First approach of *Plasmopara viticola* population biology: merging epidemiology and population genetics

A Rumbou[1] & D. Gobbin[2]
[1] National Agricultural Research Foundation, Plant Protection Institute, P.O. Box 1303, 38001, Volos, Greece
[2] Phytomedicine/Pathology, Institute of plant sciences, Swiss Federal Institute of Technology, Universitätstrasse 2, 8092 Zürich, Switzerland

The Oomycete *Plasmopara viticola* (Berk. et Curt.) Berl. et de Toni, the causal agent of grapevine downy mildew disease, constitutes the most destructive pathogen in viticultural regions with rainy springs/summers. The pathogen infects all the green tissues of the plant producing yellowish lesions. Losses are caused through fruit destruction, killing of leaf tissue and weakening of shoots (Agrios, 1997). Current concepts regarding the pathogen‘s epidemiology postulate that the sexual spores (oospores) only play a role at the initiation of the disease early in the grapevine vegetative season. The explosive progress and dispersal of the disease are attributed to the asexual spores (secondary sporangia), which are assumed to migrate in long distances within short time (Lafon & Clerjeau, 1988). The study conducted aimed to investigate the previous assumptions regarding the pathogen’s epidemiology and, specifically, the qualitative and quantitative contribution of oosporic versus clonal infections and the mode of disease spread. Combining epidemiological and population genetics data, a broader and more encompassing perspective of the disease dynamics was obtained.

In five European countries (Switzerland, Germany, France, Italy and Greece) 32 plots were selected and natural downy mildew epidemics were surveyed during the years 2000-2002 (1-22 samplings/plot). About 10,000 oilspots were collected and the oomycete strains were genotyped with the use of four specific microsatellite markers (Gobbin *et al.*, 2003a). Because *P. viticola* is diploid, every strain was characterized by a genetic profile consisting in eight microsatellite alleles. Genotypes presenting the same allele pattern were considered as clones (derived from the same oospore through asexual reproduction), while the ones presenting a different allele pattern were considered to have derived from different oospores. Following this principle, oosporic infections could be differentiated from the clonal ones. The population genetics analysis provided description of the populations’ structure (patterns of genotypic diversity, genetic variation, migration, genetic drift etc.), as well as assessment of genetic distances among populations. In parallel, spatial distribution analysis of the epidemics was conducted.

One of the main outcomes acquired is that the sexual spores constitute a major source of inoculum. The abundant genotypes identified and the high genotypic diversity estimated in most of the *P. viticola* populations studied, reflects the massive occurrence of oosporic infections and, consequently, a large pool of oospores in the soil. In contrast to the existing belief that primary infections occur only at early disease stages and in limited scale, it was shown that oosporic infections play a main role at the initiation of the disease in May and continue to occur throughout the epidemiological season. However, their quantitative contribution to the epidemic decreases with the progress of time. In Greece, they are also responsible for the

disease regeneration in September, after the disease inhibition caused by heat and drought. Exception to the prior result was found in some island regions in Greece, where limited oosporic infections were observed. The climate in those regions is very dry and the disease usually appears with low severity, while occasionally bursts heavily. In conclusion, the highly genetically variable populations of the pathogen indicate high levels of sexual reproduction.

Considering the asexual spores, it was shown that their role for the epidemic was overestimated until now. The great majority of the genotypes in each population (85%, on average) were identified only once or twice throughout the survey period (genotype frequency<1%). The genotypes that underwent a relevant asexual reproduction (dominant genotypes) were only one or two per epidemic and their contribution to the total disease severity was dependent on the epidemic surveyed and on the epidemic's stage. The amount of clonal infections was low until the middle of July and increased only late in summer. However, secondary infections played a leading role in epidemics where a small number of oosporic infections occurred; in some islands (Rumbou *et al*., 2002) or isolated mountain regions (Gobbin *et al.*, 2003b), the lesions derived from the dominant genotypes represented 20-90% of the sample size. Apart from the exceptions, the overall finding is that clonal infections play only a moderate role in downy mildew epidemics, in sharp contrast to previous beliefs about the major importance of asexual inoculum.

The spatial distribution analysis showed that secondary infections were spatially localized in most cases. In one asexual cycle, the clones' dispersal usually did not cover an area wider than a few vines around the site where the oosporic infection was first identified. The only instances of widespread dispersal were observed for the rare dominant genotypes (Rumbou & Gessler, 2004). This suggests that long-distance sporangia dispersal cannot be massive and, consequently cannot play a major role in epidemics. A stepwise spatial pattern of spread is more likely rather than the wind-mediated dispersal of sporangia believed to be common up until now.

The contribution of primary versus secondary infections to epidemic development showed two general patterns. Most frequently, the role of primary infections at plot scale was major while the role of secondary infections was minor throughout the growing season. This pattern was found in all central European populations and in the mainland populations in Greece. The other epidemic pattern was characterised by the predominance of one or a few clones and was found in low severity epidemics in central Europe as well as in Greek islands and coastal plots.

The occurrence or absence of bottleneck events during the epidemic was another feature of the epidemics studied. In cases where a bottleneck did not occur (continuous epidemic), the disease started in spring, grew continuously triggered by both primary and secondary infections, and ended late in autumn. This pattern was found in central Europe and in one Greek plot. In contrast, in the remainder of the Greek plots, the disease started in spring and grew until mid-summer, and then stopped because both primary and secondary infections were not possible due to unfavorable climatic conditions. The disease started again in autumn (two-peak epidemic).

The different contributions of the primary versus secondary infections in combination with the presence or absence of bottlenecks during an epidemic led to different genetic substructures among samples within the same plot. During a single grape-growing season, either one (typical for 'continuous' epidemics in central Europe) or two *P. viticola* subpopulations were responsible for the epidemic (typical

for ‘two-peak’ epidemics in Greece). Among samples of two or more consecutive grape-growing seasons either one or more subpopulations were responsible for the epidemics. The first case occurred only in two Greek plots characterized by the predominance of one clone and low disease severity, while the second case was more common and was found in all plots where the epidemic pattern was the ‘two-peak’ type.

Genetic subdivision among populations from different vineyards was very clear. Most pair-wise comparisons between populations from geographical sites more than 5 km away revealed genetic differentiation. This means, in biological terms, that naturally occurring exchange of propagules (oospores or sporangia) is low. Furthermore, the widely held belief about long-distance secondary sporangia migration, which would cause the homogenisation of the *P. viticola* European population, is less relevant than assumed.

Another finding was the alternative overwintering of the pathogen in asexual form. This situation was observed in the Kephalonia island, presumably as mycelium in the buds or in leaves that did not fall during the winter and for the first time it was proven by molecular genetics. Furthermore, two other biological phenomena were also recorded in this work. First, a polyploid genetic profile characterized some individuals (suggesting the existence of polyploidy in this fungus). Second, in most populations, we observed the regular appearance of clusters of similar genotypes, consistent with mutation events occurring during mitosis. These two mechanisms can contribute to the genotypic diversity of the pathogen and, possibly, also constitute adaptive mechanisms to unfavorable conditions for the survival of the population.

Our recent findings challenge the existing assumptions about *P. viticola* epidemics. The relative role of sexual and asexual spores on disease development was surprisingly misconceived. The new concept obtained by this survey implies a site-related population structure and, consequently, pattern of epidemic. This signifies a leading or a minor role of oosporic infections depending on the environmental conditions. The importance of those findings consists in their value for the design of control strategies under Integrated Pest Management and biological viticulture.

Agrios GN, 1997. The downy mildews. In: *Plant pathology*, 4rth eds, pp. 278-283. Academic Press, San Diego, CA.

Lafon R & Clerjeau M,1988. Downy mildew. In: *Compendium of grape diseases* (ed. Pearson RC and AC), pp. 11-13. APS Press, St. Paul, Minnesota, USA.

Gobbin D, Pertot I, Gessler C, 2003a. Identification of microsatellite markers for *Plasmopara viticola* and establishment of high throughput method for SSR analysis. *European Journal of Plant Pathology*, **109**, 153-164

Gobbin D, Pertot I, Gessler C, 2003b. Genetic structure of a Plasmopara viticola population in an isolated Italian mountain vineyard. *Journal of Phytopathology*, 151:636-646.

Gobbin D, Raynal M, Gessler C, 2003c. Mildiou de la vigne, des oeufs à surprise. *Phytoma*, 563, 37-41.

Rumbou A, Gobbin D and Gessler C, 2002. Epidemics and genetic variability of *Plasmopara viticola* in Greek populations. *Proc. 4th Intern. Workshop on Powdery & Downy Mildew of Grapevine*, Davis, USA, Sept.30-Oct.04 2002, p. 14.

Rumbou A, Gessler C, 2004. Genetic dissection of *Plasmopara viticola* population from a Greek vineyard in two consecutive years. *European Journal of Plant Pathology*, **110**, 379-392.