

Population dynamics and dispersal of *Scaphoideus titanus* from recently recorded infested areas in central-eastern Italy

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Abstract

We investigated the presence of the leafhopper *Scaphoideus titanus* Ball in the Marche region, central Italy, during a 10-year survey. Furthermore, from 2009 to 2011, yellow sticky traps were deployed to investigate the population dynamics. Taylor's power law and distance-weighted least-squares contour maps were used to determine the leafhopper distribution within vine fields. Polymerase chain reaction was used to detect Flavescence dorée (FD) phytoplasma in insect bodies. The leafhopper was first recorded in 2007 in a commercial nursery of scion mother vines (1 specimen) in southern Pesaro-Urbino province and in 2009 in a commercial vineyard (1 specimen; the FD focus) in north-eastern Pesaro-Urbino. Adults (total, 301) were recorded from end of June to end of September, 2009-2011, with a shifted flight activity observed for females. Male captures were more abundant than females (M:F = 1.71; $p < 0.001$). In the site including the FD focus, insecticide treatments contributed to very low leafhopper population levels: only a few individuals ($n = 4$) were caught, and no phytoplasma were detected in their bodies. Comparisons of trapping data indicate significant increase in captures from 2009 to 2011 where correct pest management was not applied. Taylor's power law indicates that adults had an aggregated distribution ($R^2 = 0.99$; $b = 1.52$; $p < 0.001$), with high population density in the central part of the vine field. Leafhopper dispersal showed a maximum 600-m radius. We also discuss the pathways of *S. titanus* introduction into the Marche region, and factors that might have influenced its dispersal.

Key words: Phytoplasma vector, Flavescence dorée, survey, seasonal flight activity, Marche region.

Introduction

The leafhopper *Scaphoideus titanus* Ball (Hemiptera Cicadellidae) is the principal natural vector of the pathogen of Flavescence dorée (FD) (Schvester *et al.*, 1963). Pathogens belong to ribosomal group 16SrV, subgroups C and D, and proposed as 'Candidatus Phytoplasma vitis' (IRPCM, 2004; Lee *et al.*, 2004).

FD is the most economically important disease of the European grapevine (*Vitis vinifera* L.) (Vitaceae) in the principal wine-production areas (Boudon-Padieu, 2003). Due to its epidemic potential, FD phytoplasma (FDP) is listed as a quarantine pathogen in the European Union (Council Directive 2000/29/EC, Annex II/AII) and in the European and Mediterranean Plant Protection Organisation (EPPO) countries (EPPO/CABI, 2003).

In Europe, *S. titanus* mainly lives on European grapevine, although it can also develop on wild American grapevine (AGV) (*Vitis* spp.), which can also host FDP (Lessio *et al.*, 2007). It is a univoltine species that lays eggs mainly in the bark of wood of two or more years, where they overwinter (Vidano, 1964; Bagnoli *et al.*, 2011). In northern Italy, egg hatching usually starts in the middle of May and ends in July. The nymphs have five instars, each of which lasts a week. The adults are present from the end of June until the middle of October (Vidano, 1964).

Leafhoppers can transmit FDP persistently after a latent period that ranges from 28 to 35 days, and a minimum phytoplasma acquisition access period from 4 to 7-8 days (Schvester *et al.*, 1969). FDP does not appear to be transmitted from one generation to another by

transovarial infection in leafhoppers (Bressan *et al.*, 2005), but all feeding growth stages (nymphs and adults) can acquire FDP by feeding on FD-diseased vines (Schvester *et al.*, 1969). However, transmission to vines is significantly higher for plants exposed to late instar nymphs and adults compared to those inoculated by early instar nymphs (Bressan *et al.*, 2006). Bressan *et al.* (2006) suggested that the long latent period associated with a low probability of early instar nymphs acquiring FDP and their flight ability might explain why adults are the most important life stage involved in the spread of FDP.

Adult leafhoppers show an aggregative spatial distribution pattern within the vineyard (Jermini *et al.*, 1993; Bosco *et al.*, 1997). Lessio and Alma (2004) reported that *S. titanus* did not disperse significantly beyond 24 m from the vineyard, while Beanland *et al.* (2006) reported dispersal of at least 40 m. However, it appears that under particular wind conditions, the vector can fly to vineyards that are several kilometres away from infested sites (Steffek *et al.*, 2007). Despite this possibility, there is no question that trade in grapevine-infested propagation material has the major role in long-distance dispersal of this leafhopper, and in its introduction into previously unoccupied areas (Weintraub and Beanland, 2006). However, vehicle traffic is another relevant pathway: in the Trentino Alto-Adige region of Italy, for example, the first *S. titanus* populations were reported along the main highway heading north (Maixner, 2005).

This pest was accidentally introduced into Europe from North America, as it was unintentionally transported on imported grapevine canes (Caudwell, 1983;

Boudon-Padieu, 1999). Within Europe, *S. titanus* was reported for the first time in southern France (Bonfils and Schvester, 1960), and then its diffusion shifted towards Italy (Vidano, 1964), Switzerland (Baggiolini *et al.*, 1968), Slovenia (Seljak, 1987), Serbia (Magud and Toševski, 2004), Spain (Batlle *et al.*, 1997), Portugal (Quartau *et al.*, 2001), Austria (Zeisner, 2005), and Croatia (Budinišćak *et al.*, 2005). More recently, this leafhopper has been detected in Hungary (Dér *et al.*, 2007), Bosnia and Herzegovina (Delić *et al.*, 2007), Romania (Chireceanu *et al.*, 2011), and Montenegro (Radonjić *et al.*, 2012). In Italy, *S. titanus* is distributed throughout all of the northern regions (Alma and Conti, 2004), and it has also been detected in Tuscany (Santini and Lucchi, 1998), Umbria (Santinelli *et al.*, 2003), Basilicata (Viggiani, 2002), Campania (Viggiani, 2004), Abruzzo (D'Ascenzo and Di Giovanni, 2006) and Lazio (Bagnoli *et al.*, 2008).

Due to the high efficiency of its epidemic cycle, FD can spread epidemically where infected grapevines and high populations of *S. titanus* occur together (Bressan *et al.*, 2006). In Italy, as well as the removal of symptomatic FDP infected plants, insecticide treatments against this pest are mandatory in the districts where FD is present (Decree Ministry n. 32442 of 31/05/2000) (Barba *et al.*, 2006). Generally, two insecticide treatments are applied when most of the nymphs have hatched, and before the occurrence of the inoculative vector individuals (Carle and Schvester, 1964; Planas, 1987; Pavan *et al.*, 2005). In nurseries, a third treatment is usually applied against the adults (Barba *et al.*, 2006). The active ingredients can include insect growth regulators (i.e., buprofezin and flufenoxuron), which are active against the nymphs, and neurotoxic products (i.e., chlorpyrifos, etofenprox and indoxacarb), to control the nymphs and adults (Bosio *et al.*, 2004). However, note that flufenoxuron has been recently banned within the European Union (Regulation EU n. 942/2011). In organic farming, pyrethrum is effective in the control of the nymphs (Gusberti *et al.*, 2008) and adults (Bottura *et al.*, 2003). Nevertheless, in many vine-growing areas, there are also untreated or abandoned vineyards. Moreover, wild species of *Vitis* are generally excluded from treatments, although they can represent insect and FDP reservoirs (Bressan *et al.*, 2006; Lessio *et al.*, 2007).

FDP has been reported in France and northern Italy (Boudon-Padieu, 2003), northern Spain (Batlle *et al.*, 2000), Switzerland (Gugerli *et al.*, 2006), Serbia (Duduk *et al.*, 2003), Austria (Reisenzein and Steffek, 2011), Croatia (Šeruga Musić *et al.*, 2011), and Portugal (De Sousa *et al.*, 2009). In Italy, this grapevine yellow (GY) disease has also been reported in the Marche region (Credi *et al.*, 2002), and northern Tuscany, Umbria (Borgo *et al.*, 2005) and Campania (Ischia Island) (D.D. SIRCA n. 71/2011). In particular, in the Marche region, two FD foci were reported in southern areas (Ascoli Piceno province) (Credi *et al.*, 2002; Romanazzi *et al.*, 2005), and a third in the northern part (Pesaro-Urbino province) (D.D. ASSAM n.584/2010).

Although FD is present in the Marche region, there are no data relating to *S. titanus*. The aim of the present study was to investigate the presence of this leafhopper

throughout the Marche region over a 10-year survey, from 2001 to 2011. Furthermore, from 2009 to 2011, the population dynamics of this leafhopper, its distribution within vine fields, and its dispersal were investigated for the only two sites where it has been recorded in the Marche region.

Materials and methods

Presence of *S. titanus* throughout the Marche region from 2001 to 2011

Since 2001, annual surveys (coordinated by the Regional Plant Protection Service) have been carried out to define the presence and spread of *S. titanus* across about 150 sites. These sites are widely distributed through all of the four provinces of the Marche region, central Italy. These survey sites included vineyards for wine production (mainly with plants < 8 years old), and for rootstock and scion mother vines. They were distributed through all of the districts of the region, and with higher survey intensities in areas where GY symptoms were present. From 30 to 50% of the sites were replaced each year. For insecticide management in areas where this leafhopper was not recorded, the following treatments were used: 1) in commercial vineyards, the control measures were targeted at the second generation of the European grapevine moth, *Lobesia botrana* (Denis et Schiffermüller) (Lepidoptera Tortricidae); 2) in the young vineyards (plants < 8 years old), no treatments were applied; 3) in the rootstock mother plants, 3-4 treatments were carried out for the management of the grapevine phylloxera, *Viteus vitifoliae* (Fitch) (Hemiptera Phylloxeridae); and 4) in scion mother vines, no treatments were applied. In areas where FD and/or its vector were recorded, the ordinance on phytosanitary measures was adopted. The obligatory measures prescribed by the ordinance include declaration of disease focus zones and buffer zones, removal of infected plants, obligatory chemical control of the vector, and enhanced surveillance (D.D. ASSAM n. 584/2010).

Visual inspections of the lower surfaces of the leaves were performed from May to June, to determine the presence of the young stages of *S. titanus* (data not reported). The adults were sampled by positioning two Glutor[®] yellow sticky traps per hectare (24.5 × 13.5 cm; Intrachem Bio Italia SpA, Grassobbio, Italy) in the canopy (1.5 m above the soil) in each vineyard or nursery. The traps were replaced every 2 weeks, from the beginning of June to the end of September. The traps were wrapped inside a plastic film, labelled, and stored at -20 °C. The specimens were removed from the traps using drops of limonene (Carlo Erba, Milan), and they were identified following the taxonomic keys reported by Della Giustina (1989), and also counted and sexed. The samples were stored in Eppendorf[®] vials (Carlo Erba, Milan) at -20 °C until the phytoplasma detection analysis.

Population dynamics, distribution and dispersal from 2009 to 2011

In 2009, 2010 and 2011, additional investigations were carried out for two sites in the Pesaro-Urbino province.

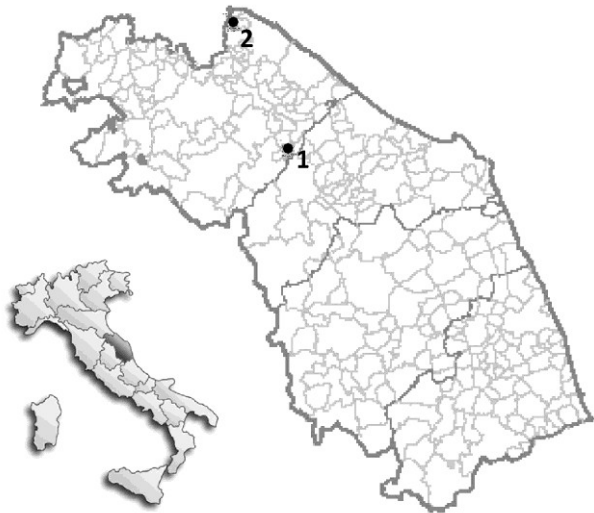


Figure 1. Map showing the locations of the two study sites in the Pesaro-Urbino province (Marche region, central-eastern Italy): 1, site 1, San Lorenzo in Campo; 2, site 2, Gradara.

Site 1 was located in the municipality of San Lorenzo in Campo (209 m a.s.l.) in a typical inland hilly agricultural landscape of the Marche region, in the mid-Cesano valley (figure 1). One rootstock mother plant nursery (site 1, nursery 1B) and 6 vineyards within a radius of 1.5 km of the scion mother plant nursery where *S. titanus* was first recorded in 2007 (site 1, nursery 1A) were surveyed (figure 2, table 1). The inter-rows and borders of the two nurseries were tilled. No FDP was detected in the grapevine plants (Credi and Nardi, unpublished data).

Site 2 was located in the municipality of Gradara (142 m a.s.l.), in a coastal hilly agricultural landscape adjacent to the A14 highway, 2 km from the Adriatic Sea and from the neighbouring province of Rimini (Emilia-Romagna region). A FD focus (site 2, commercial wine vineyard 2A) (figure 1) was reported here in 2010 (D.D. ASSAM n. 584/2010). All of the vineyards ($n = 11$) within a radius of 1.5 km from the FD focus were surveyed (table 1, figure 2).

For both sites, further surveying demonstrated the absence of AGV, hedges and woods around the vineyard/nursery borders.

The traps were positioned at distances of 30 to 60 m from each other in all of the nurseries or vineyards, and the trapping points were geo-referenced in the field using a hand-held geographic positioning system receiver (GPS 60; Garmin, Garmin International Inc., Olathe, KS, USA). The traps were replaced every 7 days to 10 days during the periods from 23 July 2009 to 12 November 2009, 23 June 2010 to 11 October 2010, and 28 June 2011 to 29 September 2011.

Phytoplasma detection

The adults captured in the sticky traps were analysed for FDP using polymerase chain reaction (PCR). The leafhoppers sampled from 2007 to 2010 were analysed individually, while those sampled in 2011 were analysed in batches, separated according to trap position and sampling date. Their DNA was extracted following the protocol proposed by Angelini *et al.* (2007). The phytoplasma gene was detected using nested PCR assays, according to conventional nested PCR assays for the diagnosis of FD, using the universal primer pair P1 (Deng and Hiruki, 1991) and P7 (Smart *et al.*, 1996). This was followed by nested PCR with primers



Figure 2. Nurseries and vineyards surveyed within site 1 (left: San Lorenzo in Campo) and site 2 (right: Gradara). White numbered shapes indicate the surveyed nurseries and vineyards. See table 1 for further details of sites.

Table 1. Details of the two study sites: site 1, San Lorenzo in Campo; site 2, Gradara (Pesaro-Urbino province). D, distance from the nursery (1A) or vineyard (2A) in which *S. titanus* was first recorded, in 2007 and 2009, respectively; Type, type of production (i.e., 1A and 1B are commercial nurseries of mother vines); Age, age of grapevine plants; untr., untreated plants; *, replacement of a part of the vineyard with photovoltaic panels; ^A, mid-August; ^J, beginning of July.

Site	Location code	N° of traps			D (m)	Type	Age (years)	Area (ha)	N° of treatments			Active ingredient
		2009	2010	2011					2009	2010	2011	
1	1A	6	6	6	0	Scions	6-8	1.5	2 ^A	1 ^A	-	Chlorpyrifos
	1B	7	7	4*	10	Rootstocks	20	3.5	2 ^A	1 ^A	-	Chlorpyrifos
	1C	2	1	1	450	Wine	6-8	0.04	untr.	untr.	untr.	-
	1D	2	2	-*	300	Wine	6-8	0.04	untr.	untr.	untr.	-
	1E	4	4	-*	650	Wine	6-8	0.3	untr.	untr.	untr.	-
	1F	4	4	4	850	Wine	6-8	0.5	untr.	untr.	untr.	-
	1G	2	2	2	600	Wine	6-8	0.4	untr.	untr.	untr.	-
	1H	2	2	2	600	Wine	6-8	0.6	untr.	untr.	untr.	-
	2	2A	10	10	10	0	Wine	10	10	1 ^J	1 ^J	1 ^J
2B		3	3	3	300	Wine	9	1	1 ^J	1 ^J	1 ^J	Flufenoxuron ^a
2C		2	2	2	300	Wine	10	0.5	1 ^J	1 ^J	1 ^J	Flufenoxuron ^a
2D		3	3	3	300	Abandoned	10	0.8	untr.	untr.	untr.	-
2E		2	2	2	600	Abandoned	8-10	0.3	untr.	untr.	untr.	-
2F		2	2	2	70	Wine	8-10	0.3	untr.	untr.	untr.	-
2G		2	2	2	1500	Wine	8-10	0.2	untr.	untr.	untr.	-
2H		2	2	2	1000	Abandoned	8-10	0.2	untr.	untr.	untr.	-
2I		2	2	2	700	Wine	7-9	0.2	untr.	untr.	untr.	-
2J		1	1	1	800	Wine	8-10	0.02	untr.	untr.	untr.	-
2K		2	2	2	1000	Wine	7-9	0.2	untr.	untr.	untr.	-

^a active ingredient banned within the European Union (Regulation EU n. 942/2011).

R16(V)F1/R1 (Lee *et al.*, 1994), which are specific for phytoplasma belonging to the 16SrV group. Elm yellow (EY1) was used as the phytoplasma reference strain.

Data analysis

Two-tailed binomial tests were performed on the trapping data, to compare the numbers of males and females, and Kruskal-Wallis tests to compare the number of specimens captured across the years, using the traps as replicates (Zar, 1999). To determine the leafhopper degree of spatial aggregation, Taylor's power law ($\log S^2 = \log a + b \log m$; where S^2 is the variance and m the mean; Taylor, 1984) was applied to the trapping data from the nurseries 1A and 1B of site 1 in the years 2010 and 2011. The sample units were represented by the sticky traps; variance, mean and regression relationships were calculated for each sampling date for the years 2010 (9 sampling dates) and 2011 (7 sampling dates). The b slope is an index of dispersion, with values of $b < 1$, $b = 1$ and $b > 1$ indicating uniform, random and aggregated distributions, respectively. To visualise the spatial distribution of the individuals in the nurseries 1A and 1B of site 1, during 2010 and 2011, the cumulative densities of the leafhopper were represented by contour maps that were drawn using Systat 11 (Systat Software Inc., SPSS 2000); these show the estimated values according to isoareas. Distance-weighted least-squares (DWLS) non-parametric interpolation was used to visualise the insect densities in the contour maps (Wilkinson *et al.*, 1996). DWLS interpolates by local quadratic ap-

proximation curves that are weighted by the distance to each non-missing point in the series. With this algorithm, all of the non-missing values in the series contribute to the missing data estimates, and thus complex local features can be modelled by the interpolant. The leafhopper numbers captured for site 1 during 2009 and for site 2 over the three years were too low to be analysed by Taylor's Power Law, and to be represented in contour maps.

Results

Presence of *S. titanus* throughout the Marche region from 2001 to 2011

During the period from 2001 to 2006, no *S. titanus* was detected in the Marche region. The first occurrence of this leafhopper in the Marche region was in 2007, in the southern part of the Pesaro-Urbino province, in a commercial nursery of scion mother vines (site 1, 1A) (figure 2), with one adult captured on 27 August. In 2008, nine adults were caught in the same nursery, from 31 July to 26 August. In 2009, on 2 September, one specimen was captured for the first time in the north-eastern part of the Pesaro-Urbino province, in a commercial wine vineyard (FD focus, site 2, 2A) (figure 2). Up to 2011, leafhopper presence was recorded only for these two specific sites in the Pesaro-Urbino province. The detailed data for the leafhopper dynamics for sites 1 and 2 from 2009 to 2011 are given in the following section.

Table 2. Trapping data for sites 1 and 2 for the years 2009 to 2011. Traps that did not have any captures are not included. -, trap not positioned due to replacement of part of the nursery with photovoltaic panels.

Site	Location code	Trap	Coordinates	Insect captures					
				2009		2010		2011	
				♂	♀	♂	♀	♂	♀
1	1A	1A1	43°36'41.0"N 12°57'49.0"E	1	0	4	0	6	3
		1A2	43°36'41.9"N 12°57'47.2"E	0	0	2	0	18	1
		1A3	43°36'41.2"N 12°57'49.3"E	1	0	6	2	26	27
		1A4	43°36'42.2"N 12°57'47.4"E	1	0	5	1	30	13
		1A5	43°36'41.5"N 12°57'49.6"E	0	1	11	11	18	21
		1A6	43°36'42.3"N 12°57'47.8"E	0	1	1	1	20	20
	1B	1B1	43°36'41.8"N 12°57'51.9"E	2	0	3	0	2	1
		1B2	43°36'42.2"N 12°57'51.2"E	0	0	15	0	4	2
		1B3	43°36'42.8"N 12°57'49.9"E	0	0	1	0	4	1
		1B4	43°36'43.6"N 12°57'48.1"E	0	0	1	2	4	0
		1B5	43°36'42.7"N 12°57'52.3"E	0	0	2	0	-	-
		1B6	43°36'43.2"N 12°57'51.2"E	0	0	3	1	-	-
	1E	1E1	43°37'01.2"N 12°57'31.4"E	0	0	1	0	-	-
		1E2	43°37'01.5"N 12°57'30.3"E	0	0	1	1	-	-
		1E4	43°37'01.4"N 12°57'31.7"E	0	0	0	1	-	-
	1H	1H1	43°36'20.7"N 12°57'48.0"E	0	0	1	0	1	0
Total captures site 1				5	2	57	20	133	89
2	2A	2A3	43°56'33.9"N 12°46'45.0"E	1	0	0	0	0	0
		2A5	43°56'25.1"N 12°46'47.1"E	0	0	0	0	1	0
	2B	2B1	43°56'45.5"N 12°46'05.8"E	0	0	0	0	1	0
		2B2	43°56'44.8"N 12°46'06.6"E	0	0	1	0	0	0
	2F	2F1	43°56'21.9"N 12°46'45.6"E	0	0	0	0	1	0
Total captures site 2				1	0	1	0	3	0

Population dynamics, distribution and dispersal from 2009 to 2011

For site 1, during 2009, seven adults were captured, from 30 July to 31 August: females were captured only on 11 August (table 1). In 2010, the population increased, with the adult flight period from 14 July to 20 August for males, and from 4 August to 6 September for females. The flight peaks were recorded on 4 August for males and on 20 August for females. In September, only females were captured (table 2, figure 3A). In 2011, the population increased dramatically, with the adult flight periods from 15 July to 8 September for males, and from 15 July to 29 September for females. The flight peak for both males and females was on 30 July, and in September, more females than males were captured (table 2, figure 3B). Overall, for the full 3 years, male captures were significantly greater than female captures (M:F, 1.71; $p < 0.001$, binomial test). The comparisons of the trapping data indicate a significant increase in the adult numbers in site 1 over the 3 years, with seven specimens captured in 2009, 77 in 2010, and 222 in 2011 ($K = 11.739$, $df = 2$, $p = 0.003$, Kruskal-Wallis test). Taylor's power law indicates that the adults had aggregated distributions in 2010 and 2011 within the site 1 nurseries 1A and 1B ($R^2 = 0.99$; $b = 1.52$; $p < 0.001$; in both years). The insect contour maps drawn indicated that in 2010 the leafhopper was almost equally distributed in both nurseries 1A and 1B, with a concentric increasing gradient towards traps 1A5 and 1B2. In 2011, there was a higher

density of individuals within nursery 1A, with a decreasing density gradient towards the south-western traps and towards nursery 1B (figure 4).

In site 2, only one male was caught in both 2009 (2 September) and 2010 (20 August), and three males were caught, from 30 July to 8 September (table 2).

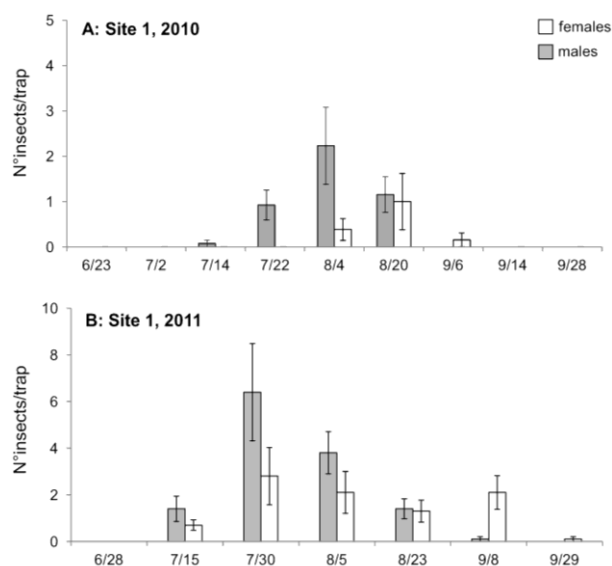


Figure 3. Seasonal flight activity of *S. titanus* males (grey bars) and females (white bars) collectively for site 1, for 2010 (A) and 2011 (B).

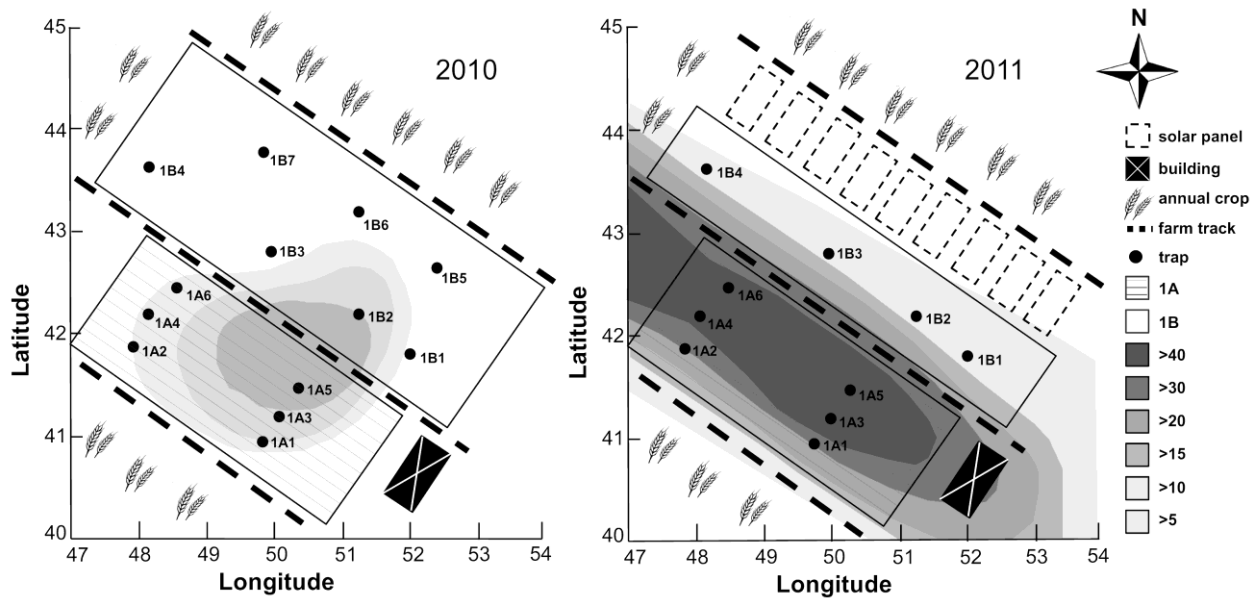


Figure 4. Contour maps of *S. titanus* adult distribution for site 1, nurseries 1A and 1B, for the years 2010 and 2011. Black numbered spots, individual trap positions; grey-scale, insect densities, as shown (right).

Phytoplasma detection

Using both of the primer pairs P1/P7 followed by R16(V) F1/R1, FD phytoplasma were not detected in any of the *S. titanus* captured. The specific band of 1100 bp according to the 16S rDNA gene was detected in the reference strain used as the positive control (data not shown).

Discussion and conclusions

In the Marche region, *S. titanus* was first recorded in a commercial nursery of scion mother vines in 2007, in the San Lorenzo in Campo municipality, which is located in the southern part of the Pesaro-Urbino province (in the mid-Cesano valley). In 2009, this leafhopper was recorded also in a commercial wine vineyard in the Gradara municipality, in the north-east of the Pesaro-Urbino province (less than 2 km from the Rimini province; adjacent to the A14 highway), where there was a FD focus.

We hypothesise that the FDP vector at the first site was accidentally introduced with trade in infested planting material or in vehicles from areas where it was present. At site 2, it might also have arrived with vehicles or through its active movement from the neighbouring province of Rimini, where its presence is known (unpublished data). Indeed, human activities have a major role in the long-distance dispersal and the introduction of this vector into previously unoccupied areas (Weintraub and Beanland, 2006). Considering the large quantity of plant material produced and exchanged commercially, it is evident that rigorous quality control of this material is imperative, both for phytoplasma infections and for leafhopper eggs.

Our data are in partial agreement with previous studies that have demonstrated that *S. titanus* does not spread

significantly outside the vineyard (Lessio and Alma, 2004; Beanland *et al.*, 2006). For San Lorenzo in Campo, this leafhopper was recorded at a maximum distance of 600 m from the nursery of scion mother plants where it was first recorded. This situation might be related to the absence of natural barriers (AGV, hedges and wood) as observed in the leafhopper *Empoasca vitis* (Goethe) (Decante *et al.*, 2009) and in different species of hover flies (Diptera Syrphidae) (Wratten *et al.*, 2003). Moreover, a particular wind situation might have enhanced leafhopper dispersal (Steffek *et al.*, 2007).

The leafhopper adults were recorded from the end of June to the end of September, with a shifted flight activity for the females, in agreement with data obtained in northern Italy by Cravedi *et al.* (1993) and Lessio *et al.* (2009). There were significantly more male captures than females, in agreement with Lessio and Alma (2004). Males of many leafhopper species are known to have high dispersion ability, while females tend to remain linked to their host plants (Weintraub and Beanland, 2006; Minuz *et al.*, 2013).

Our study also confirms that the *S. titanus* adult spatial distribution is aggregated, as has been demonstrated for both adults (Jermini *et al.*, 1993; Bosco *et al.*, 1997) and nymphs (Lessio and Alma, 2006; Rigamonti *et al.*, 2014). An aggregate spatial distribution has also been shown for other leafhoppers, such as *Euscelis lineolatus* Brulle, *Neotalitrus fenestratus* (Herrich-Schaffer) and *Psammotettix alienus* (Dahlbom) (Minuz *et al.*, 2013). Moreover, in the vineyard agroecosystem studied, a high population density was observed in the central part of the grapevine field and not along the borders, as has been reported in other studies (Pavan *et al.*, 2012). This situation should be related to the absence of AGV, hedges and woods in the crop borders, and the preference of the leafhopper for shaded and cooler leaves (Cravedi *et al.*, 1993).

A correct pest management can decrease the dispersion of leafhopper vectors, as has been observed for *Homalodisca vitripennis* (German) (Hemiptera Cicadellidae) (Park *et al.*, 2006). Indeed, few leafhopper individuals have been found in those vineyards where insecticides were applied. Nevertheless, in this same site no leafhoppers have been recorded in untreated or abandoned vineyards. This suggests that *S. titanus* populations could not only be regulated by chemical control, but also by natural enemies (Landis *et al.*, 2000).

A spatial region management strategy against FDP vector has the potential to increase its management efficiency and to ensure environmental safety, while reducing the chemical input by applying such control measures only to the areas where the leafhopper is present.

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References

- ALMA A., CONTI M., 2004.- Vettori dei fitoplasmi della vite, pp. 1-5. In: *Atti Convegno Nazionale - La vite*, Villa Gualino, Torino, Italy, 2-3 December 2004.
- ANGELINI E., BIANCHI G. L., FILIPPINI L., MORASSUTTI C., BORGIO M., 2007.- A new TaqMan method for the identification of phytoplasmas associated with grapevine yellows by real-time PCR assay.- *Journal of Microbiological Methods*, 68: 613-622.
- BAGGIOLINI M., CANEVASCINI V., CACCIA R., TENCALLA Y., SOBRIO G., 1968.- Présence dans le vignoble du Tessin d'une cicadelle nouvelle pur la Suisse, *Scaphoideus littoralis* Ball. (Hom., Jassidae), vecteur possible de la flavescence dorée.- *Mitteilungen der Schweizerischen Entomologischen Gesellschaft*, 40 (3-4): 270-275.
- BAGNOLI B., GARGANI E., 2011.- Survey on *Scaphoideus titanus* egg distribution on grapevine.- *IOBC/wprs Bulletin*, 67: 233-237.
- BAGNOLI B., FERRETTI L., TRIVELLONE V., NUCCITELLI L., PASQUINI G., 2008.- Accertamento della presenza di *Scaphoideus titanus* nel Lazio.- *Petria*, 18 (2): 304-307.
- BARBA M., FERRETTI L., PASQUINI G., 2006.- I giallumi della vite: un problema fitosanitario di rilevanza nazionale.- *Informatore Fitopatologico*, 56 (4): 4-8.
- BATLLE A., LAVIÑA A., CLAIR D., LARRUE J., KUSZALA C., BOUDON-PADIEU E., 1997.- Detection of "flavescence dorée" in grapevine in northern Spain.- *Vitis*, 36: 211-212.
- BATLLE A., MARTÍNEZ M. A., LAVIÑA A., 2000.- Occurrence, distribution and epidemiology of grapevine yellows in Spain.- *European Journal of Plant Pathology*, 106 (9): 811-816.
- BEANLAND L., NOBLE R., WOLF T. K., 2006.- Spatial and temporal distribution of North American grapevine yellows disease and of potential vectors of the causal phytoplasmas in Virginia.- *Environmental Entomology*, 35: 332-344.
- BONFILS J., SCHVESTER D., 1960.- Les cicadelles (Homoptera: Auchenorrhyncha) dans leurs rapports avec la vigne dans le Sud-Ouest de la France.- *Annales des Épiphyties*, 9: 325-336.
- BORGIO M., ANGELINI E., FILIPPINI L., BOTTI S., MARZACHÌ C., CASATI P., QUAGLINO F., ZORLONI A., ALBANESE G., LA ROSA R., TESSITORI M., PASQUINI G., BERTACCINI A., 2005.- Monitoraggio dei giallumi della vite e caratterizzazione dei fitoplasmi nell'ambito del progetto finalizzato "Gia.Vi" nel 2004.- *Petria*, 15: 161-164.
- 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.
- BOSIO G., MARTINEZ M. C., OCCHETTI P., ROVETTO I., DELLA VALLE D., LAIOLO L., VALOTA G., 2004.- Efficacy evaluation of synthetic and natural insecticides against *Scaphoideus titanus* Ball young stages on grapevine, pp. 95-102.- In: *Atti Giornate Fitopatologiche*, Montesilvano, Italy, 4-6 May 2004.
- BOTTURA N., MORI N., POSENATO G., SANCASSANI G. P., GIROLAMI, 2003.- Lotta alle cicaline nei vigneti a conduzione biologica.- *L'Informatore Agrario*, 59 (15): 75-80.
- BOUDON-PADIEU E., 1999.- Grapevine phytoplasmas. In: *First Internet Conference on Phytopathogenic Mollicutes*, 24-29 May 1999. <http://web.uniud.it/phytoplasma/conf.html>.
- BOUDON-PADIEU E., 2003.- The situation of grapevine yellows and current research directions: distribution, diversity, vectors, diffusion and control, pp. 47-53. In: *14th ICVG Conference*, Locorotondo, Italy, 12-17 September 2003.
- BRESSAN A., GIROLAMI V., BOUDON-PADIEU E., 2005.- Reduced fitness of *Scaphoideus titanus* exposed to Flavescence dorée phytoplasma.- *Entomologia Experimentalis et Applicata*, 115: 283-290.
- BRESSAN A., LARRUE J., BOUDON-PADIEU E., 2006.- Patterns of phytoplasma-infected and -infective *Scaphoideus titanus* leafhoppers in vineyards with high incidence of Flavescence dorée.- *Entomologia Experimentalis et Applicata*, 119: 61-69.
- BUDIŠČAK Ž., KRIZANAC I., MIKEC I., SELJAK G., ŠKORIĆ D., 2005.- Vektori fitoplazmi vinove loze u Hrvatskoj.- *Glasilo biljne zaštite*, 5 (4): 240-245.
- CARLE P., SCHVESTER D., 1964.- Nouvelle mise au point sur la lutte contre *Scaphoideus titanus* Ball, cicadelle vectrice de la Flavescence dorée de la vigne.- *Revue de Zoologie Agricole et Appliquée*, 63: 107-114.
- CAUDWELL A., 1983.- L'origine des jaunisses à Mycoplasme (MLO) des plantes et l'exemple des jaunisses de la vigne.- *Agronomie*, 2: 103-111.
- CHIRECEANU C., PLOAIE P. G., GUTUE M., NICOLAE I., STAN C., COMSA M., 2011.- Detection of the Auchenorrhyncha fauna associated with grapevine displaying yellows symptoms in Romania.- *Acta Phytopathologica et Entomologica Hungarica*, 46 (2): 253-260.
- CRAVEDI P., MAZZONI E., CERVATO P., 1993.- Osservazioni sulla biologia di *Scaphoideus titanus* Ball (Homoptera: Cicadellidae).- *Redia*, 76: 57-70.
- CREDI R., TERLIZZI F., STIMILLI G., NARDI S., 2002.- Flavescenza dorata della vite nelle Marche.- *L'Informatore Agrario*, 58 (22): 61-63.
- D'ASCENZO D., DI GIOVANNI R., 2006.- Bilancio fitosanitario 2005, Abruzzo.- *L'Informatore Agrario*, 62 (15): 50.
- DECANTE D., VAN LEEUWEN C., VAN HELDEN M., 2009.- Influence of plot characteristics and surrounding vegetation on the intra-plot spatial distribution of *Empoasca vitis*.- *Agricultural and Forest Entomology*, 11 (4): 377-388.
- DELLA GIUSTINA W., 1989.- *Homoptères Cicadellidae*, vol. 3 compléments.- INRA, Paris, France.
- DELIĆ D., SELJAK G., MARTINI M., ERMACORA P., CARRARO L., MYRTA A., DURIC' G., 2007.- Surveys for grapevine yellows phytoplasmas in Bosnia and Herzegovina.- *Bulletin of Insectology*, 60 (2): 369-370.
- DENG S., HIRUKI C., 1991.- Amplification of 16S rRNA genes from culturable and non-culturable mollicutes.- *Journal of Microbiological Methods*, 14: 53-61.

- DÉR Z., KOCZOR S., ZSOLNAI B., EMBER I., KÖLBER M., BERTACCINI A., ALMA A., 2007.- *Scaphoideus titanus* identified in Hungary.- *Bulletin of Insectology*, 60 (2): 199-200.
- DE SOUSA E., CASATI P., CARDOSO F., BALTAZAR C., DURANTE G., QUAGLINO F., BIANCO P. A., 2009.- Flavescence dorée phytoplasma affecting grapevine (*Vitis vinifera*) newly reported in Portugal.- *New Disease Reports*, 19: 33.
- DUDUK B., BOTTI S., IVANOVIC M., DUKIC N., BERTACCINI A., 2003.- Molecular characterization of a "flavescence dorée" phytoplasma infecting grapevine in Serbia, pp. 91-92. In: *14th ICVG Conference*, Locorotondo, Italy, 12-17 September 2003.
- EPPO/CABI, 2003.- Data sheets on quarantine pests - grapevine Flavescence dorée phytoplasma, pp. 1013-1021. In: *Quarantine pests for Europe* (SMITH I. M., MCNAMARA D. G., SCOTT P. R., HOLDERNESS M., Eds).- CABI International, Wallingford, UK.
- GUGERLI P., BESSE S., COLOMBI L., RAMEL M. E., RIGOTTI S., CAZELLES O., 2006.- First outbreak of "flavescence dorée" (FD) in Swiss vineyards, pp. 96-98. In: *Extended abstracts of the 15th meeting of the International Council for the Study of Virus and Virus-like Diseases of the Grapevine (ICVG)*, SASEV, Stellenbosch, South Africa, 3-7 April 2006.
- GUSBERTI M., JERMINI M., WYSS E., LINDER C., 2008.- Efficacy of insecticides against *Scaphoideus titanus* in organic vineyards and their side effects.- *Revue Suisse de Viticulture, Arboriculture et Horticulture*, 40: 173-177.
- IRPCM, 2004.- "Candidatus phytoplasma", a taxon for the wall-less, non-helical prokaryotes that colonize plant phloem and insect.- *International Journal of Systematic and Evolutionary Microbiology*, 54: 1243-1255.
- JERMINI M., D'ADDA G., BAUMGARTNER J., LOZZIA G. C., BAILLOD M., 1993.- Nombre des pièges englués nécessaires pour estimer la densité relative des populations de la cicadelle *Scaphoideus titanus* Ball en vignoble.- *Bollettino di Zoologia Agraria e di Bachicoltura*, 25: 91-102.
- LANDIS D. A., WRATTEN S. D., GURR G. M., 2000.- Habitat management to conserve natural enemies of arthropod pests in agriculture.- *Annual review of entomology*, 45 (1): 175-201.
- LEE I. M., GUNDERSEN D. E., HAMMOND R. W., DAVIS R. E., 1994.- Use of mycoplasma-like organism (MLO) group-specific oligonucleotide primers for nested PCR assays to detect mixed MLO infections in a single host plant.- *Phytopathology*, 84: 559-566.
- LEE I. M., MARTINI M., MARCONE C., ZHU S. F., 2004.- Classification of phytoplasma strains in the elm yellows group (16SrV) and proposal of 'Candidatus phytoplasma ulmi' for the phytoplasma associated with elm yellows.- *International Journal of Systematic and Evolutionary Microbiology*, 54: 337-347.
- LESSIO F., ALMA A., 2004.- Dispersal patterns and chromatic response of *Scaphoideus titanus* Ball (Homoptera Cicadellidae), vector of the phytoplasma agent of grapevine flavescence dorée.- *Agricultural and Forest Entomology*, 6: 121-127.
- LESSIO F., ALMA A., 2006.- Spatial distribution of nymphs of *Scaphoideus titanus* (Homoptera: Cicadellidae) in grapes and evaluation of sequential sampling plans.- *Journal of Economic Entomology*, 99: 578-582.
- LESSIO F., TEDESCHI R., ALMA A., 2007.- Presence of *Scaphoideus titanus* on American grapevine in woodlands, and infection with "flavescence dorée" phytoplasmas.- *Bulletin of Insectology*, 60: 373-374.
- LESSIO F., TEDESCHI R., PAJORO M., ALMA A., 2009.- Seasonal progression of sex ratio and phytoplasma infection in *Scaphoideus titanus* Ball (Homoptera: Cicadellidae).- *Bulletin of Entomological Research*, 99: 377-383.
- MAGUD B., TOŠEVSKI I., 2004.- *Scaphoideus titanus* Ball. (Homoptera, Cicadellidae) nova štetočina u Srbiji - *Biljni lekar, Novi Sad*, 32 (5): 348-352.
- MAIXNER M., 2005.- Risks posed by the spread and dissemination of grapevine pathogens and their vectors, pp. 141-146. In: *Introduction and spread of invasive species*, Symposium Proceedings N° 81, The British Crop Production Council, Alton, Hampshire, UK.
- MINUZ L. R., 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 (6): 2296-2309.
- PARK Y. L., PERRING T. M., YACCOUB R., BARTELS D. W., ELMS D., 2006.- Spatial and temporal dynamics of overwintering *Homalodisca coagulata* (Hemiptera: Cicadellidae).- *Journal of Economic Entomology*, 99 (6): 1936-1942.
- PAVAN F., STEFFANELLI G., VILLANI A., MORI N., POSENATO G., BRESSAN A., GIROLAMI V., 2005.- Controllo di FD attraverso la lotta contro il vettore *Scaphoideus titanus* Ball. pp. 91-116. In: *Flavescenza dorata e altri giallumi della vite in Toscana e in Italia* (BERTACCINI A., BRACCINI P., Eds).- Quaderno ARSIA 3/2005, Firenze, Italy.
- 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 (2): 281-290.
- PLANAS R., 1987.- Expérience de lutte contre la flavescence dorée dans le vignoble audois, pp. 237-247. In: *Atti del Convegno "Flavescenza dorata della vite"*, Vicenza-Verona, Italy, 28-29 May 1987.
- QUARTAU J. A., GUIMARAE J. M., ANDRÉ G., 2001.- On the occurrence in Portugal of the Nearctic *Scaphoideus titanus* Ball (Homoptera, Cicadellidae), the natural vector of the grapevine Flavescence dorée (FD).- *IOBC/wprs Bulletin*, 24 (7): 273-276.
- RADONJIĆ S., HRNČIĆ S., KRSTIĆ O., TOŠEVSKI I., JOVIĆ J., 2012.- Presence and distribution of *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae) in the vineyards of Montenegro, pp. 506-510. In: *Proceeding of International Symposium on Current Trends in Plant protection*, Belgrade, Serbia, 25-28 September 2012.
- REISENZEIN H., STEFFEK R., 2011.- First outbreaks of grapevine 'flavescence dorée' in Austrian viticulture.- *Bulletin of Insectology*, 64: 223-224.
- RIGAMONTI I. E., TRIVELLONE V., JERMINI M., FUOG D., BAUMGARTNER J., 2014.- Multiannual infestation patterns of grapevine plant inhabiting *Scaphoideus titanus* (Hemiptera: Cicadellidae) leafhoppers.- *The Canadian Entomologist*, 146 (1): 67-79.
- ROMANAZZI G., MUROLO S., TERLIZZI F., TALEVI S., BRANZANTI M. B., NARDI S., CREDI R., SAVINO V., 2005.- A second finding of Flavescence dorée in the Marche region, pp. 48-59.- In: *Atti 3° Incontro Nazionale sulle Malattie da Fitoplasmii*, Milan, Italy, 22-24 June 2005.
- SANTINELLI C., SANTONI M., BRACCINI P., BOTTI S., BERTACCINI A., 2003.- Trovato in Umbria *Scaphoideus titanus*, vettore della flavescenza dorata.- *L'Informatore Agrario*, 59 (15): 81-82.
- SANTINI L., LUCCHI A., 1998.- Presenza in Toscana del cicadellide *Scaphoideus titanus*.- *L'Informatore Agrario*, 54 (49): 73-74.
- SCHVESTER D., CARLE P., MOUTOUS G., 1963.- Transmission de la flavescence dorée de la vigne par *Scaphoideus littoralis* Ball.- *Annales des Epiphyties*, 14: 175-198.
- SCHVESTER D., CARLE P., MOUTOUS G., 1969.- Nouvelles données sur la transmission de la flavescence dorée de la vigne par *Scaphoideus littoralis* Ball.- *Annales de Zoologie et Ecologie Animale*, 1969: 445-465.
- SELJAK G., 1987.- *Scaphoideus titanus* Ball (=S. littoralis Ball) a new pest of grapevine in Yugoslavia.- *Zastita Bilja*, 38: 349-357.

- ŠERUGA MUSIĆ M., ŠKORIC D., HALUŠKA I., KRIZANAC I., PLAVEC J., MIKEC I., 2011.- First report of flavescence do-ree-related phytoplasma affecting grapevines in Croatia.- *Plant Disease*, 95 (3): 353-353.
- SMART C. D., SCHNEIDER B., BLOMQUIST C. L., GUERRA L. J., HARRISON N. A., AHRENS U., LORENZ K. H., SEEMÜLLER E., KIRKPATRICK B. C., 1996.- Phytoplasma-specific PCR primers based on sequences of the 16S-23S rRNA spacer region.- *Applied and Environmental Microbiology*, 62: 2988-2993.
- STEFFEK R., REISENZEIN H., ZEISNER N., 2007.- Analysis of the pest risk from grapevine flavescence dorée phytoplasma to Austrian viticulture.- *EPPO Bulletin*, 37 (1): 191-203.
- TAYLOR L. R., 1984.- Assessing and interpreting the spatial distribution of insect populations.- *Annual Review of Entomology*, 29: 321-358.
- VIDANO C., 1964.- Scoperta in Italia dello *Scaphoideus littoralis* Ball cicalina americana collegata alla "flavescence dorée" della vite.- *L'Italia Agricola*, 101: 1031-1049.
- VIGGIANI G., 2002.- Il vettore della flavescenza dorata trovato in Basilicata.- *L'Informatore Agrario*, 58 (36): 59.
- VIGGIANI G., 2004.- Il vettore della flavescenza dorata anche in Campania.- *L'Informatore Agrario*, 60 (18): 98-99.
- WEINTRAUB P. G., BEANLAND L., 2006.- Insect vectors of phytoplasmas.- *Annual Review of Entomology*, 51: 91-111.
- WILKINSON L., BLANK G., GRUBER C., 1996.- *Desktop Data Analysis SYSTAT*.- Prentice Hall PTR, USA.
- WRATTEN S. D., BOWIE M. H., HICKMAN J. M., EVANS A. M., SEDCOLE J. R., TYLIANAKIS J. M., 2003.- Field boundaries as barriers to movement of hover flies (Diptera: Syrphidae) in cultivated land.- *Oecologia*, 134 (4): 605-611.
- ZAR J. H., 1999.- *Biostatistical analysis*.- Prentice Hall, Upper Saddle River, NJ, USA.
- ZEISNER N., 2005.- Augen auf im Süden: Amerikanische Zikade im Anflug.- *Der Winzer*, 5: 20-21.

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