Integrated protection and production in viticulture
IOBC/ wprs Bulletin Vol. 67, 2011
pp. 131-138

Consideration of dynamical plant-pathogen interactions for an improved management of powdery mildew epidemics in grapevine

S. Schnee, J. Jolivet, A. Calonnec
INRA-Bordeaux, UMR INRA-ENITA 1065 Santé Végétale, BP 81, 33883 Villenave d'Ornon, France

Abstract: The current control of grape powdery mildew requires news strategies, able to limit the pathogen development and requesting a reduced number of chemical sprayings. This study proposes to exploit host plasticity in order to investigate the effect of a plant growth changing on either the epidemic process, or organs susceptibility. An experimental plot combining two cultivars, three rootstocks and two opposite crop management for creating a vigour differential was surveyed regularly during the vegetative growth and different variables were measured to characterise host growth and pathogen. Shoots coming from vinestocks localized in the different vigour area were sampled for organ susceptibility assessment. Two groups of vigour were obtained, not based on the established treatment but on the individual growth potential, that differ mainly by the number of secondary leaves. The vigorous group presents the higher level of disease, increasing with the appearance of secondary leaves. A low vigour modifies the susceptibility of the tissues, by an earlier appearance of ontogenic resistance phenomenon. The relevance of vigour control and the concomitant physiologist mechanisms are discussed as integrative strategy in the grape powdery mildew management.

Key words: powdery mildew, grapevine, susceptibility, vigour, epidemic

Introduction

Powdery mildew remains one of the major fungal diseases of grapevine, which requires repeated chemical applications to preserve the yield and the quality of the harvest. *Erysiphe necator*, the causal agent of powdery mildew, is a biotrophic pathogen, displaying an asexual polycyclic reproduction capable of exponential multiplication in favourable environmental conditions. The earlier discrete symptoms on leaves and the inconstant berries damages require preventive chemical sprays in classical disease control strategy without a real assessment of the infection level. Therefore, to be in accordance with sustainable agriculture, it becomes urgent to investigate more integrated strategy that would put the pathogen in a defavourable environment to its development in order to be able to control epidemics with a reduced number of applications. The occurrence of ontogenic resistance phenomenon in the cultivated grapevine (Ficke *et al.*, 2002) as well as the spatial location of primary infections on the vine stock, allows hypothesizing that dynamical changes in crop growth and architecture could be considered as key factors for explaining variability in the severity of epidemic behaviour. Then, Human interferences during the wine development through the range of crop management may be exploited to maintain epidemics at a level easy to control with fewer treatments. Management or soil-induced changes in crop growth and architecture are known to modulate significantly the course of epidemics (Calonnec *et al.*, 2009).

To investigate the effect of host growth in the epidemic process, variations of the vine development is explored. The vigour is defined as an integrative variable that reflects the metabolic activity of organs in growth and is assessed by quantifying number and rate of plant organs development. A modification of vine development may affect crop structural

characteristics as the leaves areas, the rate of shoots growth, the foliage density or the landscape of susceptible tissue. Variations of vine development can be induced through several factors: i) environment (climate, soil), ii) genetics (variety and root-stock planted), iii) cultivation factors (fertilization, irrigation, cover crop, pruning, shoot topping). The combination of all these factors should allow generating a plant growth differential that could modulate the pathogen installation and further the dynamic of epidemics. In order to assess if the plant could be considered as a key element of the protection system, combined measures of host and epidemic development are performed, as well as the measure of plant organ susceptibility.

Material and methods

Two plots were selected on the experimental site of INRA (Domaine du Grand Parc, Latresne) with the following characteristics. The P19 plot presents various levels of vigour by a combination of rootstocks (SO4, 110R and Riparia) and crop management (chemical weed control versus perennial cover crop). The plot is designed as 6 rows planted alternatively with two susceptible varieties Merlot and Cabernet-Sauvignon in 2001. The plot is shared in 8 blocks of 30 vines across the rows (6 rows x 5 vines). Each block is constituted by 6 sub-units combined each rootstock with each cultivar, randomly distributed. The first 4 blocks are conducted with perennial cover crop whereas the 4 others with weed chemical control. The P5, planted with the variety Merlot, displays two visually distinct area of vigour.

The dynamic of development of powdery mildew in relation to the plant growth was performed on the P19, whereas the susceptibility of shoots in regards to the plant development was studied on both plots.

Impact of host growth on a powdery mildew epidemic

On the P19, inside each sub-unit, one vinestock was selected based on its number of buds (7 for the Merlot and 8 for Cabernet-Sauvignon) and shoot's configuration. One shoot of this vinestock was inoculated at the stage "2 to 4 leaves" according to Calonnec *et al*. (2009). Several measurements were regularly made to characterize the vegetative growth of the vinestocks. Two to three times per week, new emerged leaves were marked by colour markers and size of shoots was measured. The different measured variables (number of leaves at flowering (NLf), number of leaves at berry pea size stage (NLps) shoot length at flowering (SLF)) and calculated variables (rate of shoot development (RSD), rate of leaf appearance (RLA)) described the dynamic of leaf appearance (Table 1).

In a point of view of disease survey, the percentage of diseased foliar surface was estimated weekly on all leaves of the selected shoot and two of its neighbours. The measured variables (number of diseased leaves at flowering (NDLf), number of diseased leaves at berry pea size stage (NDLps), conditional severity on diseased leaves at berry pea size stage (Scps)) and calculated variable (rate of diseased leaves appearance (RDLA)) described the dynamic of diseased leaf appearance (Table 1).

Susceptibility of shoots in relation to the vine vigour

For the shoots susceptibility assessments, 12 shoots coming from vinestocks localized in the different vigour area were cut early in the morning and immediately brought back to the laboratory. Before sampling, leaf petioles were marked with a colour code to specify their respective position on the shoot. In axenic conditions, leaves were disinfected (in a sterile water bath containing 65% calcium hypochlorite) and rinsed in sterile water. Three foliar

discs (Ø 22mm) were cut in each leaf to be distributed for 3 different Petri dishes corresponding to three pathogenicity tests. Leaves from a same foliar stage were distributed in Petri dishes (6 discs from 6 shoots per dish), containing an agar medium (20g l^{-1}) supplemented with benzimidazole (30mg l^{-1}). Petri dishes were placed in a settling tower and were artificially inoculated by blowing conidia from a 14 days infected leaf, according to Willocquet *et al.* (1996). The infection capacity was assessed 72 hpi by counting conidia development stage, after removal of fungal structures by a scotch application and cotton blue staining procedure. After inoculation of foliar disc by deposit of few conidia by a needle, the colony growth was measured 4, 7, 10 and 13 dpi. Sporulation assessment was performed by the measure of the quantity of generated conidia 13 dpi, by using a particle counter (Beckman Coulter) that records size cells included between 18 and 35 µm. Only sporulation data will be described in the results part.

Data were submitted to statistical analysis (PCA and clustering) with software Prism and R.

Table 1. Variables of plant development and disease measured and calculated at different plant growth stage, used for further analysis in this study.

Variables	Abbr.	Units	
Plant development			
Number of leaves at flowering	NLf	-	measured
Number of leaves at berry pea size stage	NLps	-	measured
Rate of leaf appearance	RLA	number day^{-1}	calculated
Shoot length at flowering	SLf	cm	measured
Rate of shoot development	RSD	cm day^{-1}	calculated
Disease assessment			
Number of diseased leaves at flowering	NDLf	-	measured
Number of diseased leaves at berry pea size stage	NDLps	-	measured
Conditional severity on leaves at berry pea size stage	Scps	% diseased $leaf^{-1}$	measured
Rate of diseased leaves appearance	RDLA	number day^{-1}	calculated

Results

Impact of host growth on a powdery mildew epidemic

For Merlot (and Cabernet-Sauvignon, data not shown) the plant growth is characterized by an approximately linear development of primary leaves and a linear increase of leaves appearance on secondary shoots with a strong variations between individuals at the end of the monitoring (Figure 1). The most vigorous vines presented three times more secondary leaves than the lowest vigorous. The number of diseased leaves follows an exponential curve, with an increase starting at the "flowering" stage. The last notation (68 days after contamination) presents an important variability of powdery mildew incidence on leaves.

The following variables of vigour are well correlated together according to a principal component analysis (Figure 2): the shoot growth variables (shoot length at flowering stage and rate of shoot development, $R^2 = 0.96$), the rate of leaf appearance and the leaf number at different phenological stages (at flowering stage and berry pea size stage, $R^2 = 0.88$). Individuals from the weed controlled blocks are characterized in average by a global increase of shoot development (SLf and RSD). However individuals of the two blocks overlap and

indicate that the experimental design is not able to control sufficiently a vigour differential. This fact could be explained by the high intra-group variability observed especially at the end of the monitoring (Figure 1).

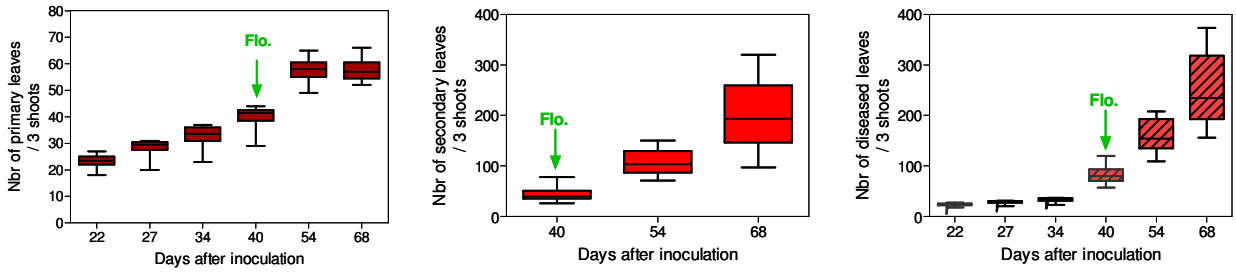

Figure 1. Distribution of the number of primary leaves, secondary leaves and diseased leaves observed on the 3 surveyed shoots on each selected vinestock function of the day after inoculation for cultivar Merlot.

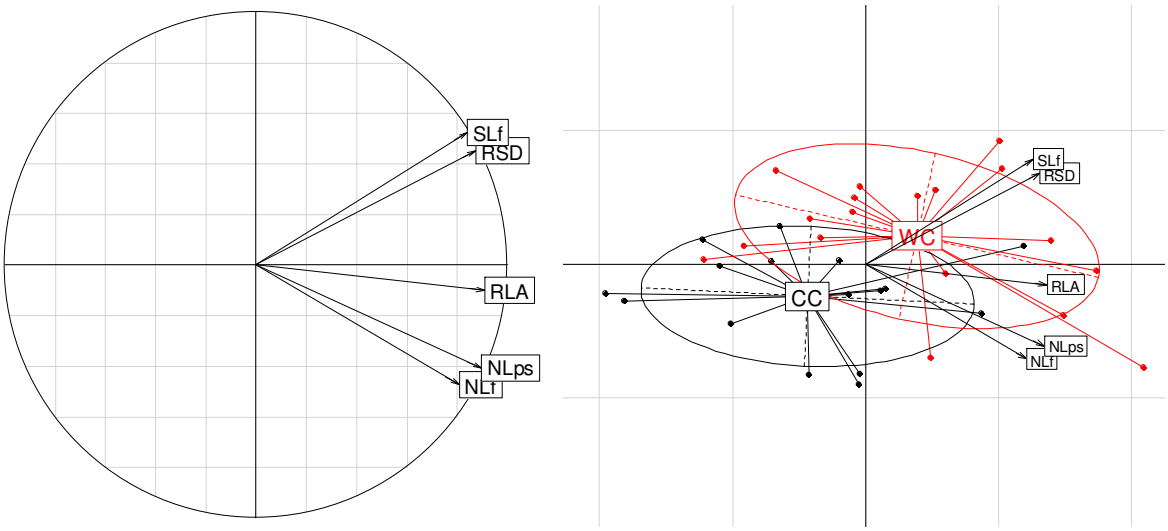

Figure 2. Correlation graph between “vigour” variables according to a principal component analysis based on: the shoot length at flowering stage (SLf), the ratio of shoot development (RSD), the rate of leave appearance (RLA), the leaf number at stage “berry pea size” (NLps) and the leaf number at flowering stage (NLf). Individuals from the same treatment are displayed by the same colour (wc: weed control area, cc: cover crop area).

A PCA preceded by a hierarchical ascendant clustering analysis was performed to group individuals the most similar according to the growth variables previously used (Figure 3). Two new clusters of individuals were based on the most relevant variables, namely the rate of leave appearance and the number of secondary leaves (Figure 3). These results were consequently used to investigate the distribution of vinestocks in relation to the disease variables. The correlation graph displays a correlation between the conditional severity at the

stage “berry pea size” and the number of diseased leaves at the stage “flowering” ($R^2 = 0.66$), and between the number of diseased leaves at the end of the survey and the rate of diseased leaves appearance ($R^2 = 0.71$) (Figure 4). The PCA graph displays a distinction between the two groups of vigour previously identified.

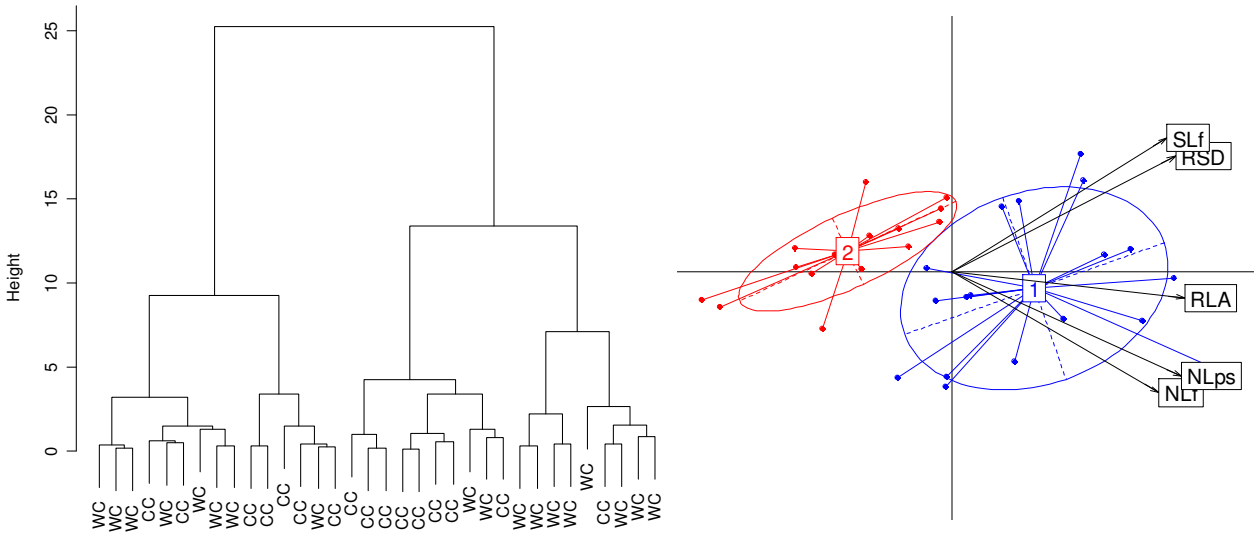

Figure 3. Hierarchical ascendant clustering analysis and principal component analysis based on the new vigour groups of the cluster analysis. Individuals from the same group are displayed by the same colour (1: high vigour individuals, 2: low vigour individuals).

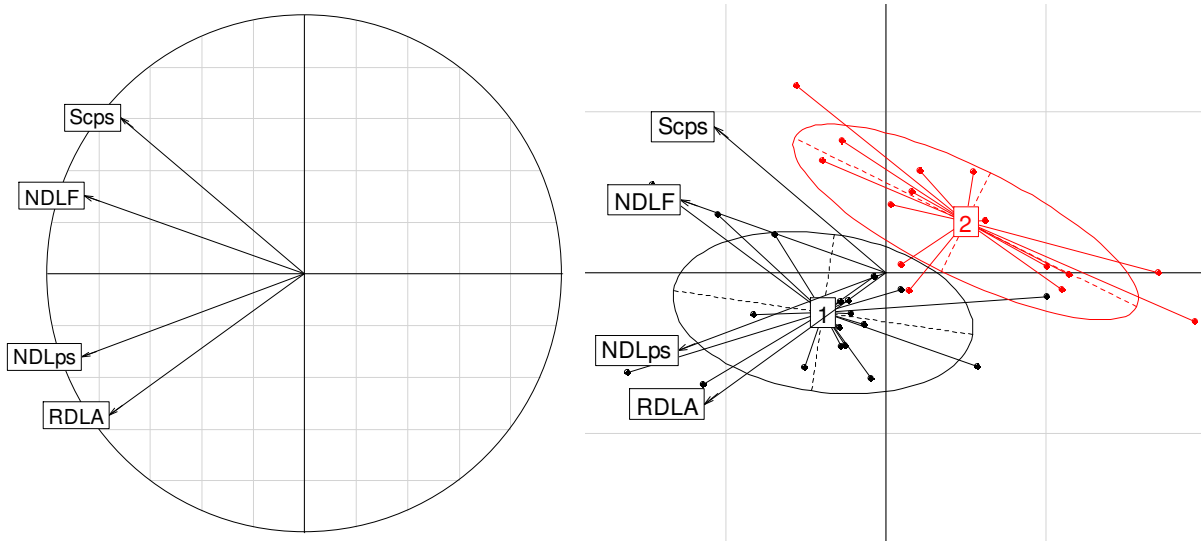

Figure 4. Correlation graph between “disease” variables according to a principal component analysis based on: the conditional severity at the stage “berry pea size” (Scps), the number of diseased leaves at the flowering stage (NDLf), the number of diseased leaves at the stage “berry pea size” (NDLps) and the rate of diseased leaves appearance (RDLA).

Susceptibility of shoots in relation to the vine vigour

The effect of wine development on tissue susceptibility was measured by comparing the intensity of sporulation per infected leaf surface (Figure 5a). On the P19, the sporulation from leaves of shoots taken from the two treatments (weed control versus cover crop) follows a comparable trend. If the sporulation amplitude and the range of diseased leaf age are identical, the maximum of sporulation in low vigour group is one day delayed in regards to the high vigour group.

Concerning the P5, only the maximal amplitude of sporulation is similar between the two groups of vigour (Figure 5b), however, the range of age for sporulating leaves is significantly lower in low vigour group (less 4 to 12 days old) than in high vigour group (from less than 4 to 18 days old). The age of maximal sporulation is also significantly earlier in the low vigour group (from less than leaf of 6-7 days old versus leaves from 8 to 10 days old in the high vigour group). The amplitude is more intense on the P19 than in the P5 ($7.5x10^4$ versus $4x10^4$ conidia produced per cm² of leaf disk).

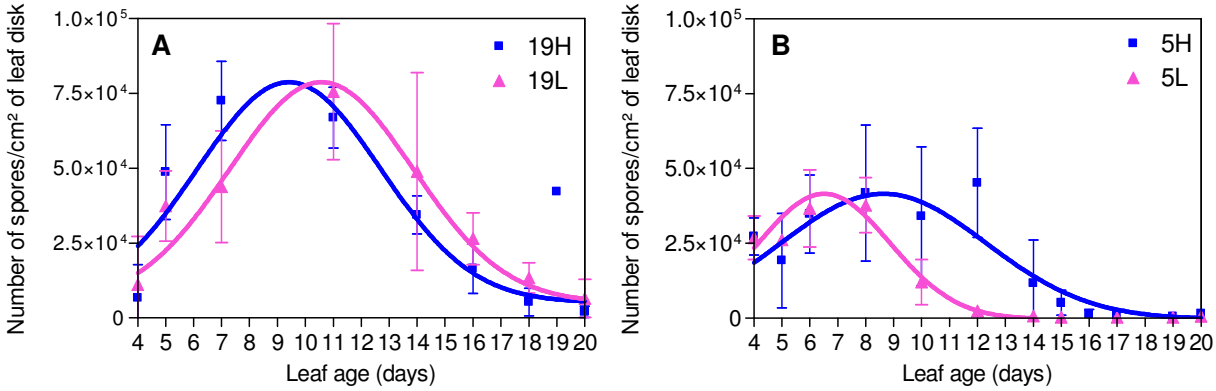

Figure 5. Sporulation level in relation to the leaf age on the surveyed shoot (A: on the P19, B: on the P5, h: high vigor level, l: low vigor level). The mean of 6 repetitions at each time point for the two distinct vigour level was adjusted by the software Prism.

Discussion

The field experiment allowed to distinct two groups of vigour which differ mainly by the number of leaves developed on secondary shoots. At flowering and berry pea size stage, the number of leaves is correlated to the total number of diseased leaves at the end of monitoring 65 days after inoculation (respectively R^2=0.89 and R^2=0.84). The emergence of secondary leaves enhance the amount of susceptibility tissues for pathogen development, explaining the rapid increase of disease level after the "flowering" stage and maintaining a high level of epidemic inside the plot, as observed by Valdes *et al.* (2007).

A higher level of vigour generates a greater amount of biomass, including a more intensive growth of secondary shoots, after primary shoot topping. The production of these new susceptible leaves explain the general high disease severity of the most vigorous vines, which accentuates with the increase of the vegetative host growth. In opposition, a low vigour level reduces the shoot apical growth, which sometimes cannot arrive to a sufficient height to be cut during the topping practice, and then limits the development of secondary shoots. For these vines early contaminated, the epidemic behaviour seems to be only dependent on the amount of tissue available.

Therefore the control of the vigour could modulate the severity of the epidemic in case of low pathogen pressure in vineyard, closely linked of the date of the primary contamination. A concrete and sustainable solution from these first results could be based on a precocious chemical intervention coupled to a limited foliage density by a reduction of secondary shoots, to prevent powdery mildew epidemic extension.

The assessment of tissue susceptibility indicates that a low vigour could modify the mechanisms of ontogenic resistance, by an earlier appearance of resistance at fixed foliar age. On the plot with the lowest difference of vigour (P19), sporulation curves are similar independently of the vigour level and the area under the curve that represents the global dispersal inoculum are identical with as consequence to maintain a high level of pathogen pressure. On the contrary on the P5, the plot with significant visually difference of vigour, a low vigour presents a narrow area under the curve of sporulation and limits the production of inoculum. The management of the global quantity of susceptible tissues (young leaves from the primary and then secondary shoots) constitutes an important factor to reduce as possible the favourable substratum for pathogen colonization.

Mechanisms of ontogenic resistance establishment remain obscure and appear specific to the considered tissue in a same variety (Gee and Gadoury, 2008). One explanatory physiological factor could be linked to the cell osmotic pressure, directly regulated by the carbohydrate metabolism. Activation of photosynthesis process and assimilation metabolism generates sugar accumulation in plant cell content, which the consequent osmotic value can promote resistance to fungal penetration (Schnathorst, 1959). The ability of leaf physiological maturity to trigger a henceforth efficient mobilization of secondary metabolism that includes the active defense reactions against pathogens attack is also closely linked to the energy provided by primary metabolic pathways (Bolton, 2009). Therefore factors limiting plant growth may modulate the balance of the plant primary metabolism that can modify the fitness of the plant-pathogen interaction. Plant growth management either quantitatively or qualitatively in sustainable agricultural strategies needs further explanations and could be an interesting prophylactic control towards powdery mildew, providing no effect on crop agronomical objectives.

Acknowledgements

This project was supported by the Agence Nationale de la Recherche, programme SYSTERRA ANR-08-STRA-04. We thank Romain Cargnelutti and Valérie Mayet for their active participation to the experimentation.

References

Bolton, M. D. 2009: Primary metabolism and plant defense – fuel for the fire. Molecular Plant-Microbe Interactions 22(5): 487-497.

Calonnec, A., Cartolaro, P., and Chadoeuf, J. 2009: Highlighting features of spatiotemporal spread of powdery mildew epidemics in the vineyard using statistical modeling on field experimental data. Phytopathology 99: 411-422.

Ficke, A., Gadoury, D. M., and Seem, R. C. 2002: Ontogenic resistance and plant disease management: A case study of grape powdery mildew. Phytopathology 92: 671-675.

Gee, T. G., Gadoury, D. M. 2008: Ontogenic resistance to *Uncinula necator* varies by genotype and tissue type in a diverse collection of *Vitis* spp. Plant Disease 92(7): 1067-1073.

Schnathorst, W. C. 1959: Resistance in lettuce to powdery mildew related to osmotic value. Phytopathology 49: 562-571.

Valdes, H. 2007: Relations entre états de croissance de la vigne et maladies cryptogamiques sous différentes modalités d'entretien du sol en région méditerranéenne. Montpellier: Ecole de Montpellier SupAgro, Thesis.

Willocquet, L., Colombet, D., Rougier, M., Fargues, J., Clerjeau, M. 1996: Effects of radiation, especially ultraviolet B, on conidial germination and mycelial growth of grape powdery mildew. European Journal of Plant Pathology 102: 441-449.