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Factors affecting the spread of "bois noir" disease in grapevine cv. Lambrusco in northern Italy

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Abstract

During 2003-2006 a research was carried out in 17 vineyards cv. Lambrusco to assess the influence on "bois noir" disease (BN) spreading of the insecticides applied on grapevine canopies and of the environment surrounding vineyards. The tuf-type I BN phytoplasma, that is associated to nettle, was clearly prevalent in the sampled vineyards. The insecticide treatments did not significantly influence either the density of the vector inside the vineyard, or BN disease spreading. The frequency distribution of symptomatic grapevines in some cases fitted to the Poisson distribution and in others to negative binomial distirbution. In the vineyards with an aggregate distribution of symptomatic grapevines, an edge effect from a border side with nettle was usually observed. The incidence of border sides not contiguous to grapevine rows on vineyard surface results associated to higher levels of BN. The border side with nettle was significantly favourable to disease in the vineyards with aggregate distribution of symptomatic grapevines. All the data support the importance of surrounding vegetation as source of inoculum of the BN phytoplasma.

Key words: 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 (Maixner, 2006). BN phytoplasmas are transmitted to grapevine by *Hyalesthes obsoletus* Signoret (Homoptera Cixiidae) from *Convolvulus arvensis* L. and *Urtica dioica* L.

In 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* and for the BN disease, would indicate that herbaceous vegetation surrounding the vineyards can be an important source of BN phytoplasmas for grapevines (Mori *et al.*, 2007).

A research was carried out in a grape-growing area of North Italy to assess the influence of the insecticides applied on grapevine canopies and of the environment surrounding the vineyards on disease spreading.

Materials and methods

In the period 2003-2006 a study was conducted in 17 vineyards cv. Lambrusco. A map of each investigated vineyard was made recording every year the symptomatic grapevines. The total length of border sides not contiguous to 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.

Symptomatic grapevines, spontaneous plants *(Urtica dioica, U. minor, Convolvolus arvensis*, *Aristolochia clematidis*, *Calistegia sepium*, *Galium aparine*, *Medicago sativa*, *Mentha* sp., *Plantago lanceolata*, *P. major*, *Salix sp.*) and *H. obsoletus* were sampled and tested by nested PCR followed by RFLP analyses on 16S ribosomal gene and on tuf gene for phytoplasma molecular characterization (Duduk *et al*., 2004).

In each vineyard the populations of *H. obsoletus* adults on herbaceous vegetation, inside and outside the vineyards, were monitored using sweep nets. Each year information about insecticides applied to grapevine canopies were collected.

Parametric and non parametric tests were used to compare respectively means of data that passed or not normality test. Relationships between two variables were studied with linear regression analysis.

To study 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. When the frequency distribution of symptomatic plants in a vineyard fitted to a negative binomial distribution, the possibility that this was the consequence of a gradient of symptomatic plants decreasing from a border with high density of *U. dioica*, was investigated. 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 (Gregory, 1968).

Results and discussion

The average percentage of symptomatic grapevines in 10 vineyards of Lambrusco cultivars, affected by BN and sampled every year, was equal to 10.6 without significant statistical differences among the years. On average the 7.5% of asymptomatic grapevines in the previous year showed the disease. On average the 64.3% of grapevines symptomatic in the previous year recovered.

The captures of *H. obsoletus* adults were significantly higher outside than inside the vineyards.

Among the grapevine samples tested 2 to 13 for each vineyard resulted to be positive to the presence of 16SrXII-A phytoplasmas. Nested-PCR amplification on tuf gene provides amplicons from the majority of BN infected grapevine samples and 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.

On the weed species collected 16SrXII-A phytoplasmas were identified 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 resulted to be infected by tuf-type II BN.

Molecular analyses performed on 270 *H. obsoletus* insects allow the detection of 16SrXII-A phytoplasmas in 105 insects, among these the 85% was carrying tuf-type I and the rest was carrying tuf-type II phytoplasmas.

The frequency distribution of symptomatic grapevines in 13 cases fitted to Poisson distribution, in 8 cases to binomial negative and in 4 cases to both distributions. Seven of the 8 vineyards that fitted to negative binomial distribution showed a gradient of diseased plants decreasing from a border side with nettle. In 6 cases the power low model significantly described this edge effect.

The density of *H. obsoletus* population on the herbaceous vegetation in the centre of the vineyards was not associated with the percentage of new symptomatic grapevines. This can be occurred because (1) the presence of the vector on grapevine canopies is not so closely related with its density on underlying herbaceous vegetation, (2) this herbaceous vegetation is not the most important source of the infectious vectors. The not significant influence on the disease spreading of the insecticides applied in the vineyards, during the vector flight, indirectly confirms this result.

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 ($Y = 158X + 2.6$, $P = 0.003$, $R^2 = 0.48$).

The relationship between the ratio length of border sides with *U. dioica*/vineyard surface and the percentage of symptomatic grapevines in the first year of sampling was significant when the vineyards with an aggregate distribution of symptomatic grapevines were considered ($Y = 486.5X + 4.45$, $P = 0.03$, $R^2 = 0.58$). No positive relationship between the percentage of ground coverage of *C. arvensis* and the percentage of symptomatic grapevines in the first year of sampling was found ($Y = - 3.53X + 17.8$, $P = 0.07$, $R^2 = 0.20$).

The influence of the border side with nettle on the disease incidence agrees with the fact that the prevalent strain of BN tuf-type I in the vineyards is reported to be associated to *U. dioica*. The absence of an 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 rarely detected in these vineyards.

In general, the influence of border sides not contiguous to other grapevine rows on tuf-type I BN incidence supports the importance of the external source of the disease. It is particularly evident in the vineyards where an edge effect from a border side with nettle occurs.

Also the ineffectiveness of the insecticides applied on grapevine canopies, indirectly confirms the important epidemic role of the external source of infectious vectors. Therefore, this research suggests that insecticide treatments in vineyards, differently from "flavescence dorée" (Pavan *et al*., 2005), are not influent for the BN control. Other two practical indications from the results of this study are: (i) to plant width and regular shaped vineyards and (ii) to not locate more susceptible cultivars on the border of a wider vineyard to reduce the border sides contiguous to spontaneous surrounding vegetation.

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