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ORIGINAL PAPER

Correlation of bois noir disease with nettle and vector abundance in northern Italy vineyards

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Abstract The phytoplasmas associated with bois noir (BN) tuf-type a are transmitted to grapevines by means of *Hyalesthes obsoletus* Signoret using *Urtica dioica* L. as the inoculum source. In the period 2003–2008, a research was carried out in six vineyards of northern Italy where a large amount of nettles in the ditches surrounding vineyards was detected. The aim was to establish the nettle control effects on the presence of *H. obsoletus* and new symptomatic grapevines. PCR and RFLP analyses showed the presence of the BN tuf-type a phytoplasmas in symptomatic grapevines of all vineyards and in the vector. Weed control strategies along ditches were always associated with decreases in nettle coverage and *H. obsoletus* numbers. A reduction in the percentage of new symptomatic grapevines occurred in all vineyards except for one vineyard where the least reduction in nettle and *H. obsoletus* was observed. The decrease in *H. obsoletus* population was significantly correlated with a decrease in nettles along ditches, and the reduction in *H. obsoletus* population was correlated with a decrease in incidence of new symptomatic grapevines compared with the incidence before the application of weed control measures. Nettle weeding can reduce the incidence of BN tuf-type a, but weed control costs and impacts need to be considered.

Keywords Grapevine yellows · Bois noir · *Hyalesthes obsoletus* · Nettle · Weed control

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Introduction

Bois noir (BN) is a grapevine yellows disease (GYs) associated with 16SrXII-A phytoplasmas, which are causing severe damage in European vineyards. BN phytoplasmas were transmitted to grapevines by means of the planthopper *Hyalesthes obsoletus* Signoret (Homoptera, Cixiidae), using *Convolvulus arvensis* L. (bindweed) (Maixner 1994; Sforza and Boudon-Padieu 1998; Sforza et al. 1999) and *Urtica dioica* L. (nettle) (Alma et al. 2002; Bressan et al. 2007) as the inoculum source. *H. obsoletus* overwinters as nymphs on the roots of these herbaceous plants and has one generation a year (Brcak 1979; Alma et al. 1988). The adults emerge from late spring, and occasionally, they feed on grapevines. Two molecularly differentiable strains of BN-phytoplasma, tuf-type a and b, are described as, respectively, associated with nettle and bindweed (Langer and Maixner 2004).

The epidemiology of BN nettle-type and BN bindweed-type shows some differences due to spatial distribution of the two herbaceous plants inside and outside vineyard areas

(Maixner 2007). *C. arvensis* spreads over both inside and outside the vineyard area (Sforza and Boudon-Padieu 1998; Maixner 2006), and the highest presence of BN tuf-type b and *H. obsoletus* is reported in vineyards where bindweed ground coverage is present (Maixner and Reinert 2000; Darimont and Maixner 2001). *U. dioica* is mainly located in the vineyard surrounding areas, and edge effects on spatial distribution of symptomatic grapevines and vector density were described, showing that nettles are an important source of BN tuf-type a phytoplasma for grapevines (Arzone et al. 1993; Cavallini et al. 2003; Credi et al. 2004; Bressan et al. 2007; Mori et al. 2008). The higher amount of nettles is associated with a higher incidence of BN, and the edge effect is more important when smaller vineyard surface areas are considered and the average distance of grapevines from phytoplasma source is shorter (Mori et al. 2008).

Control strategies against phytoplasma diseases are mostly based on vector control and roguing of infected plants (Weintraub and Beanland 2006).

Chemical control of *H. obsoletus* is not considered suitable for BN because the nymphs are hidden in the soil and the host plants grow even outside the vineyards (Sforza and Boudon-Padieu 1998; Weber and Maixner 1998; Maixner 2007). In Northern Italy, insecticides applied onto the grapevine canopy seem to influence neither the disease nor the *H. obsoletus* presence (Pavan 1989; Pavan et al. 1989; Mori et al. 1999; Cavallini et al. 2003; Mori et al. 2008).

For the BN bindweed-type, considering that *C. arvensis* is scattered throughout vineyards and uncultivated fields, chemical weed control and soil tillage were proposed (Sforza and Boudon-Padieu 1998; Weber and Maixner 1998, Langer et al. 2003; Maixner 2007). However, soil tillage in vineyards is not effective enough to reduce the incidence of BN (Pavan 1989), likely because *H. obsoletus* is attracted by sparse vegetation in open soil (Langer et al. 2003; Maixner 2007), and bindweed is a plant that easily colonizes vineyards whose ground is frequently tilled (Arzone et al. 1995). To reduce *C. arvensis* coverage and *H. obsoletus* population in the vineyards, a selective green cover appears to be a good alternative, while grubbing in the late summer and ploughing in the winter cause vector mortality in uncultivated fields (Langer et al. 2003; Maixner 2007).

For the BN nettle-type, the cultural control practices against *U. dioica* could be useful to reduce the disease (Maixner 2007; Kessler et al. 2011) as showed by decreases of *H. obsoletus* population levels (Stark-Urnau and Kast 2008; Maixner et al. 2010). In particular, the presence of *U. dioica* mostly in herbaceous vegetation surrounding the vineyard area and the edge effect in the spatial distribution of affected grapevines suggests controlling nettle in the ditches.

To evaluate the effect of nettle reduction on BN disease, one could compare treatments inside the same vineyard or among different vineyards. Both plans, however, are not suitable because in the first case vector moves from the surroundings and the diseased plants are not randomly distributed inside the vineyard (Mori et al. 2008). In the second case, many vineyards with similar characteristics of nettle abundance, disease occurrence and vector presence are difficult to find.

For these reasons, in this study, we recorded the decrease in nettle amount in farm with different nettle control strategies and verified whether it is correlated with a decrease in *H. obsoletus* population density and whether this latter is correlated with a decreasing incidence in new symptomatic grapevines.

Materials and methods

Characteristics of investigated vineyards

In the period 2003–2008, a study was conducted in six vineyards of Lambrusco cultivars affected by BN phytoplasma, located in a grape-growing area of the Emilia-Romagna region (Northern Italy) (Table 1). The ditches surrounding these vineyards were rich in nettle. In these vineyards, an edge effect in spatial distribution of symptomatic grapevines was associated with the presence of nettles along the ditches (Mori et al. 2008). Sylvoz training system as well as agronomic practices (i.e., irrigation, fertilization, pruning, weed, pest and fungi control) was the same in the six vineyards. Based on the existence of an association between the nettle amount along ditches and BN incidence (Mori et al. 2008), the phytosanitary services advised farmers that the nettle control could be useful to reduce the BN incidence. From 2004, in the investigated vineyards, owners adopted different nettle control measures along ditches characterized by different

Table 1 Characteristics of the sampled vineyards, weed management strategies adopted and incidence of the disease before and at the end of the adoption of control measures

Vineyard	Grapevines (number)	Vineyard surface area (m^2)	Symptomatic grapevines (%)	
			2003	2008
1	803	6,424	13.2	12.7
2	960	5,040	12.5	5.9
3	1,234	7,404	5.2	6.0
4	2,676	14,450	8.2	3.5
5	6,252	27,196	6.4	5.6
6	932	5,919	4.9	1.1

nettle control pressure. Five farmers used vegetation mowing (cuts): two farmers made 2–3 cuts a year before and after the *H. obsoletus* flight (vineyards N. 1 and N. 2), one farmer made cuts once a year in late June to mid July (vineyard N. 3) and two farmers made 4–5 cuts a year before and after the *H. obsoletus* flight (vineyards N. 4 and N. 5). One farmer applied herbicides in spring (glyphosate) and after harvest-before leaf falling (MCPA) (vineyard N. 6).

Sampling of symptomatic grapevines and nettle amount along ditches

A map of investigated vineyards and surrounding habitat was made. All the grapevines of each vineyard were identified according to a code comprising of two numbers indicating the row and the position along the row, respectively. The distance between the ditch and vineyard border side varied from 3 to 5 m. In 2003 and 2004–2007, the length of the border sides with *U. dioica* was measured. Each year, the grapevines were classified as symptomatic or asymptomatic depending on the presence of BN symptoms (partial or total lack of lignification of canes and shoots, rolling of leaves, sectorial discolorations of the blades). The inspection was carried out each year by the same two people. Each one inspected one side of the plants in order to double check the presence of BN symptoms and, at the same time, exclude other causes of similar symptoms (e.g., partial broken canes, *Stictocephala bisonia* Kopp e Yonke activity). From 2003 to 2008, the percentage of symptomatic grapevines was calculated. From 2004 to 2008, the percentage of new symptomatic grapevines was calculated in comparison with the asymptomatic grapevines in the previous years.

H. obsoletus samplings

From 2003 to 2008, in each vineyard, the population of *H. obsoletus* adults on weed vegetation of the ditches was monitored weekly during the flight period (from June to August), using sweep nets (diameter 40 cm). At each sampling, weed vegetation along the ditches with nettle was swept 60 times and captured specimens were counted every six sweeps.

PCR and RFLP analyses

In the investigated vineyards, 74 symptomatic grapevines, randomly collected in late August/beginning of September, and 244 *H. obsoletus* adults, captured in July during the flight period, were tested to verify phytoplasma presence and identity. Nucleic acid was extracted according to the procedures established for grapevines and herbaceous samples by Prince et al. (1993) and for insects by 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 by Duduk et al. (2004). Informative restriction enzymes employed were *Tru*I and *Taq*I on 16S rDNA gene and *Hpa*II on tuf gene.

Data analysis

For each vineyard, the variation of four parameters (nettle amount, vector density on ditch with nettle, new symptomatic grapevines and symptomatic grapevines) was calculated by comparing the situation before (pre) and after (post) the application of nettle control measures. Then, the relationships among the variations in *U. dioica* amount, *H. obsoletus* density and new symptomatic grapevines in the six vineyards were studied with linear regression analysis. In this way, it was possible to know whether the variation in new symptomatic grapevines was independent or proportional to the effect of weed control measures on nettle amount and *H. obsoletus* density.

For *U. dioica*, the variation was expressed as a ratio between the average amount of nettles (meters along ditches) in the period 2004–2007 and that recorded in 2003. For *H. obsoletus*, the percent variation of captured specimens was expressed as a ratio between the average of captured specimens in the period 2004–2007 and that recorded in 2003. For the BN disease, the percent variation of new symptomatic grapevines was expressed as a ratio between the average value in the period 2005–2008 and the value recorded in 2004. As the effect of the weed and vector reduction on the disease appears in the following year, the variation in presence of nettles and *H. obsoletus* was established with reference to 2003, while the disease variations were compared with 2004. The effect of weeding strategies was considered as an average of more years so as to have a more stable effect, considering also that the different weed control measures have a different interaction with nettle and *H. obsoletus* feeding on (see also "Discussion").

The statistical analysis was performed with GraphPad Instat 3.0 program.

Results

Identification of GY

PCR and RFLP analyses detected BN phytoplasmas in 38 of 74 grapevines with GY symptoms (Table 2). BN tuf-type a phytoplasma occurred in all vineyards and in 20 of 74 vines with GY symptoms. The BN tuf-type b phytoplasma was much rarer than BN tuf-type a, in fact it was detected only in

Table 2 Phytoplasmas identified in symptomatic grapevines and *Hyalesthes obsoletus* adults collected on nettle

Vineyard	Symptomatic grapevines				*Hyalesthes obsoletus*			
	No. of analyzed	No. positive to BN	No. positive to BN tuf-type a	No. positive to BN tuf-type b	No. of analyzed	No. positive to BN	No. positive to BN tuf-type a	No. positive to BN tuf-type b
1	10	4	3	0	63	10	1	6
2	8	5	2	0	67	40	20	10
3	10	7	4	1	19	6	4	2
4	8	4	2	0	28	12	4	8
5	30	15	7	0	40	10	3	0
6	8	3	2	0	27	8	6	0

2008 in one grapevine of the vineyard N. 3. Therefore, half of the samples from the BN infected grapevines were negative for either BN tuf-type a or tuf-type b, and this is related to the lower sensitivity of primers employed to determine the BN tuf-type (Carnevale et al. 2008).

Molecular assays on *H. obsoletus* adults detected 16SrXII-A phytoplasmas in all vineyards, with 16–60% of the captured specimens testing positive. Among the positive insects, 44% were positive for tuf-type a and 30% positive for tuf-type b.

Nettle, *H. obsoletus* and new symptomatic grapevines amount

In all vineyards, the presence of nettles along the ditches decreased after the adoption of weed control measures (Table 3). During 2004–2007, the average reduction varied from 18 to 96%. The highest reduction was observed where 4–5 cuts a year were made (vineyards N. 4 and N. 5).

During 2004–2007, the presence of *H. obsoletus* decreased on average from 58 to 94% (Table 3). The decrease in number of captured *H. obsoletus* along the ditches surrounding the vineyards was significantly correlated with the decrease in nettle coverage (Fig. 1).

The percentage of symptomatic and new symptomatic grapevines decreased after the adoption of weed control measures along ditches in all sampled vineyards, except for vineyard N. 3 (Tables 1, 3). Only in this vineyard, characterized by the lower decrease in nettle covering, an increase in the incidence of new symptomatic grapevines was observed.

The variation in the incidence of new symptomatic grapevines with respect to the incidence before the application of weed control measures was correlated with the reduction in *H. obsoletus* population (Fig. 2). Instead, there was no significant correlation between variations in *H. obsoletus* captures and variations in total number of symptomatic grapevines. The variation in the incidence of new symptomatic grapevines with respect to the incidence before the application of weed control measures was also significantly correlated with the decrease in nettle coverage independently from the effect of this latter on *H. obsoletus* population density (Fig. 3).

Discussion

In this study, we demonstrate that nettle reduction is associated with a decreasing incidence of new symptomatic grapevines infected by BN tuf-type a with respect to the incidence recorded before the application of weed control measures. The decrease in nettles along ditches surrounding the vineyards also reduced the population levels of

Table 3 Ditch border length with *U. dioica*, number of captured *H. obsoletus* and percentage of new symptomatic grapevines

Vineyard	*U. dioica* along ditches (m)		*H. obsoletus* (number)		New symptomatic grapevines (%)	
	Pre (2003)	Post (2004–2007 average)	Pre (2003)	Post (2004–2007 average)	Pre (2004)	Post (2005–2008 average)
1	115	27.5	488	106.8	18.2	5.7
2	75	25	69	24.0	5.0	3.6
3	52	42.5	117	49.0	2.5	4.0
4	191	10	173	11.0	12.3	3.9
5	118	5	374	24.0	11.4	2.5
6	20	5	51	6.5	3.0	2.1

For each variable, the data before (pre) and after (post) the adoption of weed control measures were reported

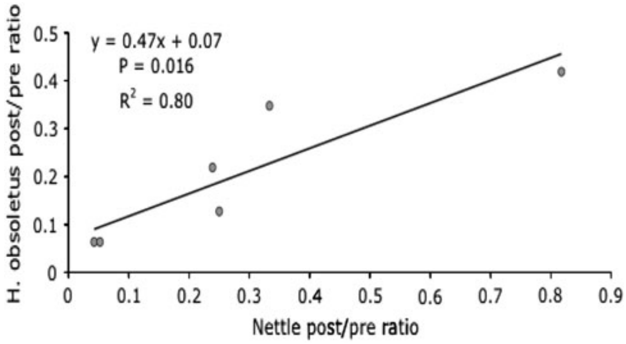

Fig. 1 Effect of the reduction in borders with *U. dioica* (meters) on the *H. obsoletus* captures. *Pre* values before adoption of weed control measures; *post* average values after adoption of weed control measures

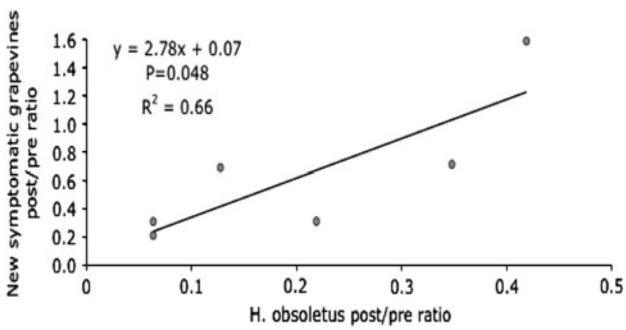

Fig. 2 Effect of the reduction in *H. obsoletus* captures on the percentage of new symptomatic grapevines. *Pre* values before adoption of weed control measures; *post* average values after adoption of weed control measures

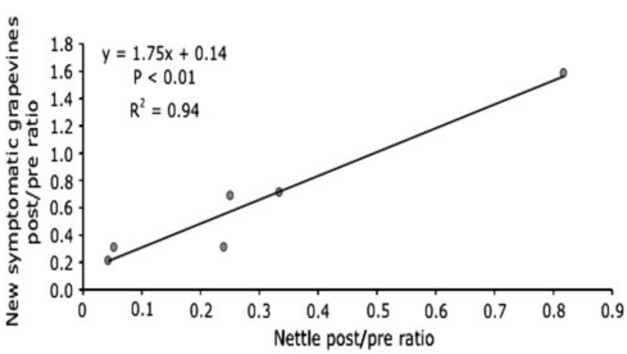

Fig. 3 Effect of the reduction in borders with *U. dioica* (meters) on percentage of the new symptomatic grapevines. *Pre* values before adoption of weed control measures; *post* average values after adoption of weed control measures

H. obsoletus as previously observed (Stark-Urnau and Kast 2008; Maixner et al. 2010).

In the vineyards where grass cuts were applied on the ditches, a higher decrease in nettles was observed where a higher number of cuts a year was carried out. Frequent cuts of herbaceous vegetation along ditches can be an alternative strategy to chemical weeding for nettles and, as a consequence, for the reduction in *H. obsoletus* population. However, frequent cuts can induce the migration of adults emerged from nettle roots toward the surrounding habitats. This can occur because: (1) nettle plants are not immediately killed by cuts, and therefore, there is not a negative effect on *H. obsoletus* nymphs which feed on nettle roots; (2) cuts, reducing nettle canopy, induce the dispersal of emerged adults to search for uncut host plants. On the contrary, the elimination of the host plant by spring herbicide causes the mortality of *H. obsoletus* nymphs. The positive effect of frequent cuts of herbaceous coverage on the reduction in the vector population can be shown only in the following years. However, it is essential to highlight that weed control can have negative effects when it is carried out during vector flight because adults could be encouraged to colonize grapevines (Maixner and Langer 2006; Maixner 2007).

Comparing the incidence of BN tuf-type a, before and after weed control measures, a decrease in the disease was recorded in five out of six vineyards where the BN nettle-type was present. In the vineyard where nettle was cut once a year during *H. obsoletus* flight, the reduction in nettle was negligible and the incidence of new symptomatic grapevines increased, probably because the timing of cut was favorable to migration of vector toward grapevines as observed by Mori et al. (2010).

The weed control showed a significant effect on the number of new symptomatic grapevines, but not on the total number of symptomatic ones. That is why the total number of symptomatic grapevines, differently from the new ones, depends on former infections and on incidence of recovery that differs among vineyards independently on nettle control (Credi 1989; Mutton et al. 2002; Bellomo et al. 2007). Therefore, to evaluate the effect of control strategy against phytoplasma diseases of woody plants, it is more correct to consider the new infections. In any case, efficacy evaluations based on total symptomatic plants would require more years of observation.

Before implementing nettle control to reduce the spread of BN tuf-type a, costs and benefits have to be carefully evaluated, as well as the possible adverse environmental effects of weeding. Moreover, the timings of cuts or herbicide applications need to be optimized to minimize the emergence of *H. obsoletus* adults and their vineyard colonization.

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