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Border effect in spatial distribution of Flavescence dorée affected grapevines and outside source of *Scaphoideus titanus* vectors

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Abstract

Flavescence dorée (FD) is a severe Grape Yellows Disease of European vineyards whose phytoplasma causal agent is transmitted from grapevine to grapevine by the leafhopper *Scaphoideus titanus* Ball. Grapevines untreated with insecticides are considered potential sources of infectious vectors for treated vineyards. Research was carried out: (i) to study the spatial distribution of grapevines affected by FD, (ii) to compare the *S. titanus* population levels between treated and untreated vineyards and (iii) to verify if there are decreasing gradients of *S. titanus* adult captures in treated vineyards from contiguous untreated grapevines. In two vineyards with potential external sources of infected *S. titanus* populations in the near vicinity, a significant decreasing gradient of grapevines affected by FD was observed. In two other vineyards without untreated grapevines potential sources of infectious *S. titanus* in surrounding areas, an edge effect was not observed. The vineyards untreated with insecticides, both cultivated and abandoned, showed much higher population levels of *S. titanus* than treated vineyards. In vineyards treated against the vector, a decreasing gradient of *S. titanus* adult captures was observed from contiguous untreated grapevines. This occurrence shows that the vector moves from untreated grapevines to treated vineyards. So, if adult vectors moving from untreated grapevines are infected by FD phytoplasmas, they can represent a real risk for the surrounding vineyards treated with insecticides. Therefore, the strategy for effective FD control needs to also include: (i) insecticide treatments effective against *S. titanus* in all the grape-growing areas with vineyards affected by FD and (ii) the eradication of abandoned vineyards and American grapevines growing wild in spontaneous vegetation.

Key words: Grape Yellows, abandoned vineyard, grapevine grown wild, edge effect, vector dispersal.

Introduction

Flavescence dorée (FD) is a Grape Yellows Disease causing severe damage in European vineyards. '*Candidatus* Phytoplasma vitis', the causal agent of the disease, is transmitted from grapevine to grapevine by the leafhopper *Scaphoideus titanus* Ball (Rhynchota Cicadellidae) (Schvester *et al*., 1963; Carraro *et al*., 1994; Bianco *et al*., 2001; Mori *et al*., 2002).

Cultivated vineyards not treated with insecticides effective against *S. titanus* support the highest populations of the vector and the highest percentage of symptomatic grapevines (Pavan *et al*., 2005a; Bressan *et al*., 2005). For this reason, insecticide treatments against the leafhopper are compulsory in cultivated vineyards of grape-growing areas where FD is widespread (Planas, 1987; Barba, 2005; Pavan *et al*., 2005b).

The vector and the disease were detected also in abandoned vineyards and in American grapevines growing wild in hedgerows or groves (Schvester *et al*., 1962; Carle and Schvester, 1964; Vidano, 1964; Moutous and Fos, 1977; Caudwell *et al*., 1994). Recent surveys carried out in abandoned vineyards and in wild American grapevines showed that they can sometimes support high population levels of *S. titanus*, similar to cultivated vineyards that have not been treated with insecticides (Pavan *et al*., 2005a; Lessio and Alma, 2006; Lessio *et al*., 2007). In North America, higher *S. titanus* population levels were observed on *Vitis riparia* Michaux in adjacent woodlands than on *Vitis vinifera* L. in commercial vineyards (Maixner *et al*., 1993; Beanland *et al*., 2006). Therefore, abandoned vineyards and wild American grapevines are considered potential sources of infectious *S. titanus* adults. Unfortunately, they cannot be treated because the use of insecticides is not permitted in natural areas and the conditions in abandoned vineyards often do not allow access of machineries. For this reason, there is a lot of pressure in France and in Italy to remove them (Planas, 1987; Barba, 2005). However, there is sometimes reluctance to enforce the removal of abandoned vineyards as there are conflicting information on their role in the epidemiology of FD: (1) the low population density of the vector sometimes observed on uncultivated grapevines (Pavan *et al*., 2005a; Lessio and Alma, 2006), (2) the low mobility of *S. titanus* adults (Lessio and Alma, 2004) and (3) the absence of an edge effect in the captures of *S. titanus* observed in vineyards contiguous to American grapevines in woodland vegetation (Lessio *et al*., 2007). However, a high incidence of FD phytoplasma was detected in nymphs and adults of *S. titanus* collected on American grapevines in woodlands (Lessio *et al*., 2007), suggesting a potential role of these plants as a source of infectious adult vectors.

In North America, migration of *S. titanus* from woodlands to the peripheral part of commercial vineyards was shown (Maixner *et al*., 1993; Beanland *et al*., 2006). Even though it is unknown whether *S. titanus* is the vector of the causal phytoplasma of North America

Grapevine Yellows Disease, both the detection of a phytoplasma on wild grapevines and the occurrence of diseased grapevines mostly near vineyard edges bordering woodland vegetation support the concept that a vector moves from wild to cultivated grapevines.

To evaluate the potential risk for cultivated vineyards due to the presence of outside sources of vectors: (i) gradients of symptomatic grapevines between and along rows in vineyards affected by FD were verified, (ii) the population density of *S. titanus* in abandoned and in cultivated, treated or not against the vector, vineyards was compared and (iii) captures of *S. titanus* in treated vineyards as a function of distance from contiguous untreated vineyards were verified.

Materials and methods

Gradients in the incidence of grapevines affected by FD in vineyards

Four northern-Italian vineyards with an incidence of grapevines affected by FD more than 5% were investigated during 2004-2009 for the presence of gradients of symptomatic grapevines between and along rows. The vineyards were treated at least once a year with insecticides against the vector. Three vineyards (No 1-3) were located in northeastern Italy (Friuli Venezia Giulia region, cultivar Merlot, Sylvoz training system; No 1, 45°96'N, 12°45'E, 576 grapevines; No 2, 45°96'N, 12°44'E, 334 grapevines; No 3, 45°88'N, 12°53'E, 842 grapevines) and one (No 4) in northwestern Italy (Piedmont region, cultivar Barbera, Guyot training system; 44°77'N, 8°45'E, 1442 grapevines). In the symptomatic grapevines of these vineyards only the "Flavescence dorée" phytoplasma ('*Candidatus* Phytoplasma vitis') was detected by PCR analysis (at least five grapevines per vineyard).

A map of each investigated vineyard (rows and grapevine along rows) and of the surrounding habitats, to identify potential sources of infectious *S. titanus*, was made (figure 1). Near the east side of the vineyard No 2, a vineyard with a percentage of FD infected grapevines higher than 50% and untreated with insecticides was present. Two sides of the vineyard No 4 were bordered by a grove with many American grapevines grown wild. In this last case, during summer 2008, 69 *S. titanus* adults were captured with yellow sticky traps on grapevines growing wild, 6 in the row 2 and 1 in the row 14 of vineyard despite of four insecticide applications against the vector in the cultivated vineyard (W. Biasi, personal communication). No potential sources of infectious *S. titanus* were found in the proximity of the vineyards No 1 and 3. In late August/early September of each sampling year the grapevines of the four vineyards were classified as symptomatic or asymptomatic depending on the presence of typical symptoms (partial or total lack of lignification of canes and shoots, rolling of leaves, sectorial discolorations of the blades). The plants that showed symptoms, at least once during the sampling years (No 1, 2004-2007; No 2, 2004-2005; No 3, 2004-2007; No 4, 2008-2009), were considered infected.

The occurrence of gradients within the vineyards in FD affected grapevines was studied. For this purpose, the percentage of diseased plants in the different rows

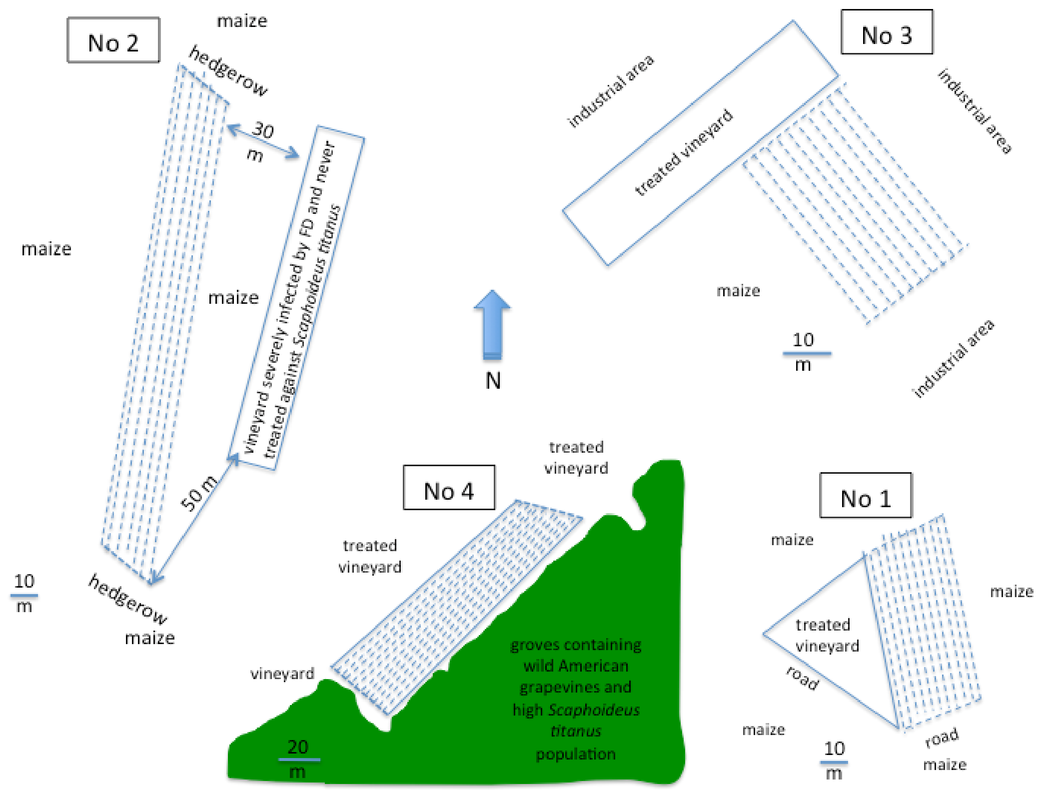

Figure 1. Maps of four vineyards (No = vineyard number) sampled for FD incidence.

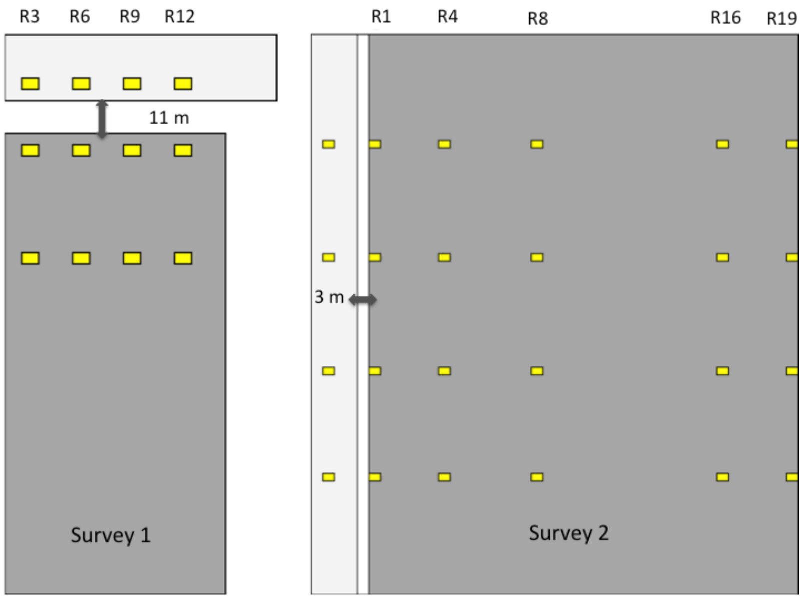

Figure 2. Maps of vineyards for surveys on *S. titanus* colonization (dark grey = vineyards treated with insecticides against *S. titanus*; light grey = vineyard untreated with insecticides; R = row; the small rectangles within the vineyard areas show the spatial distribution of yellow sticky traps).

and in an equal number of plots across the rows was calculated. The percentage of diseased plants in each plot was plotted as a function of distance from vineyard borders associated with the higher percentage of diseased plants. The observed data were compared with those predicted by the power law model by calculating the log-log regression between the percentage of infected grapevines and the distance from vineyard borders. Moreover, symptomatic plants and external potential source of infectious vectors were reported in a map.

Population density of *S. titanus* in abandoned and cultivated vineyards

The research was carried out during 2004-2007 in a flatland grape-growing area of northeastern Italy (Gorizia district, Friuli Venezia Giulia region) not affected by FD.

A yearly sampling of *S. titanus* adults was made with yellow sticky traps in cultivated vineyards, treated or untreated with insecticides against the leafhopper, and in abandoned vineyards. Each year between 32 and 54 vineyards were considered. The traps (11.5 × 24 cm) were obtained by a plastic sheet (Plastibor s.r.l., Ponte San Nicolò, Padova, Italy) and were smeared with Temoocid (Kollant s.r.l., Vigonovo, Venice, Italy). Two traps per sampling site were placed at the beginning of August, when higher amounts of captures were usually observed (Pavan *et al*., 2005b) and remained in the field for 14 days. The two traps were placed within the grapevine canopies, approximately 1 m above ground level, in a central row of the sampling vineyards at about 10 and 30 m from a border side. All the captured *S. titanus* adults were counted under a dissection microscope. For the vineyards treated with insecticides the active ingredients sprayed and the proximity to grapevines untreated against the vector was recorded.

To compare the captures of *S. titanus* in abandoned and cultivated vineyards in the different years, Kruskal-Wallis test and Dunn's multiple comparisons test were used. To compare the captures of *S. titanus* in vineyards treated with different insecticides against the vector throughout the four years, Friedman version of the Kruskal-Wallis test and Dunn's multiple comparisons test were used (Mack and Skillings, 1980; Groggel and Skillings, 1986).

S. titanus ability to colonize cultivated/treated vineyards

Two surveys were carried out in 2005-2007 in two flatland vineyard habitats, located in northeastern Italy (Friuli Venezia Giulia region), to verify the ability of *S. titanus* adults to move from abandoned or cultivated, but untreated against *S. titanus*, vineyards to vineyards treated against the vector (figure 2).

In the first habitat (45°96'N, 13°45'E; survey 1), the ability of *S. titanus* to colonize a cultivated treated vineyard moving from a cultivated untreated vineyard was tested. The treated vineyard (cultivar Sauvignon Blanc, Guyot training system, grapevines trained at a distance of 2.5 × 1 m) was placed at the distance of 11 m from the untreated vineyard (cultivar Sauvignon Blanc, Guyot training system, grapevines trained at a distance of 3 × 0.8 m).

In the second habitat (45°93'N, 13°51'E; survey 2), the ability of *S. titanus* to colonize a cultivated treated vineyard moving from an abandoned vineyard was tested. The cultivated vineyard (cultivar Pinot gris, Guyot training system, grapevines trained at a distance of 2.5 × 0.6 m) was placed at the distance of 3 m from the abandoned vineyard (three rows of European grapevine and American grapevines grown from graft root stock mixed with bramble and other woody plants (e.g. *Ulmus minor* Miller, *Cornus sanguinea* L., *Sambucus nigra* L.).

The insecticides applied in the treated vineyards were:

clorpyriphos-ethyl in early July (survey 1), flufenoxuron in mid June (surveys 2/2005 and 2/2006) and pyrethrum in mid June (survey 2/2007).

The adults of *S. titanus* were monitored with yellow sticky traps, as those described above, from late June/early July to September. Groups of four traps were placed on untreated grapevines and in different positions of the contiguous treated vineyards (figure 2). In survey 2/2005 all the five positions were considered, while in surveys 2/2006 and 2/2007 only R1, R4 and R19. The traps were placed within the grapevine canopies, approximately 1 m above ground level, and replaced every one (survey 2/2007) or two weeks (surveys 1/2005, 2/2005, 2/2006). All the captured *S. titanus* adults were counted under a dissection microscope.

To compare the captures of *S. titanus* in abandoned and cultivated vineyards in the three years of survey 2, Mann-Whitney test was used. To compare the captures of *S. titanus* in different positions inside cultivated vineyards (surveys 1 and 2) repeated measures ANOVA for matched observations and Tukey-Kramer multiple comparisons test were used considering sampling dates as repeated measures. Prior to the analysis, data were transformed to log10 (x + 1) to respect ANOVA assumptions. In survey 2/2005, when the captures of *S. titanus* were monitored in five different positions inside the treated vineyards, the *S. titanus* adult spatial distribution was compared with those predicted by the power law model that has previously been shown to describe disease gradients by a natural source of infectious vectors (Gregory, 1968; Purcell, 1974; Bressan *et al*., 2007; Mori *et al*., 2008). To establish the statistical significance of the power law function, the log-log regression between captures and distance from the *S. titanus* source was calculated. Statistical analysis was performed with GraphPad Instat 3 for Macintosh.

Results

Gradients in the incidence of grapevines affected by FD in vineyards

In the vineyards No 2 and 4 significant decreasing abundances of grapevines affected by FD were observed as the distance from a border row or a border across rows increased (figure 3; table 1). In the vineyard No 2

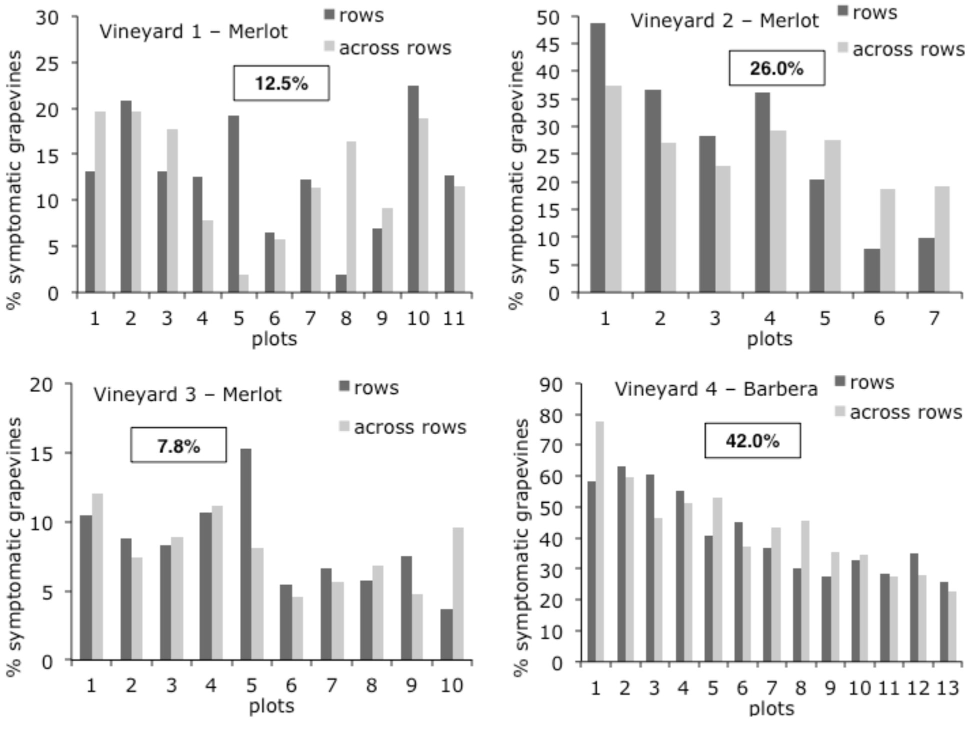

Figure 3. Percentage of grapevines affected by FD observed in each row and in an equal number of plots across rows (plot 1 and the plot with the highest number are the two border rows or the two border plots across row).

Table 1. Comparison of the observed percentages of infected grapevines between and across rows with those predicted by the power law model by calculating the log-log regression between the percentage of infected grapevines and the distance from vineyard borders.

Vineyard No	Between rows	Across rows
1	$Y = 29.8X^{-0.32}$; $P = 0.37$; $R^2 = 0.09$	$Y = 23.3^{-0.22}$; $P = 0.45$; $R^2 = 0.06$
2	$Y = 205.9X^{-0.85}$; $P < 0.01$; $R^2 = 0.70$	$Y = 54.1^{-0.29}$; $P < 0.03$; $R^2 = 0.65$
3	$Y = 18.7^{-0.31}$; $P = 0.090$; $R^2 = 0.32$	$Y = 19.4^{-0.27}$; $P = 0.088$; $R^2 = 0.32$
4	$Y = 181.5X^{-0.52}$; $P < 0.001$; $R^2 = 0.82$	$Y = 257.0X^{-0.47}$; $P < 0.001$; $R^2 = 0.83$

the gradients were decreasing from east to west and from north to south and appeared associated with the distance from the severely FD-infected vineyard because the highest incidence of FD are in the two borders nearest to this vineyard (figure 4). In the vineyard No 4 the gradients were decreasing from south-east to north-west and from south-west to north-east and appeared associated with the distance from the grove with grapevines growing wild and infested by *S. titanus* (figure 4).

On the contrary, in the vineyards No 1 and 3, where no potential sources of infectious *S. titanus* were found in surrounding areas, no significant gradients from borders were observed (figures 3 and 5; table 1).

Population density of *S. titanus* in abandoned and cultivated vineyards

The population density of *S. titanus* adults in abandoned and cultivated/untreated vineyards was significantly higher than in cultivated/treated vineyards (figure 6A). In three out of four years the captures in abandoned vineyards were on average higher than in cultivated/untreated vineyards but the differences were not significant due to the high variability in the captures of *S. titanus* observed in the two vineyard groups.

The type of active ingredients applied against *S. titanus* influenced the captures of the vector (figure 6B). In particular, the lowest captures were observed for

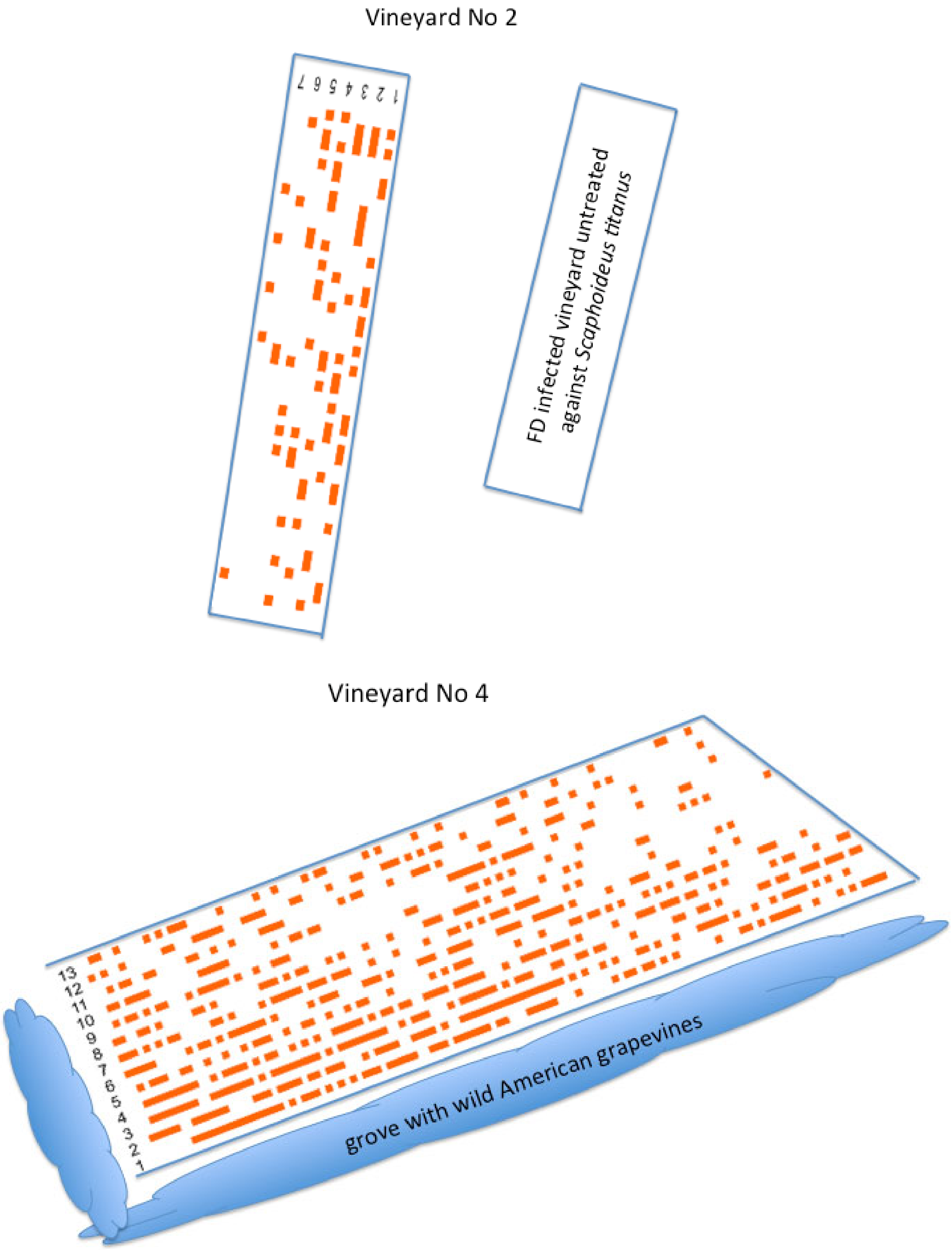

Figure 4. Maps of vineyards No 2 (upper) and 4 (bottom) with spatial distribution of symptomatic grapevines and potential sources of infected *S. titanus*.

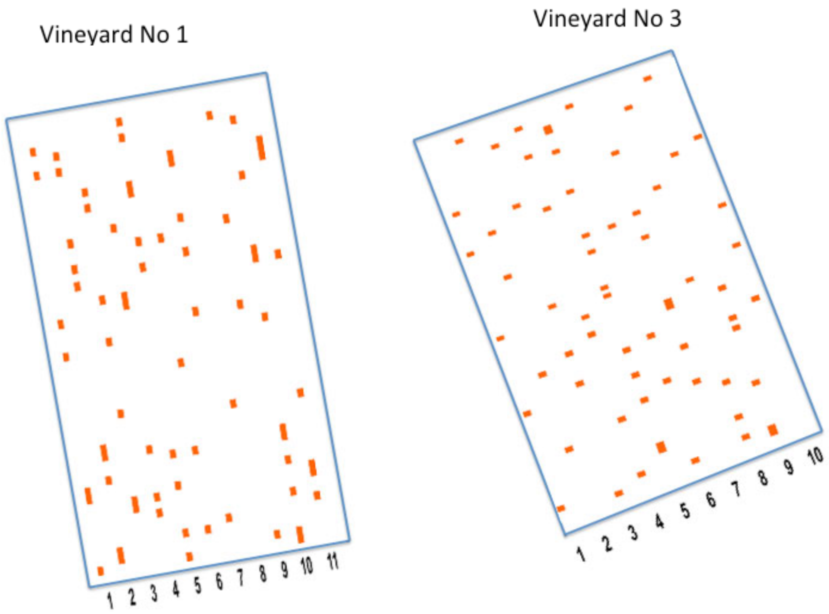

Figure 5. Maps of vineyards No 1 (left) and 3 (right) with spatial distribution of symptomatic grapevines.

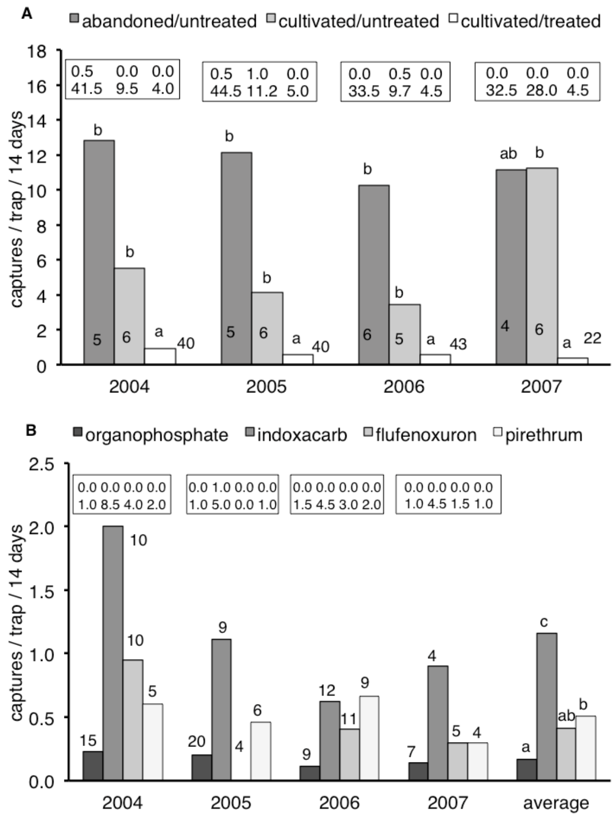

Figure 6. Captures of *S. titanus* adults recorded during 2004-2007 in vineyards located in a grape-growing area of northeastern Italy. (A) Comparison among abandoned and cultivated (treated or untreated against the vector) vineyards. (B) Comparison among different active ingredients applied in the vineyards treated against the vector. The numbers inside or above the columns indicate the number of sampled vineyards. Different small letters above the columns indicate significant differences at the 0.05 level (Dunn's multiple comparisons test). The numbers in the upper part of the figures indicate the min and max of captures in the different types of sampled vineyards.

organophosphate and the highest for indoxacarb. However, for all the active ingredients vineyards without captures were observed. During 2004-2007 in only 15 cases out of 145 the vineyards treated with insecticides against *S. titanus* showed more than two captures/trap/14 days. In four cases the sampled vineyards were bordered by abandoned or cultivated/untreated vineyards, with another four cases bordered by groves containing wild American grapevines, and in the remaining seven cases the insecticides applied were indoxacarb or pyrethrum. The first active ingredient is little effective against *S. titanus*, while the second shows a high knock-down effect but has a low residual activity (Pavan *et al*., 2005b).

S. titanus ability to colonize cultivated/treated vineyards

Survey 1

In the two sampling positions inside the treated vineyard the captures of *S. titanus* were significantly lower than in the untreated vineyards (figure 7). Considering the total captures, no differences were observed between the two positions inside the treated vineyard.

Considering the dynamics of captures over time, during the two weeks after the insecticide application a significant reduction in the number of *S. titanus* adults was observed in the treated vineyard (figure 7). The next two weeks the differences remains significant only between the untreated and treated inside. By one month after the treatment, the differences were not longer significant.

Survey 2

In all the three years the captures were significantly higher in the abandoned vineyard than in the contiguous cultivated/treated vineyard (figure 8). In 2005 inside the treated vineyard the total captures in the border row contiguous to the abandoned vineyard were significantly higher than in the other sampled rows (figure 9A). The variation in *S. titanus* captures according to the distance from the abandoned vineyard was described well by a power law curve showing the existence of a gradient of

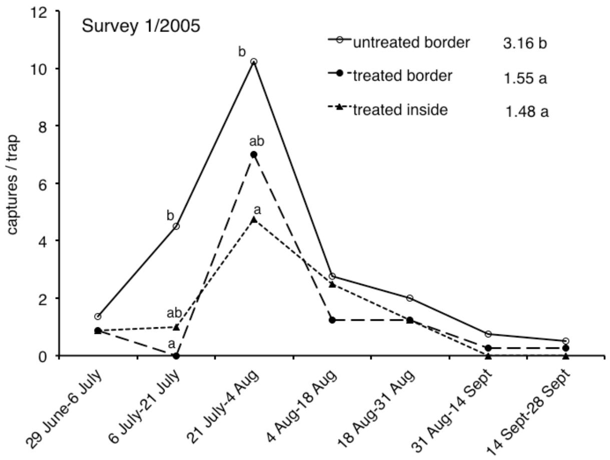

Figure 7. Captures of *S. titanus* adults recorded in 2005 from late June to late September in an untreated vineyard and in a contiguous treated vineyard at different distances from the former. In the treated vineyard clorpyriphos-ethyl was sprayed on July 6th. The traps were placed in both vineyards at 4 m from the opposite border sides and also located 36 m inside the treated vineyard. Different small letters among positions near legend (the values show captures/trap/sampling period) indicate statistical differences at the 0.05 level (Tukey's test). Different small letters among positions within the same sampling period indicate statistical differences at the 0.05 level (Tukey's test).

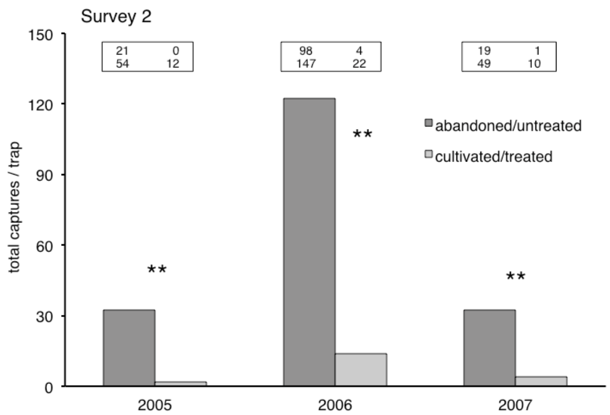

Figure 8. Captures of *S. titanus* adults during 2005-2007 in an abandoned vineyard and in the inside part of a contiguous cultivated vineyard (traps on the borders are not considered). The cultivated vineyard was treated every year against the vector. For each year the asterisks indicate significant differences at the 0.01 level (Mann-Whitney test). The numbers in the upper part of the figure indicate the min and max of captures in the traps.

captures from the border side contiguous with the abandoned vineyard to the opposite border side (figure 9B). Considering the dynamics of captures over the time, in all the sampling periods the number of *S. titanus* adults recorded in the vineyard row contiguous to the abandoned vineyard was higher than in the other rows, even though

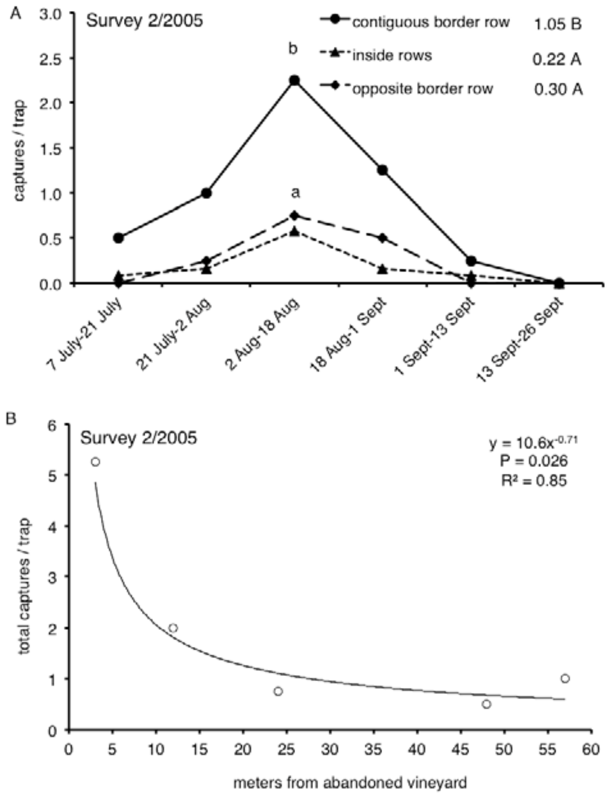

Figure 9. Captures of *S. titanus* adults recorded during the summer in survey 2/2005 in a cultivated/treated vineyard contiguous to an abandoned vineyard. (A) Dynamic of captures from early July to late September in the two border rows and in the inside rows (average of R4, R8, R16). In the treated vineyard flufenoxuron was sprayed on June 23th. Different capital letters among positions near legend (the values show captures/trap/sampling period) indicate statistical differences at the 0.01 level (Tukey's test). Different small letters among positions within the same sampling period indicate statistical differences at the 0.05 level (Tukey's test). (B) Gradient of total adult captures from the abandoned vineyard. The relationship was described by a significant power law function.

the differences were significant only in August (figure 9A).

Also in the following years (surveys 2/2006 and 2/2007) the captures from border rows contiguous with the abandoned vineyard were significantly higher than those observed in the opposite border side (figures 10A and 10B). Considering the dynamics of captures over the time, both years in all the sampling periods the number of *S. titanus* adults recorded in the vineyard row contiguous to the abandoned vineyard was higher than in the other sample rows, even though the differences were significant only in July of survey 2/2006 (figure 10A).

Discussion

The occurrence of decreasing gradients of FD infected grapevines from vineyard borders shows that external sources of infectious *S. titanus* can play an important

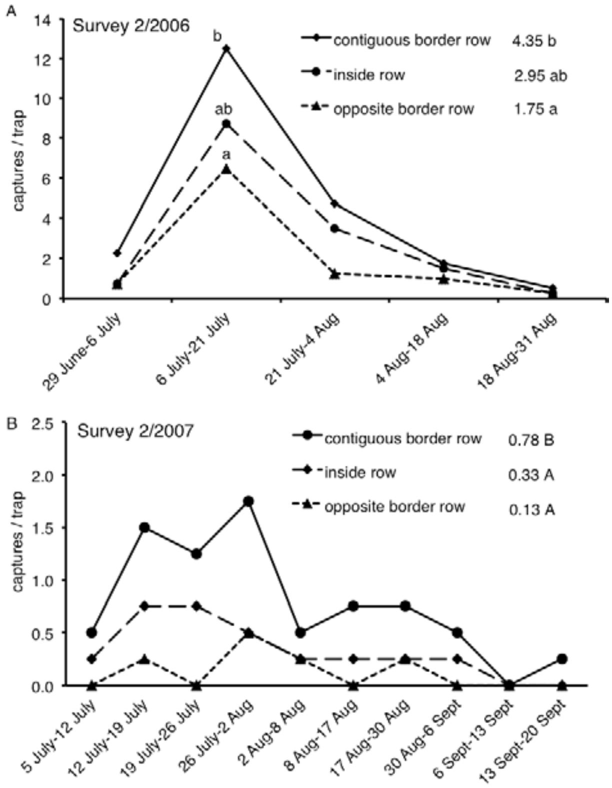

Figure 10. Captures of *S. titanus* adults recorded during the summer in survey 2/2006 (A) and survey 2/2007 (B) in three different positions of a cultivated/treated vineyard (flufenoxuron on June 22^{th} 2006, pyrethrum on June 25^{th} 2007) contiguous to an abandoned vineyard. Different small and capital letters among positions near legend (the values show captures/trap/sampling period) indicate statistical differences at the 0.05 and 0.01 levels, respectively (Tukey's test). Different small letters among positions within the same sampling period indicate statistical differences at the 0.05 level (Tukey's test).

role in the epidemiology of FD. Besides, the fact that the decreasing gradients were observed in vineyards with the highest incidence of infected grapevines suggests that external sources of infectious vectors can be more dangerous than internal ones. These high levels of FD occurred even though the vineyards No 2 and 4 were treated at least twice a year with insecticides active against *S. titanus*. Analogies with another European Grape Yellows Disease, Bois noir nettle type, are evident. In fact, most of the vineyards affected by this phytoplasmosis are characterized by: (i) external sources of infectious *Hyalesthes obsoletus* Signoret vectors (usually nettle along ditches surrounding vineyards), (ii) the occurrence of decreasing gradients of infected grapevines and (iii) the ineffectiveness of insecticide treatments when applied to these vineyards (Maixner, 2006; Mori *et al*., 2008; 2012).

The decreasing gradient of FD infected grapevines from vineyard borders is reflected in a decreasing gradient of *S. titanus* captures from borders contiguous to external sources of the vector. These can be represented by cultivated vineyards untreated against *S. titanus*, abandoned vineyards and grapevines growing wild in woodland. In these last vegetational structures the population levels of *S. titanus* can be comparable to or higher than those recorded in cultivated vineyards untreated against the vector. Since the vector can move from untreated vineyards to contiguous vineyards treated with insecticides, the efficacy of the same active ingredient against *S. titanus* appears lower if the treated vineyard is contiguous to an external source of the leafhopper. The migration of *S. titanus* adults from abandoned vineyards to cultivated ones could be favoured by the fact that the leaves of uncultivated European grapevines are usually severely affected by fungus diseases during the summer. However, the captures remain very higher in the vineyards source of *S. titanus* than in those adjacent. This indirectly confirmed the low mobility of *S. titanus* adults (Lessio and Alma, 2004).

If untreated vineyards, both cultivated and abandoned, are FD infected, they can be a potential source of infectious *S. titanus* adults for other cultivated vineyards, particularly for plants placed on contiguous border sides. Therefore, the results of this research confirm not only the importance of treating all vineyards against *S. titanus*, but they also suggest the need to remove abandoned vineyards.

With wild American grapevines growing in hedgerows or groves as the potential source of *S. titanus*, the movement of the vector from woodlands to vineyards has been observed in North America (Maixner *et al*., 1993; Beanland *et al*., 2006), whereas it did not apparently occur in a grape growing-area of northwestern Italy (Lessio *et al*., 2007). In any case, in grape-growing areas affected by Grape Yellows Disease, the high incidence of infectious *S. titanus* specimens detected on wild growing grapevines (Maixner *et al*., 1993; Lessio *et al*., 2007) and the border effect in symptomatic grapevines, both in North America (Maixner *et al*., 1993; Beanland *et al*., 2006) and in Italy (this research), suggest the removal of these plants. Also, the unsatisfactory efficacy of insecticide treatments in the control of FD occurring in some vineyards contiguous to woodland vegetation supports this decision. However, the complete removal of hedgerows or groves in the near vicinity of vineyards is not advisable, as *S. titanus* is able to complete its biological cycle only on *Vitis* spp. (Vidano, 1964; Maixner *et al*., 1993) and spontaneous plants can harbour natural enemies of many pests.

If it is not possible to remove grapevines that are a potential source of infectious *S. titanus*, an appropriate separation between uncultivated grapevines and cultivated vineyards could be advisable, as suggested in North America (Beanland *et al*., 2006). Research on the mobility of *S. titanus* would indicate that distance between uncultivated grapevines and cultivated vineyards has to be at least 40 m (Lessio and Alma, 2004; Beanland *et al*., 2006). When it is not profitable to respect this distance, and taking into consideration the different cultivar susceptibility to FD (Caudwell, 1990; Posenato *et al*., 1996; Pavan *et al*., 1997; 2012; Bellomo *et al*., 2007), a valid alternative could be to plant the less susceptible grape varieties near woodland vegetation.

Nowadays, in the context of FD control strategies, the recovery of infected plants plays an important role (Osler *et al*., 2000; Bellomo *et al*., 2007; Pavan *et al*., 2012). Recovery can be favoured by resistance inducers and, in the future, by the use of fungi and bacteria antagonists of phytoplasmas (Martini *et al*., 2009; Romanazzi *et al*., 2009; Bulgari *et al*., 2011; Musetti *et al*., 2011; Bianco *et al*., 2012). However, recovery is a useful tool for FD control only if new infections are prevented. This can be obtained with traditional approaches based on chemical control of *S. titanus* and removing of external sources of infectious *S. titanus*. In the future, the vector could be also controlled with a symbiotic approach (Marzorati *et al*., 2008) and with mating disruption based on substrate-borne vibrational signals (Mazzoni *et al*., 2009; Eriksson *et al*., 2012).

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