Analysis of selection pressure exerted on *Plasmopara viticola* by organically based fungicides

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Downy mildew is one of the most important grape diseases world-wide. The pathogen is a genetically highly diversified organism with a high capacity of adaptation. A monitoring of changes in population structure of *P. viticola* subjected to new copper replacing products or strategies, studied and developed within REPCO (Replacement of Copper Fungicides in Organic Production of Grapevine and Apple in Europe) is important for assessing selection pressure which could lead to a reduction of efficacy of these new measures. Therefore *P. viticola* lesions collected on untreated and treated vines were analyzed by means of microsatellite markers. No significant differences in the populations structure were determined among untreated and treated populations, indicating that the applied products didn't exerted any selection pressure on the *P. viticola* populations.[1]

Introduction

Downy mildew, caused by the oomycete *Plasmopara viticola* (Berk. & Curt.) Berl & de Toni, is one of the most important grape (*Vitis vinifera*) diseases world-wide. *P. viticola* is a genetically highly diversified organism with a high capacity of adaptation. In organic agriculture, protection strongly depends on copper containing fungicides. However, the use of copper does not fully fulfil the requirement of sustainability due to the severe pressure exerted on the habitat where copper is present in high amounts. REPCO (Replacement of Copper Fungicides in Organic Production of Grapevine and Apple in Europe) aims to contribute to the replacement of copper fungicides in organic agriculture by studying and developing new organically based fungicides and potentiators of resistance, new biocontrol agents and new integrated management systems.

A monitoring of changes in population structure of *P. viticola* subjected to the copper replacing products or strategies will reveal if selection pressure, which could lead to forced evolution, and in the time in a reduction of the efficacy of the measure, is exerted on the pathogen. Two possible scenarios are expected: (1) the *P. viticola* population is composed from a large number of different genotypes present at low frequency, indicating that no selection pressure is exerted; (2) the population is composed from a reduced number of genotypes present at high frequency, indicating that the most fit genotypes have been selected by the pressure exerted.

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Material and methods

Products or products combinations applied on schedule consisted in Aliette (ALI), Copper (COP), Plant extract 1 (PE1), Tri-40 (TRI), Chitoplant (CHI), Sonata (SON), Mycosin plus Sulfur (STR), and Chitoplant plus Sonata plus KBV (COM). *P. viticola* lesions were collected from infected treated and untreated grapevines (UNT) in an experimental vineyard at Fibl in Frick (Aargau, Switzerland) in 2005 following five important infective events. Automated high-throughput DNA extraction, PCR amplification of the four polymorphic *P.viticola*-specific SSR loci, BER, CES, GOB and ISA, and sequencer-based fragment analysis were used for genotyping the collected samples (Gobbin et al., 2003). Downy mildew genotypes were defined as strains that differed by at least one allele in their SSR profiles (Gobbin et al. 2005). Each oospore was assumed to contain a single nucleus (Burruano, 2000; Gobbin et al., 2003) that was asexually inherited by the progeny (secondary lesion). Therefore, two oil spots showing identical allele pattern (same genotype) were interpreted as clonal progeny. Different genotypic profiles of two oil spots were interpreted as being derived from independent oosporic infections (Gobbin et al., 2005).

Table I. Total number of samples (tN_{obs}), total number of genotypes (tN_{gen}) and Shannon index (H) calculated for individuals collected on untreated vines (UNT) and on vines treated with Aliette (ALI), Chitoplant (CHI), Chitoplant plus Sonata plus KBV (COM), Sonata (SON), Copper (COP), Mycosin plus Sulfur (STR), Tri-40 (TRI), and Plant extract 1 (PE1).

	tN_{obs}	tN_{gen}	H
UNT	123	83	4.1
ALI	72	52	3.7
CHI	48	47	3.8
COM	36	33	3.5
SON	50	47	3.8
COP	87	76	4.3
STR	69	58	4.0
TRI	56	52	3.9
PE1	101	79	4.3

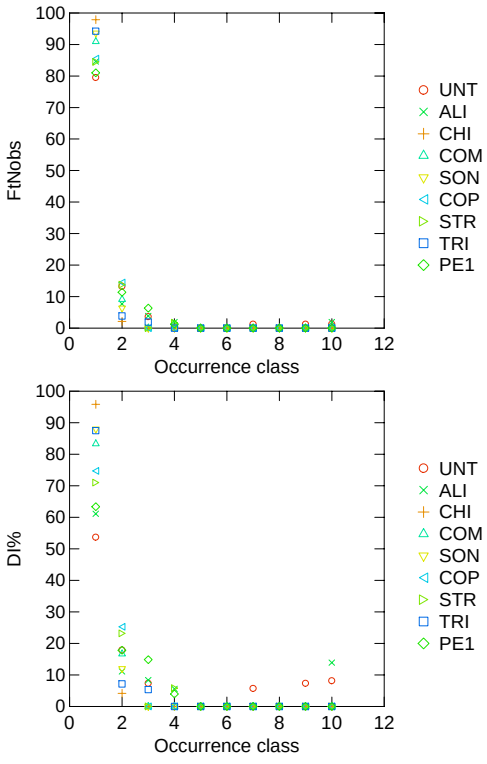

Figure 1. Assessment of frequency (F_{tNobs}= X) of Plasmopara viticola on untreated vines (UNT) and on vines treated with Aliette (ALI), Chitoplant (CHI), Chitoplant plus Sonata plus KBV (COM), Sonata (SON), Copper (COP), Mycosin plus Sulfur (STR), Tri-40 (TRI), and Plant extract 1 (PE1), and percentage of total disease incidence ($DI_{\%}$) of genotype occurrence classes. The genotypes that were identified the same number of times throughout the survey period were classified in occurrence classes.

For each product and product combination and for the untreated control, all the data corresponding to the sampling dates were pooled. Total number of samples (tN_{obs}) and total number of genotypes (tN_{gen}) were calculated. The number of genotypes that were identified the same number of times (tN_{obs}=X) throughout the survey period was determined and the frequency of every occurrence class (F_{tNobs}= X) was calculated. The genotypes occurring once throughout the survey period (tN_{obs}=1) were defined as single genotypes. The contribution of any genotype to the total disease incidence was calculated by dividing the number of lesions generated by the tN_{obs} and expressed as a percentage ($DI_{\%}$) (Gobbin et al., 2005). The diversity of the *P. viticola* populations for each product and product combination and for the untreated control where measured with the Shannon index, calculated with: $H = -\Sigma\ p_i * \ln(p_i)$, where p_i is the proportion of samples in the *i*th clonal genotype (Magurran, 1988).

Results

A comparison among the 642 totally coded individuals revealed the presence of 429 different genotypes over all treatments and dates. Single genotypes (tN_{obs}=1) were shown to constitute the greatest percentage of all individuals collected in all treatments, on average, 88.0% (SD 6.4%). The lowest percentage was recorded for the individuals collected on the untreated (UNT) vines, where single genotypes made up 79.5% of all genotypes; the highest was for CHI, when single genotypes comprised 97.9% of all genotypes. On average, over the nine treatments, single genotypes were responsible of 75.4% of the total disease incidence (SD 14.3%), with the lowest percentage recorded for UNT (53.7%) and the highest for CHI (95.9%). Genotypes that were identified twice (tN_{obs}=2) in the same treatment throughout the season (either twice on the same sampling date or once on two sampling dates) were on average 9.1% (SD 4.5%). For CHI and COP respectively the lowest (2.1%) and the highest (14.5%) value. The proportion of disease incidence caused by those genotypes was on average 15.0% (SD 7.0%), with values for CHI (4.1%) and for COP (25.3%) as the lowest and highest, respectively. The treatments that included genotypes identified more than 8 times ($tN_{obs}\geq 9$) were collected on the untreated (UNT) vines (tN_{obs}=9, 7.3%; tN_{obs}=10, 8.1%) and on them collected on ALI (tN_{obs}=10, 13.9%). F_{tNobs}= X and $DI_{\%}$ data distribution within occurrence class (tN_{obs}=X) were equally described for the nine treatments (Fig. 1). The average diversity calculated with the Shannon index was 3.9 (SD 0.3), with the highest value for COP and E73 (4.3) and the lowest for COM (3.5) (Table I).

Discussion

All nine analyzed treatments were characterized by high population diversity. No differences among the treatments were observed in the distribution of the frequency (F_{tNobs}= X) and percentage of total disease incidence ($DI_{\%}$) of genotype occurrence classes. In all treatments the greatest percentage of individuals was constituted by single genotypes, with values in accordance with the ones of a study performed by Gobbin et al. (2005), grouping 4685 *P. viticola* single lesions samples collected from 18 plots across central Europe, in which on average 71% of all genotypes were identified only once throughout the season. All analyzed treated and untreated *P. viticola* populations were composed from a large number of different genotypes present at low frequency, it could therefore be concluded that none of the eight analyzed products or product combinations exerted a selection pressure on *P. viticola* populations.

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