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Competitive Fitness and Adaptation of QoI-Resistant *Plasmopara viticola* Strains

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Abstract
Quinone outside inhibiting fungicides (QoIs) represent one of the most important fungicide classes used to control *Plasmopara viticola*, the causal agent of downy mildew in grapevine. Soon after the introduction of QoI fungicides in vineyard, resistant isolates have been detected. To manage resistance evolution, it is important to understand how resistant populations emerge, spread and survive. One major approach in understanding these processes is to measure the pathogen fitness in the absence of fungicide pressure and to investigate the competitiveness of resistant isolates. In this study, different isolates of *P. viticola* were included to measure the Composite Index of Fitness. It was also investigated whether there is a fitness cost in QoI-resistant isolates. No fitness cost was detected for the tested isolates under controlled conditions suggesting that only highly fit isolates are selected under field conditions leading to a balance between sensitive and resistant isolates.

Introduction

Delaying resistance to fungicides in pathogen populations is an important goal in sustainable agriculture. A major issue in understanding resistance evolution is to measure the pathogenic fitness of isolates in absence of fungicide selection. The capacity of populations to spread resistant genotypes and their ability to infect host plants and survive can be evaluated with various methods. Several studies have focused on the fitness of pathogenic bacteria (Andersson, 2006) and their individual contribution to the gene pool of the next generation by measuring the growth rate with a Malthusian fitness index (Pringle and Taylor, 2002). One difficulty in filamentous fungi is the existence of different reproduction types in their life cycle, i.e. asexual (clonal) and/or sexual phases. During the clonal phase, fitness may be measured through the growth rate. Nonetheless, other parameters such as infection efficiency appear to be equally important for fungi (Kadish and Cohen, 1988; Antonovics and Miller Alexander, 1989).

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In this study, the fitness of QoI-resistant and –sensitive strains (18 each) of *Plasmopara viticola* was evaluated under controlled conditions. QoI fungicides act as mitochondrial respiration inhibitors of cytochrome b. Resistance usually results from the replacement of glycine by alanine at codon 143 (G143A) in cytochrome b, thereby compromising ATP production (Bartlett *et al.*, 2002; Gisi *et al.*, 2002; Chen *et al.*, 2007). The fitness parameters investigated in this study included infection frequency and efficiency, sporulation capacity, latent period and sporangia size, with which a fitness index was developed to characterize downy mildew isolates. In addition, the competition between sensitive and resistant isolates in different mixtures was evaluated. In this way the competitive fitness of resistant isolates could be assessed in the absence of fungicide pressure.

Material and Methods

Isolates collected in French vineyards from 2003 to 2006 were maintained on detached leaves of grape cv. Cabernet Sauvignon in Petri dishes under controlled conditions (21°C, photoperiod of 16 h). The QoI fungicide used for resistance assessment in a bioassay was famoxadone.

Fitness measurement (according to Tooley *et al.*, 1986). Parameters studied were: 1) **S**= sporangia size; 2) **L**= latent period = period necessary to obtain 50 and 100% of sporulation intensity, respectively; 3) **M**= Malthusian fitness (parameter for growth rate of a population), $M = \ln(N_t/N_0)/t$, where N_t is the number of sporangia at day 7, N_0 the number of inoculated sporangia; 4) **IF**= infection frequency = proportion of infection points out of 45 points at 7 days post-inoculation; 5) **IE**= infection efficiency = rate of germinated zoospores reaching stomata versus amount of zoospores not germinated or germinated outside stomata and 6) **Composite index of fitness** (F_{ic}) calculated as: $F_{IC} = S \times L \times M \times IF \times IE$

Competition test (according to Kadish & Cohen, 1988). In order to evaluate the change in proportion of resistant and sensitive isolates in the mixture, 4 pairs of resistant and sensitive isolates were inoculated at initial proportion of 20, 50 or 80% of resistant isolates and tested for sensitivity to famoxadone (100 mg/L^{-1}) after each asexual cycle.

All data for each fitness component were subjected to an analysis of variance. Means for each isolate within sensitivity groups and among sensitivity groups were compared using Duncan's Multiple Range Test at $P= 0.05$.

Results

Results for fitness parameters are given in Table 1. Sporulation capacity was the only parameter that was significantly different between QoI-sensitive and –resistant isolates. For the 36 isolates sporulation capacity expressed as Malthusian index ranged from 0.032 (BOM06) to 0.042 (PIC59), exhibiting a 1.28-fold increase for the most efficient compared to the least efficient isolate. The mean Malthusian index was 0.036 ± 0.002 for sensitive and 0.038 ± 0.002 for resistant isolates ($P < 0.05$). Resistant

isolates had an average sporulation equivalent of 960 ± 340 sporangia per inoculated sporangium, while sensitive isolates produced only 730 ± 300 sporangia per inoculated sporangium. This difference is significant at $P < 0.05$. QoI-sensitive isolates exhibited a 1.3-fold lower sporulation than QoI-resistant isolates suggesting that resistant isolates have an advantage over sensitive isolates.

F_{IC} values were not significantly different for sensitive and resistant isolates. Therefore, there is no overall advantage of QoI-resistant isolates and the predicted cost of resistance is only very minimal if at all. Indeed, the sensitive isolates did not exhibit a higher F_{IC} than the QoI-resistant isolates. Figure 1 shows the F_{IC} values for the 36 isolates sorted by decreasing size.

Table 1. Fitness components as mean values for 18 sensitive and 18 resistant isolates of *P. viticola*. S = size of sporangia (µm), L= latency index, M= Malthusian index, IF= infection frequency, IE=infection efficiency and F_{IC}=composite index of fitness).

Component	S Isolates	R Isolates	Significance
S	17.0 ± 2.8	17.2 ± 2.1	*P=0.82*
L	0.79 ± 0.11	0.81 ± 0.11	*P=0.72*
M	0.036 ± 0.002	0.038 ± 0.002	*P=0.004*
IF	0.90 ± 0.14	0.96 ± 0.06	*P=0.15*
IE	0.42 ±0.12	0.43 ± 0.12	*P=0.78*
F_{IC}	0.19 ± 0.09	0.21 ± 0.07	*P=0.35*

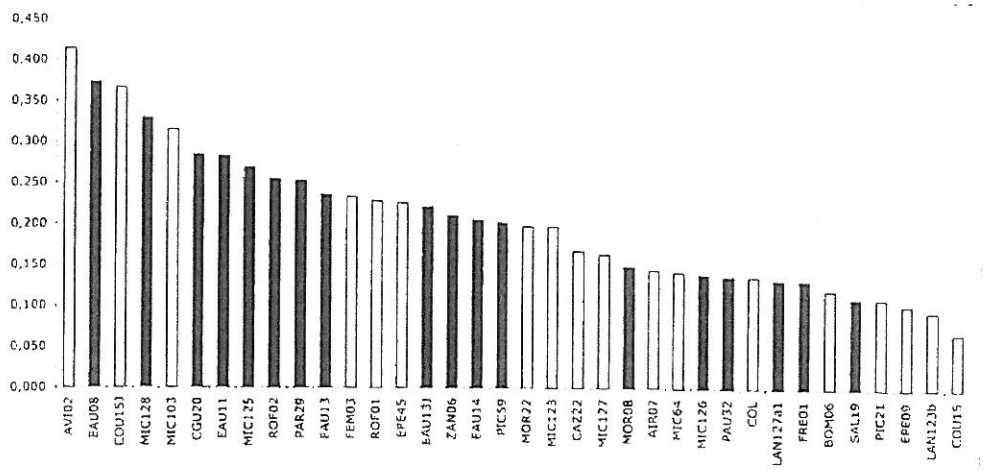

Figure 1. Composite Index of Fitness (F_{IC}) for 36 *P. viticola* isolates sorted by decreasing size. QoI-resistant isolates represented by solid bars, QoI-sensitive isolates by empty bars.

Whatever was the initial concentration of resistant isolate in the mixture, the proportion tended to be stable over 8 cycles, the observed decrease was not significant (Figure 2). As with fitness components, there is a balanced competitiveness between QoI-resistant and -sensitive isolates.

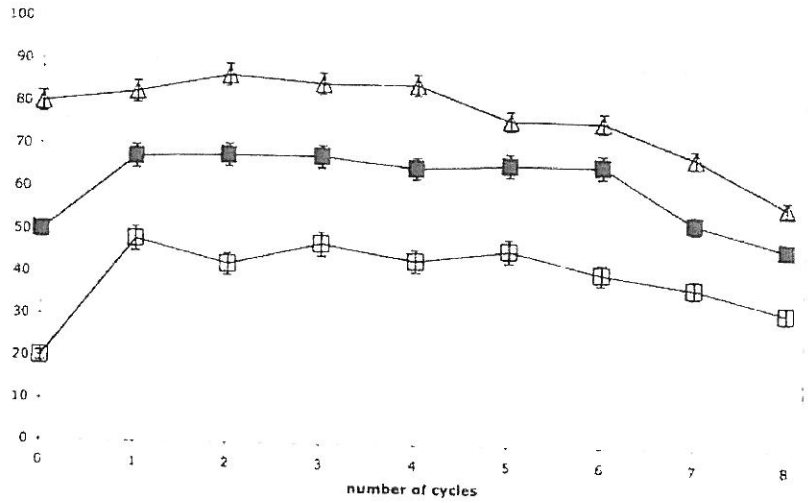

Figure 2. Evolution of resistant isolates in mixture with sensitive isolates at different initial proportions (80:20, 50:50, 20:80) over 8 asexual cycles. Initial proportion of resistance 80% Δ, 50% □, 20% ■

Discussion

Some authors (e.g. Pringle and Taylor, 2002) consider the Malthusian index as representative for measuring fitness of fungi during the asexual phase. In this study, it provided only a partial picture of fitness suggesting that QoI-resistant isolates were fitter than QoI-sensitive isolates. However, by integrating other parameters, QoI-resistant isolates did not show fitness advantages over sensitive isolates. Furthermore, no fitness cost was detected by using the Fic index and no significant difference was recorded with regard to competitiveness. Therefore, the acquisition of QoI resistance has no or at best little influence on fitness of resistant isolates. Three explanations are possible: 1) Under natural selection pressure, only very fit strains are selected and can survive after mutation at codon 143. 2) The G143A exchange induces no fitness cost. 3) The cost of resistance may be compromised by other mutations. The overall fitness of isolates is difficult to predict on the basis of an individual fitness component. For this reason, several fitness components were combined by calculating the composite fitness index thus approximating the overall fitness. Similar composite indices combining various parameters have been used by Haymer & Hartl (1983) and Tooley *et al.*, (1986).

Under laboratory conditions, no differences were detected in fitness parameters or competitiveness between QoI-resistant and -sensitive strains originating from the field. Unlike for artificial mutations, only highly fit isolates are selected under conditions of

natural selection and mutation (G143A) (Schoustra *et al.*, 2006). However, other components such as oospore production or overwintering were not determined which could also play a role in the overall fitness of resistant strains in vineyards. In this study, an appropriate combination of parameters provided a good estimation of competitiveness of *P. viticola* isolates in the asexual phase. At present, investigations on population genetics of *P. viticola* in vineyards without QoI treatment are in progress in order to monitor the evolution of resistance.

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DPG | Spectrum Phytomedizin

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