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Factors affecting the spread of "Bois Noir" disease in north Italy vineyards

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Summary

To define control strategies for "Bois Noir" disease (BN) it is necessary to know factors favouring its spreading by the vector *Hyalesthes obsoletus* Signoret. During 2003-2006 a research was carried out in 18 vineyards of a grape-growing area of North Italy to assess the influence of insecticides, applied on grapevine canopies, and environment surrounding the vineyards on disease spreading. The vector population density was higher outside than in the centre of the vineyards. Insecticides applied to grapevine canopies did not significantly influence the vector population level in the centre of the vineyards. The majority of vineyards showed randomized distribution of symptomatic grapevines. Seven vineyards had an aggregate distribution due to an edge effect from a border side with nettle. The incidence of border sides not contiguous to other grapevine rows on vineyard surface was positively related to higher levels of BN. The incidence of border sides with nettle on vineyard surface was positively correlated to disease incidence in the vineyards with aggregate distribution of symptomatic grapevines. All the data support the importance of surrounding vegetation as source of inoculum of BN phytoplasma. Molecular analyses on ribosomal and tuf genes show that 16 out of the 18 vineyards were affected only by BN: in 13 only tuf-type I was identified, in 2 only tuf-type II, in 1 both tuf-types, and in 2 it was not possible to identify the tuf-type of phytoplasmas detected. In the weeds tested only tuf-type II phytoplasmas were identified while *H. obsoletus* was carrying both phytoplasma tuf-types.

K e y w o r d s : Grapevine yellows, *Hyalesthes obsoletus*, phytoplasma, epidemiology, chemical control.

Introduction

Bois noir (BN) is a grapevine yellows (GY) associated with 16SrXII-A phytoplasmas causing severe damages in European vineyards. BN phytoplasmas were transmitted to grapevine by *Hyalesthes obsoletus* Signoret (Homoptera, Cixiidae) using as source of inoculum *Convolvulus arvensis* L. (Maixner 1994, Sforza *et al*. 1998) and *Urtica dioica* L. (Alma *et al*. 2002, Bressan *et al*. 2006). However, these phytoplasmas were also detected in other plants and auchenorrhyncha species that are supposed to be involved in BN epidemiology (Maixner 2006).

Recently, within the 16SrXII-A ribosomal subgroup (stolbur phytoplasmas), in Germany three different strains were distinguished on the basis of tuf gene sequences (Langer and Maixner 2004). These strains show specificity for different plant species: type I was detected only in *U. dioica*, type II was found in *C. arvensis* and in *Calystegia sepium* L. and type III was recorded only in *C. sepium*.

In Germany the population level of the vector inside the vineyards is influenced by the ground coverage of *C. arvensis* and highest levels of BN - type II were observed in the grape-growing areas where *H. obsoletus* is more abundant in the vineyards (Maixner and Reinert 2000; Darimont and Maixner 2001). However, in the grape-growing areas of central Europe, where BN is mostly associated to *C. arvensis* (Maixner *et al*. 1995; Sforza *et al*. 1998), the chemical control of the vector is considered not suitable because the nymphs are hidden in the soil and the planthopper host plants grow also outside the vineyards (Sforza and Boudon-Padieu 1998; Weber and Maixner 1998).

In some grape-growing areas of North Italy the high density of the vector on nettle outside the vineyards, and the edge effect observed both for *H. obsoletus* (Credi *et al*. 2004, Bressan *et al*. 2006) and for BN disease (Arzone 1993, Cavallini *et al*. 2003, Credi *et al*. 2004), would indicate that herbaceous vegetation surrounding the vineyards can be an important phytoplasma source for grapevines. In North Italy insecticides applied on the grapevine canopy seem to influence neither the disease (Pavan 1989, Pavan *et al*. 1989) nor the captures of *H. obsoletus* in grapevine canopy (Mori *et al*. 1999, Cavallini *et al*. 2003).

To define control strategies, factors favouring BN spreading in the different grape-growing areas must be well investigated. Therefore, a research was carried out in a grape-growing area of North Italy to assess the influence of insecticides, applied on grapevine canopies, and of environment surrounding the vineyards on disease spreading.

Material and Methods

In the period 2003-2006 a study was conducted in 18 vineyards (13 in the first year) located in a grape-growing area of the Emilia-Romagna region (North Italy) (Tab. 1). In the first year of study only BN phytoplasmas were detected in the investigated vineyards. In 2006 vine-

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Table 1

Characteristics of the sampled vineyards

Vineyard N. (*)	Area (m^2)	Border sides not contiguous to grapevine rows (m)		Ground covered by *C. arvensis* (classes **)	Ratio border side length / vineyard surface		Spatial distribution of symptomatic grapevines		Phytoplasmas identified in grapevine (N. samples)		
		total	with *U. dioica*		total	with nettle	frequency distribution	edge effect	16Sr XII-A	type I	type II
1	15513	336	64	1	0.025	0.005	Poisson	no	1	1	0
2	22482	570	130	3	0.025	0.006	Poisson	no	3	2	0
3	1960	265	15	2	0.135	0.008	Poisson	no	2	2	0
4	8169	426	10	1	0.052	0.001	Poisson	no	2	0	1
5	7734	226	0	2	0.029	0.000	Poisson	no	5	4	0
6	30478	554	10	4	0.018	0.000	Poisson	no	5	3	0
7	6424	176	52	2	0.027	0.008	Poisson, negative binomial	yes	1	0	0
8	5502	291	6	2	0.053	0.001	Poisson	no	13	6	1
9	12072	576	192	1	0.048	0.016	negative binomial	yes	2	2	0
10	5040	288	75	4	0.057	0.015	Poisson, negative binomial	yes	2	1	0
11	9778	302	40	3	0.031	0.004	Poisson	no	1	0	0
12	14450	564	191	3	0.039	0.013	negative binomial	yes	1	1	0
13	9596	177	55	1	0.018	0.006	Poisson	no	2	0	1
14	27196	478	118	2	0.018	0.004	negative binomial	yes	11	6	0
15	16428	270	59	3	0.016	0.004	negative binomial	yes	5	3	0
16	7404	190	52	2	0.026	0.007	Poisson, negative binomial	yes	8	2	0
17	3877	354	0	3	0.091	0.000	Poisson, negative binomial	no	6	3	0
18	5219	253	20	2	0.048	0.004	Poisson	no	3	2	0

(*) all the vineyards are cv. 'Lambrusco', except N. 9 that is 'Chardonnay'. (**) 1 = < 10 %; 2 = 10-20 %; 3 = 20-30 %; 4 = > 30 %.

yard 9, the only of 'Chardonnay', was removed since it was not anymore productive.

Map of the vineyards: A map of each investigated vineyard (symptomatic and asymptomatic grapevines) and of the surrounding habitat (border sides contiguous or not to other grapevine rows) was made. The border sides not contiguous to other grapevine rows were rich in spontaneous herbaceous vegetation, mostly along edgerows and ditches. The area of the mapped vineyards, the total length of border sides not contiguous to other grapevine rows and the length of the border sides with *U. dioica* were measured. The percentage of vineyard ground covered by *C. arvensis* was estimated and the vineyards grouped in four covering classes (1 = < 10 %; 2 = 10-20 %; 3 = 20-30 %; 4 = > 30 %). The percentage of symptomatic grapevines was estimated for four consecutive years; from the second year the percentage of new symptomatic grapevines and the percentage of recovered grapevines were calculated respectively on the basis of the asymptomatic and symptomatic grapevines in the previous year.

PCR and RFLP analyses: In the investigated vineyard habitats symptomatic grapevines, spontaneous plants with suspected symptoms of phytoplasma disease *(Urtica dioica, Ulmus minor, Convolvolus arvensis*, *Aristolochia clematidis*, *Calistegia sepium*, *Galium aparine*, *Medicago sativa*, *Mentha* sp., *Plantago lanceolata*, *P. major*, *Salix sp.*) and *H. obsoletus* were collected and tested to verify phytoplasma presence and identity. Nucleic acid was extracted following described procedures for grapevines and herbaceous samples (PRINCE *et al*. 1993) and insects (ANGELINI *et al*. 2001). Nested-PCR followed by restriction fragment length polymorphism (RFLP) analyses on 16S ribosomal gene and on tuf gene for phytoplasma molecular characterization was performed as described (DUDUK *et al*. 2004). Informative restriction enzymes employed were *Tru*I and *Taq*I on 16S gene and *Hpa*II on tuf gene.

H. obsoletus samplings: In each vineyard the population of *H. obsoletus* adults on herbaceous vegetation, inside (central part) and outside (around) the vineyards, were weekly monitored from June to August using sweep nets. In 2003 the sampling was carried out only on herbaceous vegetation outside the vineyard. At each sampling the herbaceous vegetation was swept 60 times, both inside and outside the vineyards, collecting the specimens captured in the net every six sweeps.

Each year in the autumn, information about insecticides applied to grapevine canopies were collected, and the vineyards were grouped according to the number of neurotoxic insecticides sprayed during the flight of *H. obsoletus* adults.

Data analysis: The means of the data (number symptomatic, new symptomatic or recovered grapevines and number of vector captures) that passed the normality test were compared with ordinary ANOVA (repeated measured ANOVA for matched observations) and Tukey-Kramer multiple comparisons test.

The means of the data that did not pass the normality test were compared with Mann-Whitney U-test (two unmatched samples), Kruskal-Wallis test (more than two unmatched samples) or Friedman test (more than two matched samples). For multiple comparisons Dunn's post-test was used.

Relationships between two variables were studied with linear regression analysis. To test the relationship between *H. obsoletus* population density, or chemical control intensity, and the disease, the number of new symptomatic grapevines in each year was related to the number of vector captures, or to the number of insecticide treatments, both in the previous and in the same year.

To study the spatial distribution of the disease in the vineyards, the grapevines were grouped in sampling units of nine plants. The observed frequencies of symptomatic plants per sampling unit were compared with the expected frequencies generated according Poisson and negative binomial models using the chi-square test (Fowler *et al*. 1998). When the frequency distribution of symptomatic plants in a vineyard fitted to a negative binomial distribution, it was investigated if this was the consequence of a gradient of symptomatic plants decreasing from a border side with high density of *U. dioica*. The vineyards were divided in six zones of equal size characterized by increasing distance from the considered border side. For each zone the number of grapevines symptomatic in the first sampling year and in at least one of the sampling years was counted. The existence of a gradient was determined comparing the percentage of symptomatic grapevines in the six zones with the chi-square test. The observed disease gradients were compared with those predicted by power low model, that well describes disease gradients by a natural source of infectious vectors (Gregory 1968, Purcell 1974).

Results

H. obsoletus density and flight period: In 2003 the captures outside the vineyards were higher than in the following years, but the data are not comparable because of the different number of sampled vineyards (13 in 2003 and 18 in the other years) (Fig. 1). Considering the 18 vineyards monitored during the period 2004-2006 (17 in 2006), the total captures of *H. obsoletus* on herbaceous vegetation were higher in the first two years, even if the differences were not significant because of the high variability among vineyards within each year (Fig. 1). In the three years in which *H. obsoletus* was sampled in two different positions, the captures were always higher outside than inside the vineyards (significant differences at Mann-Whitney U-test in 2004 and 2005).

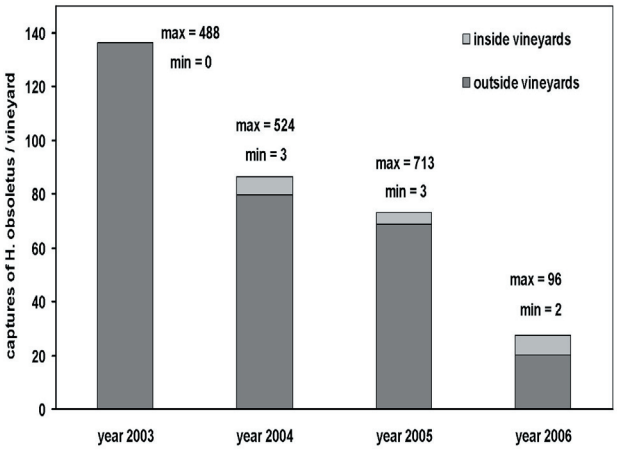

Fig. 1: Average captures recorded with sweep net from June to September in 18 vineyards (13 in 2003) sampled during 2003-2006. inside = herbaceous vegetation in the centre of the vineyards (non considered in 2003); outside = herbaceous vegetation surrounding the vineyards. Max and min of the total catches recorded in the sampled vineyards are reported.

In the four years of the study the captures of *H. obsoletus* outside the vineyards began in mid June-early July and ended in early-late August. The peak of captures was always observed in July (Fig. 2).

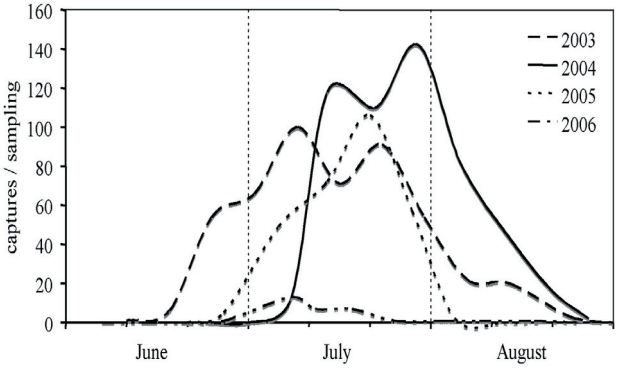

Fig. 2: Flight of *H. obsoletus* recorded on *U. dioica* and *C. arvensis* surrounding the vineyards using sweep net.

Identification of GY: Among the grapevine samples tested 2 to 13 for each vineyard resulted positive to the presence of 16SrXII-A phytoplasmas (Tab. 1); in vineyards 1 and 11 "Flavescence dorée" phytoplasmas were also identified (data not shown). Nested-PCR amplification on tuf gene provides amplicons from the major-

ity of BN infected grapevine samples; only two vineyards (7 and 11) were providing no amplification with this gene (Tab. 1). RFLP analyses showed the clear prevalence of tuf-type I BN phytoplasmas (Langer and Maixner 2004), only in three vineyards tuf-type II was identified (Tab. 1).

The tests performed on the spontaneous plant species collected allow the identification of 16SrXII-A phytoplasmas in *U. dioica*, *C. arvensis*, *M. sativa*, *P. lanceolata* and *Salix* sp.; only in *C. arvensis* and *M. sativa* it was possible to obtain amplification of tuf gene, and both species were infected by tuf-type II "Bois Noir" phytoplasmas (data not shown). Molecular analyses performed on 270 *H. obsoletus* insects allow the detection of 16SrXII-A phytoplasmas in 105 insects, among these 85 % were carrying tuf-type I and the rest was carrying tuf-type II phytoplasmas.

Symptomatic grapevines over the years: During 2003-2006 the average percentage of symptomatic grapevines in the 10 vineyards with 'Lambrusco', affected by BN and sampled every year, was equal to 10.6 without significant statistical differences among the years (Fig. 3). On average the 7.5 % of asymptomatic grapevines in the previous year showed the disease (Fig. 3). In 2006 the percentage of new symptomatic grapevines was significantly lower than in 2004. On average the 64.3 % of grapevines symptomatic in the previous year recovered (Fig. 3). In 2006 the incidence of recovery decreased significantly in comparison with previous years.

Border sides and herbaceous vegetation: The ratio length of border sides not contiguous to other grapevine rows/vineyard surface was highly variable (Tab. 1) in relation to the width of the mapped plot and to its belonging to a wider vineyard. It was maximum when the sampled plots were short, of irregular shape and not contiguous to rows of other grapevine cultivars.

The ratio length of border sides with nettle/vineyard surface was very variable being influenced by both the presence of *U. dioica* and by the same factors mentioned above. *C. arvensis* was present on the ground inside and outside the vineyards; in some vineyards it was rare, whereas in others it covered a wide part of the ground (Tab. 1).

Spatial and frequency distribution of symptomatic grapevines: Frequency distribution of symptomatic grapevines, recorded in the first year of sampling, fitted only to Poisson distribution in 10 cases, only to negative binomial distribution in 4 cases and to both distributions in 4 cases (Tab. 1). The two vineyards affected also by FD (1 and 11) fitted to Poisson model. The vineyards in which the symptomatic grapevines showed an aggregate distribution were, except one (17), also those with a high presence of *U. dioica* along at least one border side of the vineyard. The zone of the vineyard nearest to this border side, showed a percentage of symptomatic grapevines significantly higher of that in the inner zones (Tab. 2). The vineyard 17 did not show an edge effect, but in this case the distribution of symptomatic grapevines did not reject either Poisson model (Tab. 1). The power low model significantly described the edge effect in six of the seven considered cases (Tab. 2). In the vineyard 16 the distribution of the symptomatic plants would suggest the existence of two sources of inoculum opposite each to other.

Factors influencing the captures of *H. obsoletus* on herbaceous vegetation in the centre of the vineyards: *H. obsoletus* captures on the herbaceous vegetation inside the vineyards was apparently not influenced by those on herbaceous vegetation outside the vineyards (in the four years of the study the P of the regression analyses ranged from 0.15 to 0.54).

The number of insecticide treatments applied on grapevine canopies during the flight of *H. obsoletus* did not significantly influence planthopper captures inside the vineyards (in the four years the P, at Kruskal-Wallis test, range from 0.19 to 0.78) (Fig. 4). However, considering the classes one and two treatments per year, the captures were on average lower where two times insecticides were sprayed. The amount of *H. obsoletus* captured inside the vineyards was not related to *C. arvensis* coverage of the ground: in the three years the P of the regression was respectively 0.07, 0.96 and 0.70.

Factors influencing BN disease: Vineyards 1 and 11 were not included in the analyses because affected also by FD, as well as vineyard 9 because of a different cultivar. Therefore, only the 15 vineyards of cultivar Lambrusco and affected only by BN were considered.

The percentage of new symptomatic grapevines was not related to the insect captures inside the vineyards recorded either in the following year or in the same year (Tab. 3). The relationship between vector captures out-

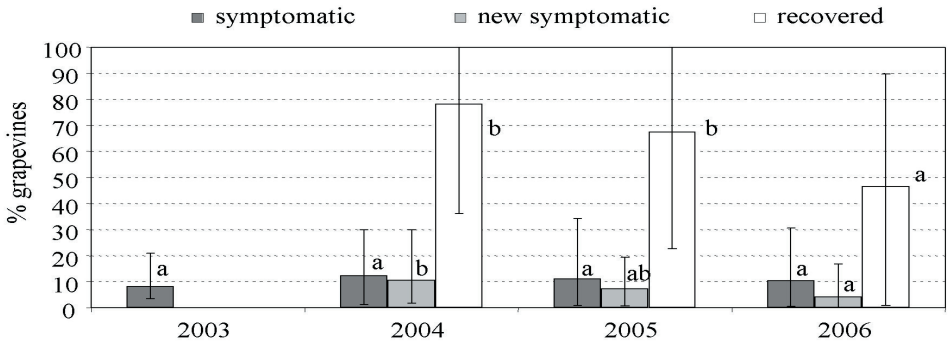

Fig. 3: Average percentage of grapevines symptomatic, new symptomatic (calculated on the asymptomatic in the previous year) and recovered (calculated on the symptomatic in the previous year) recorded in during 2003-2006 in the 10 Lambrusco vineyards sampled all the four years and infected by BN phytoplasma. Bars indicate the range observed in the sampled vineyards. Different small letters indicate significant differences per $p \leq 0.05$.

Table 2

Percentage of symptomatic grapevines observed in the six zones characterized by increasing distance by a border side with *U. dioica* (1= border zone) and fitting of the data to power law model

Vineyard N. (m^2/zone)	Zone						Power law regression	
	1	2	3	4	5	6	P	R^2
7 (1071)								
1st year	29.1 d	17.9 c	3.7 a	10.5 b	9.0 ab	8.2 ab	0.13	0.46
total	78.5 d	47.8 c	27.6 a	31.4 ab	40.3 bc	24.7 a	0.03	0.74
9 (2012)								
1st year	25.0 c	21.5 c	12.5 b	8.9 b	8.3 b	0.6 a	0.08	0.57
total	45.3 e	29.8 d	20.9 cd	14.9 bc	8.3 b	1.2 a	0.04	0.88
10 (840)								
1st year	16.5 b	6.7 a	7.1 a	4.3 a	6.3 a	5.9 a	0.04	0.69
total	23.5 c	9.8 ab	11.4 ab	8.6 ab	8.2 a	14.1 b	0.17	0.41
12 (2408)								
1st year	19.7 c	10.1 b	6.3 a	4.5 a	4.0 a	5.4 a	0.004	0.90
total	42.4 d	41.7d	24.0 c	19.3 b	7.2 a0	10.8 a	0.02	0.77
14 (4033)								
1st year	18.0 d	10.1 c	3.2 b	2.8 b	2.2 ab	1.4 a	< 0.001	0.96
total	51.3 f	40.7 e	19.4 d	14.5 c	10.7 b	6.9 a	0.002	0.93
15 (2738)								
1st year	9.2 c	5.5 b	3.5 ab	3.1 ab	3.3 ab	2.4 a	< 0.001	0.98
total	34.0 d	20.4 c	13.4 ab	10.7 a	12.9 ab	15.3 b	0.03	0.73
16 (1234)								
1st year	13.1b	2.4 a	2.4 a	3.9 a	5.8 a	3.4 a	0.33	0.24
total	25.8 d	9.7 ab	5.8 a	7.8 ab	13.1 bc	15.1 c	0.45	0.15

1st year = percentage of grapevines symptomatic in the 1st sampling year; total = percentage of grapevines symptomatic in at least one of the sampling years; P = statistical significance of the regression; R^2 = coefficient of determination

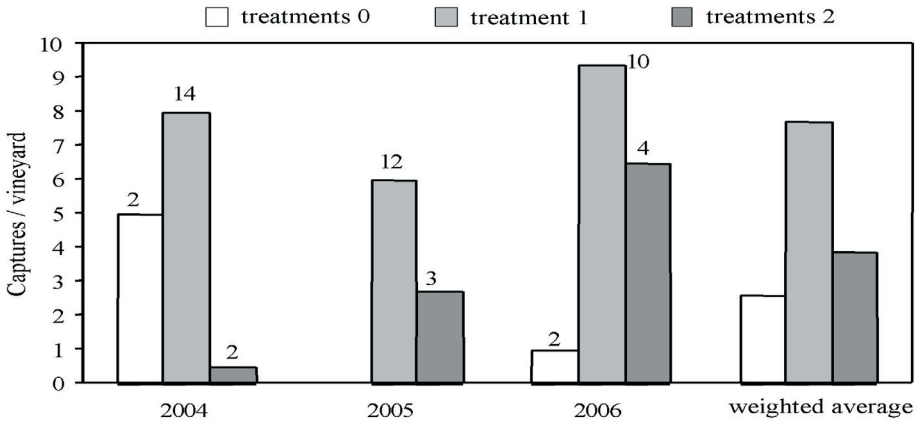

Fig. 4: Average captures of *H. obsoletus* using sweep net on herbaceous vegetation present in the central part of vineyards differently treated with insecticides (0, 1, 2 times) in the period of the adult flight. Only the group with at least two vineyards where considered. On the top of the columns the number of the vineyards within each group is reported. No statistical differences were observed.

side the vineyards and the percentage of new symptomatic grapevines in the next year was positive, even if it was significant only in one of the three considered years.

The number of insecticide treatments applied during *H. obsoletus* flight did not significantly influence the percentage of new symptomatic grapevines either in the same year or in the following year (Fig. 5).

A significant relationship between the incidence of the border sides not contiguous to other grapevine rows on vineyard surface, and the percentage of symptomatic grapevines in the first sampling year was observed considering all the sampled vineyards (Tab. 4). This relationship was still significant if only the vineyards whose frequency distribution fitted to Poisson model were considered, whereas it was not significant in relation the vineyards whose frequency distribution fitted to negative binomial model (Tab. 4). The relationship between the incidence of the border sides with *U. dioica* on vineyard surface, and the percentage of symptomatic grapevines in the first year of sampling was not significant considering both all the vineyards and those whose frequency distribution fitted to Poisson model; whereas, it became significant when the

Table 3

Regressions between the captures of *H. obsoletus* on herbaceous vegetation inside and outside the vineyards and percentage of new symptomatic grapevines in the same and in the next year. year/year = year of captures/year regarding new symptomatic grapevines

Capture year/new symptomatic year	N. vineyards	equation	P	R^2
inside the vineyards				
2004/2004	10	Y = 13.31-0.44X	0.42	0.08
2005/2005	15	Y = 9.75-0.52X	0.08	0.21
2006/2006	15	Y = 5.04-0.08X	0.36	0.07
2004/2005	15	Y = 9.99-0.31X	0.24	0.16
2005/2006	15	Y = 5.41-0.25X	0.14	0.16
outside the vineyards				
2004/2004	10	Y = 8.84+0.025X	0.25	0.16
2005/2005	15	Y = 7.46-0.0003X	0.99	< 0.001
2006/2006	15	Y = 3.15+0.05X	0.17	0.14
2003/2004	10	Y = 7.08+0.025X	0.23	0.18
2004/2005	15	Y = 6.85+0.008X	0.59	0.02
2005/2006	15	Y = 2.65+0.02X	0.04	0.29

Table 4

Regression between incidence of border sides not contiguous to other grapevine rows, total or with *U. dioica*, on vineyard surface and percentage of symptomatic grapevines observed in the first sampling year considering both all the vineyards and only those whose frequency distribution of symptomatic grapevines did not rejected per $p \leq 0.05$ two theoretical frequency distributions (Poisson and negative binomial ones)

	N. vineyards	equation	P	R^2
all vineyards				
total border sides	15	Y = 2.46+157.31X	0.004	0.48
border sides with *U. dioica*	15	Y = 7.02+450.08X	0.31	0.08
only vineyards that fitted to Poisson distributions				
total border sides	12	Y = 2.39+158.44X	0.016	0.46
border sides with *U. dioica*	12	Y = 7.23+612.58X	0.28	0.11
only vineyards that fitted to negative binomial distributions				
total border sides	7	Y = 7.56+8.57X	0.89	0.004
border sides with *U. dioica*	7	Y = 4.58+454.6X	0.049	0.46

vineyards whose frequency distribution fitted to negative binomial distribution were considered (Tab. 4). No positive relationship between percentage of ground coverage of *C. arvensis* and the percentage of symptomatic grapevines in the first year of sampling was found (Y = 16.3 -2.83X; P = 0.20, R^2 = 0.12).

Discussion

The *H. obsoletus* population density on herbaceous vegetation in the centre of the vineyards was not associated with the percentage of new symptomatic grapevines. The not significant influence of the insecticides, applied in the vineyards during the vector flight, on the disease spreading, indirectly confirms this result. This can be due

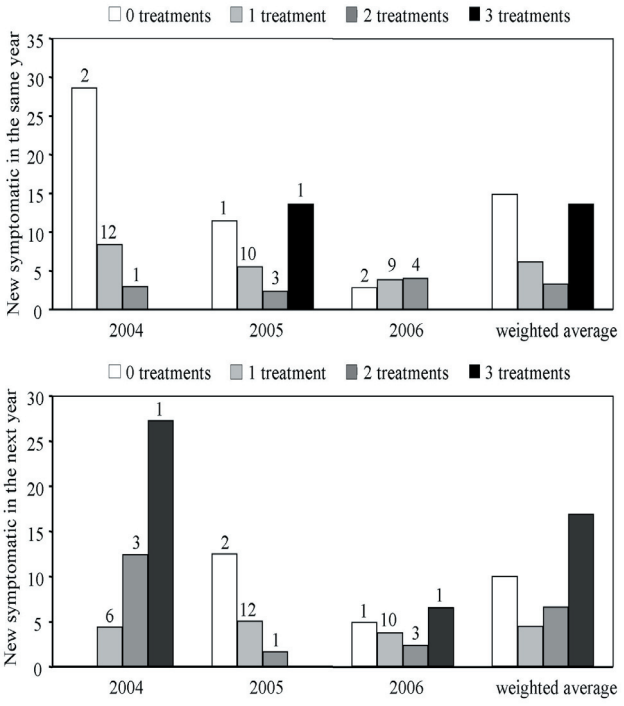

Fig. 5: Relationship between insecticide treatment numbers and new symptomatic grapevines in the same year and in the next one. On the top of the columns the number of the vineyards within each group is reported. For statistical analysis only the groups with at least two vineyards were considered. No statistical differences were observed.

to the occasional occurrence of vectors on the grapevine canopies, therefore it can be not closely correlated with underlying herbaceous vegetation, or to the fact that this herbaceous vegetation is not the most important source of infectious vectors. Three results of this research support this second hypothesis: (i) the highest is the incidence of the border sides not contiguous to other grapevine rows on the surface of the vineyards, the highest was the percentage of symptomatic grapevines in the first year of sampling; (ii) a significant positive relationship between the density of *H. obsoletus* outside the vineyards in 2005 and the new symptomatic grapevines in 2006; (iii) a significant edge effect in symptomatic grapevines from a border side with *U. dioica* observed in 7 of the sampled vineyards.

The influence of the border sides with nettle on the disease incidence agrees with the fact that the prevalent BN strain (tuf-type I) in the vineyards is reported to be associated to *U. dioica* and that this plant is localized mostly in the herbaceous vegetation surrounding vineyards. The absence of association of the disease with *C. arvensis* is in agreement with the fact that the strain of BN associated with bindweed (tuf-type II) was not very frequently identified in these vineyards. In the three vineyards with BN tuf-type II the symptomatic grapevines were randomly distributed according to literature (Maixner 2006).

The influence of border sides not contiguous to other grapevine rows on the vineyard symptom appearance supports the importance of the external disease source not only in the vineyards where the disease edge effect was observed, but in the case of BN tuf-type I it is important also in vineyards where the symptomatic grapevines were randomly distributed. In the considered grape-growing areas ineffectiveness of the insecticide treatments applied on grapevine canopies, indirectly confirms the important epidemic role of infectious vector external sources.

Therefore where BN is associated to *U. dioica*, the control strategies must mainly consider external sources of vectors and phytoplasmas. In the grape-growing areas where BN is associated to *C. arvensis*, the relationship between bindweed density inside the vineyards and disease incidence (Weber and Maixner 1998, Pavan and Stefanelli 2000) indicates that control strategies must consider also herbaceous vegetation inside the vineyard (Maixner 2006).

This research suggests that insecticide treatments in vineyards, differently from "Flavescence dorée" (Pavan *et al*. 2005), are not influent for BN control, and therefore no specific applications have to be advised. Other two practical indications from the results of this study are: (i) to plant width and regular shaped vineyards and (ii) to locate susceptible cultivars in the central part of a wider vineyard to reduce the border sides contiguous to spontaneous surrounding vegetation.

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