineyard and vice-versa occurs. However, since different vectors have different ecological needs, the results concerning each species will be discussed separately.

One of the most abundant species was *N. fenestratus,* which lives mainly on Asteraceae (Landi *et al.*, 2013; Minuz *et al.*, 2013) but may also feed on grapevine as an adult (Bosco *et al.*, 1997; Landi *et al.*, 2013). High captures were observed on the strip in Canelli, where a high presence of *P. hieracioides* was noted, and also in the inter-rows in Serralunga d'Alba, where other weeds of the same family were abundant. In Europe, *N. fenestratus* is a vector of group 16SrII-E phytoplasmas to *P. hieracioides* (Mitrović *et al.*, 2012), and it is also associated to phytoplasmas of subgroups 16SrI-B and -C (Landi *et al.*, 2013), and group 16SrXII (Orenstein *et al.*, 2003); in Iran it is an acknowledged vector of 16SrIX phytoplasmas causing lettuce and wild lettuce phyllodies (Salehi *et al.*, 2007); in the present research, it was found positive to 16SrIX-group phytoplasmas, which however do not seem harmful to grapevine, although they were recently identified in vine leaves in Turkey (Bianco, 2013). Nevertheless, since *N. fenestratus* may be associated to Stolbur as well, a big population of this leafhopper close to vine growing areas may become a problem, although its vector ability has not been proved yet.

D. europaea is another species of a certain concern: in the present research it was found positive to 16SrV, and it can transmit this phytoplasma from infected *C. vitalba* to grapevine (Filippin *et al*., 2009). It was also associated to Stolbur phytoplasmas in Serbia (Cvrković *et al*., 2011), but this aspect was not confirmed here. However, at present, it may be considered the most threatening species settled on the strips: in fact, *D. europaea* is common in xerothermic habitats with isolated grass patches and portions of bare soil, used for laying eggs (Nickel and Remane, 2002), and may move on shrubs such as vine (Lessio and Alma, 2008) and *C. vitalba* (Krstić *et al*., 2016) during the dry season. This species which is polyphagous and feeds on many weeds including pigweed, *A. retroflexus* (Lessio and Alma, 2008; Krstić *et al.*, 2016) may have taken advantage of the presence of plant patches with bare soil in the site of Canelli, especially during the second year of study. Moreover, 3 specimens out of 10 were positive to 16SrV phytoplasmas. Such an infection rate (30%) is quite high if compared to data obtained from North-Eastern Italy and Serbia, where positive individuals were 3-4% of the total collected (Filippin *et al*., 2009). On the other hand, it is consistent with (unpublished) data from specimens (N≈40) which we have collected from other vine growing areas of Piedmont, especially in the Asti Province. For these reasons, *D. europaea* may be considered as a harmful species, and therefore foraging crops along vineyards must be correctly managed in order to avoid this species to settle.

The species of the subfamily Agallinae were quite well represented, although not in very high numbers: association with 16SrXII phytoplasmas have been reported for the following: *A. laevis* (Orenstein *et al*., 2003; Drobnjaković *et al.*, 2010), *A. venosa*

(Drobnjaković *et al.*, 2010) and *A. ribauti* (Riedle-Bauer *et al*., 2008; Drobnjaković *et al.*, 2010), the last one being also a vector under laboratory conditions (Riedle-Bauer *et al.*, 2008). On the other hand, phytoplasmas of group 16SrI were detected in *A. laevis* (Drobnjaković *et al*., 2010). However, Agallinae are mainly associated with weeds (Nickel and Remane, 2002) and do not seem up to now harmful to grapevines. *A. venosa* is reported on *L. corniculatus* in Germany (Nickel and Remane, 2002), which is a part of the seeding mixture.

The species of the subfamily Aphrodinae were not caught in abundance. *Aphrodes makarovi* Zachvaktin is known to harbour 16SrXII phytoplasmas (Sanna *et al*., 2016), whereas it was not able to transmit 16SrV phytoplasmas after micro-injections (Bressan *et al.*, 2006). Given also the few specimens captured on the strips, species in this subfamily may not be considered a threat to grapevine.

The presence of *H. obsoletus* and *R. panzeri* - two acknowledged vectors of '*Ca.* Phytoplasma solani' (16SrXII) to grapevines (Lessio *et al.*, 2007; Cvrković *et al*., 2014) - was quite low. *H. obsoletus* relies on weeds such as stinging nettle, *U. dioica* (Lessio *et al*., 2007), bindweed (*C. arvensis*) (Weber and Maixner, 1998), and lavender (Johannesen *et al*., 2008). *U. dioica* is not likely to become invasive in other plant communities, mainly because of its light needs (it grows better under half-shade conditions) (Olsen, 1921). Therefore, in the vineyard agro-ecosystem it is typical of field margins close to ditches and/or under tree vegetation (Mori *et al*., 2015), and is not likely to become invasive within strip crops. On the other hand, bindweed is common in vineyard inter-rows and has a wide distribution with little ecological needs (Lososova *et al*., 2003; Jurado-Exposito *et al*., 2004): its presence on the flowering strips should be therefore considered a risk factor. Without its host plants, *H. obsoletus* does not become a threat: similar results were obtained by Burgio *et al.* (2016), who did not find many specimens on sweet alyssum [*Lobularia maritima* (L.) Desv.]. Concerning *R. panzeri*, it is hosted mainly by trees and shrubs (Picciau *et al*., 2008), and does not seem to build up great populations in meadows (and therefore strip crops). Other *Reptalus* species associated to 16SrXII phytoplasmas are *R. cuspidatus* (Skorić, 2013), not acknowledged as a vector yet, and *R. quinquecostatus*, which is able to transmit to artificial feeding medium (Pinzauti *et al*., 2008), and to periwinkle (Chuche *et al*., 2016). Since *R. quinquecostatus* and *R. panzeri* females could not be distinguished by observing morphological features (along with *R. melanochaetus*) (Bertin *et al*., 2010), the presence of *R. panzeri* may have been underestimated. Anyway, very few individuals of *Reptalus* spp. were captured and should not be therefore deemed important in this context.

E. variegatus showed no differences between positions, probably because its high degree of polyphagia (Nickel and Remane, 2002) that has permitted its development on a wide range of host plants either in the strip or in the vineyard inter-row and in the surroundings. *E. incisus*, which lives mainly on Gramineae and Fabaceae (Nickel and Remane, 2002), was found in high number only in the surrounding areas, especially on Gramineae (e.g. meadows and winter cereals). Both species are not usually caught in high numbers on the grapevine canopy (Bosco *et al*., 1997), and their presence is therefore not considered a threat.

Finally, *A. fuscovenosus* was found in relatively high numbers on the strips: the results obtained are in accordance with the fact that this species relies on trees and shrubs for egg-laying, but develops and feeds on herbaceous plants (Alma, 1995). This species is able to transmit 16SrV phytoplasmas under laboratory conditions, following micro-injections on adults (Bressan *et al*., 2006). However, its vector ability (especially concerning acquisition on host plants) has not been proved yet.

S. titanus was never found neither on the strips nor on weeds in the vineyard: this is not surprising, since it is a grapevine specialist (Chuche *et al*., 2014; Alma *et al*., 2015). This leafhopper is abundant on stands of wild grapevines in woods, which act as reservoirs and pathways when adults disperse moving into vineyards (Pavan *et al*., 2012; Lessio *et al*., 2014). Breaking its ecological corridors may result therefore in a decreased spread capability (Alma *et al*., 2015). For this reason, the application of agroecology by the use of strip crops to replace stands of wild vines may be applied in an area-wide IPM perspective, and this aspect calls for further investigation. At present, the settlement of foraging strips in the proximity of vineyards should not be considered a risk for the transmission of phytoplasmas to grapevine, provided strips themselves are correctly managed avoiding the development of dangerous weeds hosts of potentially harmful leafhoppers.

Acknowledgements

We are grateful to Federica Tota for help in molecular analyses of specimens, to Chiara Morone for providing data on the spread of FD and LN, to Syngenta Italia SpA for providing Operation Pollinator® seed mixture and operational sites and to all the farmers that provided suitable sites for the experiments.

References

ABOU-JAWDAH Y., DAKHIL H., EL-MEHTAR S., LEE I. M., 2003.- Almond witches'-broom phytoplasma: a potential threat to almond, peach, and nectarine.- *Canadian Journal of Plant Pathology*, 25: 28-32.

ALMA A., 1995.- Ricerche bio-etologiche su *Anoplotettix fuscovenosus* (Ferrari) (Cicadellidae Deltocephalinae).- *Bollettino di Zoologia Agraria e Bachicoltura*, 27: 45-52.

ALMA A., ARNÒ C., ARZONE A., VIDANO C., 1988.- New biological reports on Auchenorrhyncha in vineyards, pp. 509-516.- In: *Proceedings of the 6th Auchenorrhyncha meeting* (VIDANO C., ARZONE A., Eds), Turin, Italy, 7-11 September 1987. CNR, Turin, Italy.

ALMA A., TEDESCHI R., LESSIO F., PICCIAU L., GONELLA E., FERRACINI C., 2015.- Insect vectors of plant pathogenic Mollicutes in the European-Mediterranean region.- *Phytopathogenic Mollicutes*, 5: 53-73.

ALTIERI M. A., 2002.- Agroecology: the science of natural resource management for poor farmers in marginal environments.- *Agriculture, Ecosystems and Environment*, 93: 1-24.

BERTIN S., BOSCO D., 2013.- Molecular identification of phytoplasma vector species, pp. 87-108. In: *Phytoplasma*: *methods and protocols, methods in molecular biology* vol. 938 (DICKINSON M., HODGETTS J., Eds).- Clifton, NJ, USA.

BERTIN S., PICCIAU L., ACS Z., ALMA A., BOSCO D., 2010.- Molecular differentiation of four *Reptalus* species (Hemiptera: Cixiidae).- *Bulletin of Entomological Research*, 100: 551-558.

BIANCO P. A., 2013.- Phytoplasmas and phytoplasma vectors in Greece, Israel, Italy and Turkey, pp. 17-18. In: *COST Action FA0807 Final Meeting: integrated management of phytoplasma epidemics in different crop systems* (BERTACCINI A., Ed.), Carcavelos, Lisbon, Portugal, 30 September-1 October 2013. *Alma Mater Studiorum* University of Bologna, DipSA, Plant Pathology, Bologna, Italy.

BIEDERMANN R., NIEDRINGHAUS R., 2004.- Die Zikaden Deutschland.- Wissenschaftlich Akademischer Buchvertrieb, Germany.

BOSCO D., ALMA A., ARZONE A., 1997.- Studies on population dynamics and spatial distribution of leafhoppers in vineyards (Homoptera: Cicadellidae).- *Annals of Applied Biology*, 130: 1-11.

BRESSAN A., CLAIR D., SEMETEY O., BOUDON-PADIEU E., 2006.- Insect injection and artificial feeding bioassays to test the vector specificity of flavescence dorée phytoplasma.- *Phytopathology*, 96: 790-796.

BURGIO G., FERRARI R., POZZATI M., BORIANI L., 2004.- The role of ecological compensation areas on predator populations: an analysis on biodiversity and phenology of Coccinellidae (Coleoptera) on non-crop plants within hedgerows in Northern Italy.- *Bulletin of Insectology*, 57: 1-10.

BURGIO G., FERRARI R., BORIANI L., POZZATI M., VAN LENTEREN J., 2006.- The role of ecological infrastructures on Coccinellidae (Coleoptera) and other predators in weedy field margins within northern Italy agroecosystems.- *Bulletin of Insectology*, 59: 59-67.

BURGIO G., MARCHESINI E., REGGIANI N., MONTEPAONE G., SCHIATTI P., SOMMAGGIO D., 2016.- Habitat management of organic vineyard in Northern Italy: the role of cover plants management on arthropod functional biodiversity.- *Bulletin of Entomological Research*, 106: 759-768.

CHUCHE J., THIÉRY D., 2014.- Biology and ecology of the Flavescence dorée vector *Scaphoideus titanus*: a review.- *Agronomy for sustainable development*, 34: 381-403.

CHUCHE J., DANET J. L., SALAR P., FOISSAC X., THIERY D., 2016.- Transmission of '*Candidatus* Phytoplasma solani' by *Reptalus quinquecostatus* (Hemiptera: Cixiidae).- *Annals of Applied Biology*, 169: 214-223.

CVRKOVIĆ T., JOVIĆ J., MITROVIĆ M., KRSTIĆ O., KRNJAJIĆ S., TOŠEVSKI I., 2011.- Potential new hemipteran vectors of stolbur phytoplasma in Serbian vineyards.- *Bulletin of Insectology*, 64: 129-130.

CVRKOVIĆ T., JOVIĆ J., MITROVIĆ M., KRSTIĆ O., TOŠEVSKI I., 2014.- Experimental and molecular evidence of *Reptalus panzeri* as a natural vector of bois noir.- *Plant Pathology*, 63: 42-53.

DROBNJAKOVIĆ T., PERIĆ P., MARCIĆ D., PICCIAU L., ALMA A., MITROVIĆ J., DUDUK B., BERTACCINI A., 2010.- Leafhoppers and cixiids in phytoplasma-infected carrot fields: species composition and potential phytoplasma vectors.- *Pesticidi i phytomedicina*, 25: 311-318.

FILIPPIN L., JOVIĆ J., CVRKOVIĆ T., FORTE V., CLAIR D., TOŠEVSKI I., BOUDON-PADIEU E., BORGO M., ANGELINI E., 2009.- Molecular characteristics of phytoplasmas associated with Flavescence doree in clematis and grapevine and preliminary results on the role of *Dictyophara europaea* as a vector.- *Plant Pathology*, 58: 826-837.

GALETTO L., BOSCO D., MARZACHÌ C., 2005.- Universal and group-specific real-time PCR diagnosis of flavescence dorée (16Sr-V), bois noir (16Sr-XII) and apple proliferation (16Sr-X) phytoplasmas from field-collected plant hosts and insect vectors.- *Annals of Applied Biology*, 147: 191-201.

GUNDERSEN D., LEE I. M., 1996.- Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs.- *Phytopathologia mediterranea*, 35: 144-151.

HOLZINGER W. E., KAMMERLANDER I., NICKEL H., 2003.- The Auchenorrhyncha of Central Europe.- Brill NV, Leiden, The Netherlands.

JOHANNESEN J., LUX B., MICHEL K., SEITZ A., MAIXNER M., 2008.- Invasion biology and host specificity of the grapevine yellows disease vector *Hyalesthes obsoletus* in Europe.- *Entomologia Experimentalis et Applicata,* 126: 217-227.

JURADO-EXPOSITO A., LOPEZ-GRANADOS F., GONZALEZ-ANDUJAR J. L., GARCIA-TORRES L., 2004.- Spatial and temporal analysis of *Convolvulus arvensis* L. populations over four growing seasons.- *European Journal of Agronomy*, 21: 287-296.

KEVAN P. G., 1999.- Pollinators as bioindicators of the state of the environment: activity and diversity.- *Agriculture, Ecosystems and Environment*, 74: 373-393.

KRSTIĆ O., CVRKOVIĆ T., MITROVIĆ M., TOŠEVSKI I., JOVIĆ J., 2016.- *Dictyophara europaea* (Hemiptera: Fulgoromorpha: Dictyopharidae): description of immatures, biology and host plant associations.- *Bulletin of Entomological Research*, 106: 395-405.

LANDI L., ISIDORO N., RIOLO P., 2013.- Natural phytoplasma infection of four phloem-feeding auchenorrhyncha across vineyard agroecosystems in Central-Eastern Italy.- *Journal of Economic Entomology*, 106: 604-613.

LESSIO F., ALMA A., 2008.- Host plants and seasonal presence of *Dictyophara europaea* in the vineyard agro-ecosystem.- *Bulletin of Insectology*, 61: 199-200.

LESSIO F., TEDESCHI R., ALMA A., 2007.- Population dynamics, host plants and infection rate with Stolbur phytoplasma of *Hyalesthes obsoletus* Signoret in north-western Italy.- *Journal of Plant Pathology*, 89: 97-102.

LESSIO F., TOTA F., ALMA A., 2014.- Tracking the dispersion of *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae) from wild to cultivated grapevine: use of a novel mark-capture technique.- *Bulletin of Entomological Research*, 104: 432-443.

LESSIO F., PICCIAU L., GONELLA E., MANDRIOLI M., TOTA F., ALMA A., 2016.- The mosaic leafhopper *Orientus ishidae*: host plants, spatial distribution, infectivity, and transmission of 16SrV phytoplasmas to vines.- *Bulletin of Insectology*, 69: 277-289.

LOSOSOVA Z., DANIHELKA J., CHYTRY M., 2003.- Seasonal dynamics and diversity of weed vegetation in tilled and mulched vineyards.- *Biologia Bratislava*, 58: 49-57.

MARCONE C., RAGOZZINO A., SCHNEIDER B., LAUER U., SMART C. D., SEEMULLER E., 1996.- Genetic characterization and classification of two phytoplasmas associated with spartium witches'-broom disease.- *Plant Disease*, 80: 365-371.

MINUZ R. L., ISIDORO N., CASAVECCHIA S., BURGIO G., RIOLO P., 2013.- Sex-dispersal differences of four phloem-feeding vectors and their relationship to wild-plant abundance in vineyard agroecosystems.- *Journal of Economic Entomology*, 106: 2296-2309.

MITROVIĆ M., JOVIĆ J., CVRKOVIĆ T., KRSTIĆ O., TRKULJA N., TOŠEVSKI I., 2012.- Characterisation of a 16SrII phytoplasma strain associated with bushy stunt of hawkweed oxtongue (*Picris hieracioides*) in south-eastern Serbia and the role of the leafhopper *Neoaliturus fenestratus* (Deltocephalinae) as a natural vector.- *European Journal of Plant Pathology*, 134: 647-660.

MORI N., POZZEBON A., DUSO C., REGGIANI N., PAVAN F., 2015.- Vineyard colonization by *Hyalesthes obsoletus* (Hemiptera: Cixiidae) induced by stinging nettle cut along surrounding ditches.- *Journal of Economic Entomology*, 109: 49-56.

NICHOLLS C. I., ALTIERI M. A., 2013.- Plant biodiversity enhances bees and other insect pollinators in agroecosystems. A review.- *Agriculture for Sustainable Development*, 33: 257-274.

NICKEL H., REMANE R., 2002.- Check list of the planthoppers and leafhoppers of Germany, with notes on food plants, diet width, life cycles, geographic range and conservation status (Hemiptera, Fulgoromorpha and Cicadomorpha).- *Beiträge zur Zikadenkunde*, 5: 27-64.

OLSEN C., 1921.- The ecology of *Urtica dioica*.- *Journal of Ecology*, 9: 1-18.

ORENSTEIN S., ZAHAVI T., NESTEL D., SHARON R., BARKALIFA M., WEINTRAUB P. G., 2003.- Spatial dispersion patterns of potential leafhopper and planthopper (Homoptera) vectors of phytoplasma in wine vineyards.- *Annals of Applied Biology*, 142: 341-348.

PAVAN F., MORI N., BIGOT G., ZANDIGIACOMO P., 2012.- Border effect in spatial distribution of Flavescence dorée affected grapevines and outside source of *Scaphoideus titanus* vectors.- *Bulletin of Insectology*, 65: 281-290.

PICCIAU L., LESSIO F., ALMA A., 2008.- Preliminary data on the Cixiid fauna of the vineyard agro-ecosystem in Piedmont (North-Western Italy).- *Bulletin of Insectology*, 61: 197-198.

PIGNATTI S., 1982.- *Flora d'Italia*.- Edagricole, Bologna, Italy.

PINZAUTI F., TRIVELLONE V., BAGNOLI B., 2008.- Ability of *Reptalus quinquecostatus* (Hemiptera: Cixiidae) to inoculate stolbur phytoplasma to artificial feeding medium.- *Annals of Applied Biology*, 153: 299-305.

PONTI L., RICCI C., VERONESI F., TORRICELLI R., 2005.- Natural hedges as an element of functional biodiversity in agroecosystems: the case of a Central Italy vineyard.- *Bulletin of Insectology*, 58: 19-23.

RIEDLE-BAUER M., SARA A., REGNER F., 2008.- Transmission of a stolbur phytoplasma by the Agalliinae leafhopper *Anaceratagallia ribauti* (Hemiptera, Auchenorrhyncha, Cicadellidae).- *Journal of Phytopathology*, 156: 687-690.

SALEHI M., IZADPANAH K., NEJAT N., SIAMPOUR M., 2007.- Partial characterization of phytoplasmas associated with lettuce and wild lettuce phyllodies in Iran.- *Plant Pathology*, 56: 669-676.

SANNA F., QUAGLINO F., FILISETTI S., CASATI P., FACCINCANI M., BIANCO P. A., MORI N., 2016.- Preliminary results on putative vectors of '*Candidatus* Phytoplasma solani' in Bois noir-affected vineyards in Franciacorta (Lombardy region, North Italy), pp. 28-31. In: *4th European Bois noir workshop* (EDER R., RIEDLE-BAUER M., ROSNER F. G., Eds), Klosterneuburg, Austria, 9-11 March 2016. Federal College and Research Insitute for Viticulture and Pomology, Klosterneuburg, Austria.

SKORIĆ D., 2013.- Overview of the phytoplasma and vector research in Austria, Croatia and Slovenia since 2009, pp. 10-11. In: *COST Action FA0807 Final Meeting: integrated management of phytoplasma epidemics in different crop systems* (BERTACCINI A., Ed.), Carcavelos, Lisbon, Portugal, 30 September-1 October 2013. *Alma Mater Studiorum* University of Bologna, DipSA, Plant Pathology, Bologna, Italy.

WEBER A., MAIXNER M., 1998.- Survey of populations of the planthopper *Hyalesthes obsoletus* Sign. (Auchenorrhyncha, Cixiidae) for infection with the phytoplasma causing grapevine yellows in Germany.- *Journal of Applied Entomology*, 122: 375-381.

Authors' addresses: Alberto ALMA (corresponding author, e-mail: alberto.alma@unito.it), Federico LESSIO, Rosemarie TEDESCHI, Dipartimento di Scienze Agrarie, Forestali e Alimentari, University of Torino, largo P. Braccini 2, 10095 Grugliasco (TO), Italy.

Received January 24, 2017. Accepted June 12, 2017.