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Epidemiology of 'bois noir' disease and molecular variability of associated phytoplasmas in organic vineyards in Tuscany (Italy)

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Abstract

The spread of 'bois noir' disease in grapevine was monitored in three organic vineyards in Tuscany (Italy). Annual incidence, infection and recovery rates were calculated to describe changes in the epidemic with time. In general symptomatic plants showed the recovery in a relatively short time, meanwhile the infection rate decreased rapidly after the second year of assessments. The temporal dynamics of infection and recovery rates were satisfactorily fitted to a power and polynomial models, respectively. Survey on the herbaceous vineyard weeds allow to identify 16SrXII phytoplasmas, polymorphisms in 16Sr and tuf genes were detected.

Key words: epidemiology, disease, molecular detection, weeds.

Introduction

Among the phytoplasmas diseases affecting grapevine 'bois noir' (BN) associated with stolbur phytoplasmas (16SrXII) is present in all grape growing areas worldwide. It presence is often endemic but in some cases it is associated with severe epidemic, such as in several Italian regions in the last fifteen year.

The spread of 'bois noir' (BN) disease of grapevine was monitored to describe changes in the epidemic with time, and to determine the spatial pattern of the disease in selected vineyards in Tuscany (Italy) (Marchi *et al*., 2011). Molecular analyses were also carried out to verify presence of BN phytoplasmas strains in the infected vineyards and in weeds in the same vineyards in order to verify relevance of identified polymorphisms in the BN epidemic studied.

Materials and methods

To study temporal dynamics of BN epidemics, data were collected over a 6 year period from experimental plots established within three organic vineyards of cv. Sangiovese (Cs, Fd and Fc) in the province of Florence (Italy). Vineyards Fc and Fd were established in 2001 and 2002, respectively, and are separated by a 4 meters stretch of non cultivated land. Vineyard Cs was established in 2001 in a different locality approximately 10 km apart. Bidimensional maps with locations of all symptomatic, recovered (vines with disease symptoms at least once in the previous years but asymptomatic in the current year) and healthy (disease symptoms never observed) vines were prepared from survey results for each plot and each year of assessments.

To describe and compare epidemics the following indices were calculated: disease incidence (number of symptomatic vines divided by the total number of plants in the plot in each year), infection rate (number of newly affected plants the current year/total number of plants showing symptoms at least once in the previous period) and recovery rate (number of recovered plants the current year/total number of plants showing symptoms at least once in the previous period (Morone *et al*., 2007).

At the end of summer 2010 all vines that were showing grapevine yellows symptoms as well as samples of the most represented dicotyledonous herbaceous species (weeds) that were present in vineyards Cs and Fd, screened for phytoplasmas presence by real time PCR (Angelini *et al*., 2007). Samples resulted infected by stolbur were further tested by nested PCR with P1/P7 and R16F2/R2 (Deng and Hiruki, 1991; Schneider *et al*., 1995; Lee *et al*., 1995) primer pairs followed by RFLP analyses with *Tru*I, *Mbo*II, *Hpy188*I, and *Alu*I restriction enzymes (Fermentas, Vilnius, Lithuania). Obtained patterns were compared with those of relevant stolbur phytoplasma strains (Contaldo *et al.,* 2011) on same size amplicons.

Results

Temporal analysis of BN epidemics showed similarities between the 3 vineyards. After an initial progressive phase, in the third year of assessments (2007) it begun a regressive phase that lasted until 2009. In 2010 disease incidence increased again. Changes in the annual disease incidence values over time were somehow less marked in vineyard Cs compared to vineyard Fc and Fd. The infection rate decreased rapidly over time from the maximum observed in 2006 (figure 1a). In vineyards Fc and Fd, recovery rate increased until 2009 (figure 1b) and then

decreased in 2010; meanwhile in the case of vineyard Cs increased in 2007, was nearly stationary in the two following years and then increased again in 2010. In all three vineyards a power model (Figure 1a) was appropriate to describe change in infection rate over the whole period (Fc, R^2=0.99; Fd, R^2=0.99; Cs, R^2=0.97) meanwhile change in recovery rate fitted well to a polynomial model (Figure 1b) although less consistently in the case of vineyard Cs (Fc, R^2=0.96; Fd, R^2=0.97; Cs, R^2=0.88). Real time PCR results indicated that both in grapevines and weeds only 16SrXII phytoplasmas could be detected.

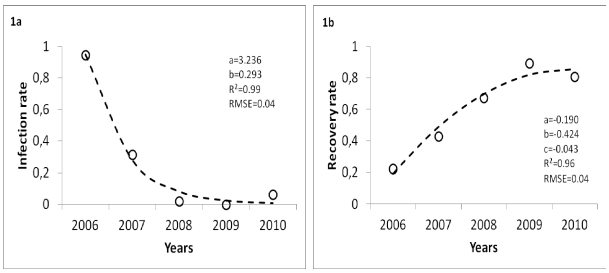

Figure 1. Relationship of the infection rate (1a) and recovery rate (1b) from 2006 to 2010 in vineyard Fc, estimated using a power model ($y=a*b^x$) and a polynomial model ($y=a+bx+cx^2$), respectively. Open circles, observed values. R^2 statistic, coefficient of determination and RMSE is the square root of mean square error. a, b and c (figure 1b) are the equation parameters.

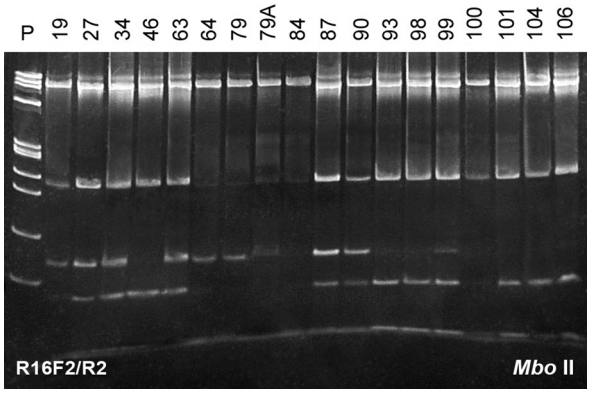

Figure 2. RFLP profiles of R16F2/R2 amplicons of weeds collected in the surveyed vineyards after *Mbo*II digest. P, marker ΦX174 *Hae*III digested.

Nested PCR/RFLP analyses showed that the following 16SrXII infected weed species contained tuf-type a: *Daucus carota*, *Lactuca saligna*, *Bupleurum tenuissimum*, *Mercurialis annua*, and *Medicago lupulina*; while tuf-type b was identified in *Lactuca sp.*, *Pichiris hieracioides, Convolvolus* spp., and *Cuscuta* spp.; both types were found in *Linaria vulgaris* and in grapevine samples (data not shown). Polymorphisms were detected in R16F2/R2 amplicons with *Mbo*II (figure 2), and *Hpy188*I, while *Alu*I, did not show any polymorphism. In particular the *Mbo*II polymorphisms were commonly detected in both weeds and grapevine samples, while the one with *Hpy188*I was only present in weeds. The latter enzyme also shows the presence of profiles compatible with interoperon heterogeneity presence or mixed phytoplasmas lineages population in both weeds and in grapevine.

Discussion

The phases of BN epidemic over time were very similar in all vineyards, suggesting that the same insect vector is present and that its interactions with the grapevine host are more variable between years than between different vineyards in the same year. Nevertheless as the spatial distance between the monitored vineyards increased, the goodness of fit of the data to the proposed models decreased, suggesting that other factor/s, strictly related to the agroecosystem, may significantly influence the shape of disease curves over time. Analyses of tuf and 16S rDNA genes of both weed and grapevines show presence of tuf-type a and b in both cases and also of possible mixed BN lineages presence. Further research is in progress to evaluate the possible relationship of these molecular findings with the evolution of BN disease in the studied agroecosystems.

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