Dynamics of ontogenic resistance and growth variation in the interaction powdery mildew-grapevine

S. Schnee, J. Jolivet, A. Calonnec

INRA-Bordeaux, UMR INRA-ENITA 1065 Santé Végétale, BP 81, 33883 Villenave d'Ornon, France

Introduction

Powdery mildew is an ubiquitous fungal disease of cultivated grapevine (*Vitis vinifera* L.). The causal agent, *Erysiphe necator* Schwein., is an obligate biotrophic pathogen producing specialised infection structures in host epidermal cells to take up nutrients. Favourable environmental conditions insure repeated asexual reproduction, involving foliage and berries damage. In the vineyard, epidemic development is initiated by primary infections on young vegetative shoots, resulting from the dispersion of ascospores after cleistothecium dehiscence and/or conidia from latent mycelium conserved in dormant buds (Rumbolz *et al.*, 2000). The extent of the following epidemic is closely linked to the dynamic interaction between the pathogen, the host and their environment (Deytieux-Belleau *et al.*, 2009). Environmental factors influence the biological process, generating simultaneous fungal and vegetative growth. On one hand, the plant host produces a vegetative biomass, defined by a growth rate and a maximum potential quantity of tissues. On other hand, pathogen development and dispersion depend on infection efficiency linked to the pathogen's fitness, the ability to produce an important quantity of dispersal spores, and the probability of contact with a neighbouring organ in space and time. However biotrophic pathogens are strongly dependant on subtrate quality, defined by a quantity of susceptible tissues. Ontogenic resistance defined by the intrinsic acquisition of resistance with increased organ age, is a key factor limiting repetition of the infection process. Therefore the window of pathogen colonization is restricted to the range of susceptible leaves, determined by vegetative growth of the host plant. Consideration of the dynamic change of crop architecture in plant disease management constitutes an alternative integrated strategy to counteract epidemic development (Calonnec *et al.*, 2009; Schnee *et al.*, 2010). To date, main investigations of ontogenic changes have been undertaken on the grape berry (Ficke *et al.*, 2002), but relatively little information is available on the mechanisms associated with leaf resistance-age dependence (Doster and Schnathorst, 1985). On the model powdery mildew-grapevine, we investigated 1) the dynamics of the ontogenic resistance mechanism on leaves, 2) the effect of host growth variation on the susceptibility of foliar tissues to powdery mildew infection.

Methods

Two sets of data were analysed. 1) *For the investigation of ontogenic resistance mechanism*: This experiment was carried in 2005 (experimental site of Couhins) on shoots sampled at two different dates during the vegetative growth of grapevines cv. Cabernet Sauvignon (May 12th, stage 5-6 leaves; May 26th, stage 10-12 leaves). 2) *For the study of the impact of a plant growth variation on ontogenic resistance:* This experiment was carried in 2009 (experimental site of Latresne) on shoots sampled from vine-stocks localized in different vigour areas (cv. Merlot). For the two experiments, the following methodology was performed.

Shoots 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 22 mm) were cut in each leaf to be distributed in 3 different Petri dishes corresponding to three pathogenicity tests. Six foliar discs (Ø 8 mm) were cut at the same time for glucose analysis. Leaves from the same foliar level were distributed in Petri dishes (6 discs from 6 shoots per dish), containing an agar medium (20g l^{-1}) supplemented with benzimidazole (30 mg l^{-1}). Petri dishes were placed in a settling tower and were artificially inoculated by blowing conidia from a 14 day infected leaf, according to Cartolaro (1990). All leaf levels were inoculated together in one settling tower. The infection capacity was assessed 72 hours post inoculation (hpi) by counting conidial development stages, after removal of fungal structures by a scotch application and cotton blue staining procedure. After inoculation of each foliar disc by deposition of a few conidia using a needle, the colony growth was measured 4, 7, 10 and 13 days post inoculation (dpi). Sporulation assessment was performed by measuring the quantity of generated conidia 12 dpi (first date of sampling) and 14 dpi (second date of sampling) in the 2005 experiment and 13 dpi in the 2009 experiment. Sporulation was quantified by using a particle counter (Beckman Coulter) that recorded cells sized 18 to 35 µm. Determination of glucose and starch concentration in the foliar discs was performed by enzymatic microdosage (kit Biosentec). Data were statistically analysed with Prism software.

Results

Dynamics of ontogenic resistance in relation to foliar age

The appearance of ontogenic resistance was assessed by comparing the intensity of sporulation per infected leaf surface in relation to the leaf age.

In 2005, the two dates of sampling presented an identical optimum of sporulation for leaves 5-6 days old. Older leaves displayed decreased sporulation in both cases (Figure 1). The amplitude of inoculum produced was two fold higher at the late sampling date. The range of cell glucose content varied from less than 1 (% in dry weight) for the young leaves to more than 4 for the older leaves. Simultaneously with the sporulation decrease, leaves 8-9 days old displayed a drastic increase in cell glucose content. This trend in the susceptibility tissue pattern was also observed for infection efficiency (data not shown).

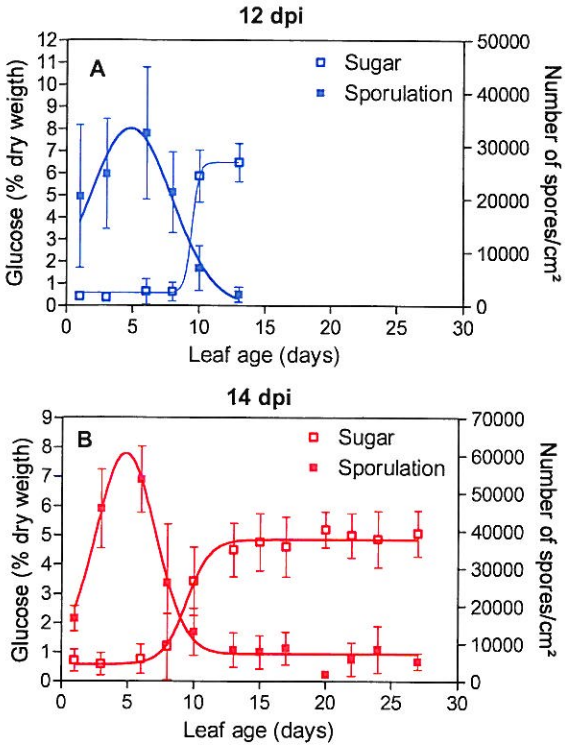

Figure 1: Intensity of sporulation and glucose cell content in relation to foliar age. Shoots were sampled at phenological stages "5-6 leaves" (May 12th) (A) and "10-12 leaves" (May 26th) (B). Each point corresponds to an average of 6 repetitions, and bars indicate standard errors.

Effect of host growth on the susceptibility of foliar tissues

The effect of vine development on tissue susceptibility was measured by comparing the intensity of sporulation per infected leaf surface in relation to leaf age. The maximum amplitude of sporulation was similar between the two levels of vigour (Figure 2). However, the range of age of sporulating leaves was significantly lower at the low vigour level (from 4 to 10 days old) than at the high vigour level (from 4 to 15 days old). The age for maximum sporulation was also significantly earlier at the low vigour level (between 6-8 days old versus 8 to 12 days old at the high vigour level). The glucose concentration increased with the leaf age and a clear distinction appeared for the 12 day old leaves between the two levels of vigour (Figure 3). The observed variability at some points could be attributed to individual dynamics of the selected shoots.

Discussion

This study showed that the establishment of ontogenic resistance with regard to foliar age is correlated with a steep increase in cell glucose concentration. Moreover ontogenic resistance appearance is linked to host vegetative growth performance.

On grapevine, foliar tissue susceptibility occurs on the youngest leaves and is rapidly modified by the appearance of ontogenic resistance. Mechanisms of ontogenic resistance establishment remain hypothetical and the investigation of associated physiological factors could allow a better understanding of the appearance of this age-related resistance. Young leaves are considered to be a sink organ corresponding to a limited activation of the photosynthetic process. The time of physiological maturity of the leaf appears to be approximately between 6 and 8 days old, when cellular glucose content increases. This physiological leaf evolution corresponded to the progressive decrease in sporulation. The underlying causes of the observed relationship between a restricted range of sporulation and an increase of leaf glucose content with foliar age are subject to speculation, but sugar accumulation is known to modify osmotic value that can promote resistance to fungal penetration (Schnathorst, 1959).

A low vigour may modify the susceptibility of tissues, by an earlier appearance of ontogenic resistance at a fixed foliar age. This trend was already observed in different experiments, in which vigorous vines had a significantly higher number of diseased leaves (Schnee *et al.*, 2010) and a higher percentage of diseased berries (Valdes, 2007). The plastic behaviour of grapevines supports the possibilities of alternative host growth management to limit epidemic progress.

Further experiments in 2010 will be performed to: 1) explore determinism of the ontogenic resistance in relation to dosage of secondary metabolism compounds; 2) understand the ontogenic resistance dynamics with regard to different host growth; and 3) test a model of causal relationship between variables by PLS-path modelling (Tennenhaus *et al.*, 1999) to identify variables that contribute significantly to an explanation of the pathosystem interaction.

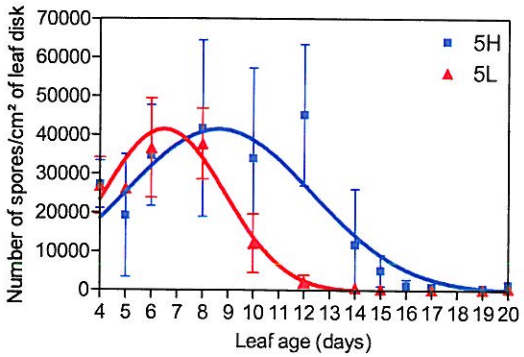

Figure 2: Sporulation level in relation to leaf age and associated vigour level (5H: high vigour level, 5L: low vigour level). Each point corresponds to an average of 6 repetitions, and bars indicate standard errors.

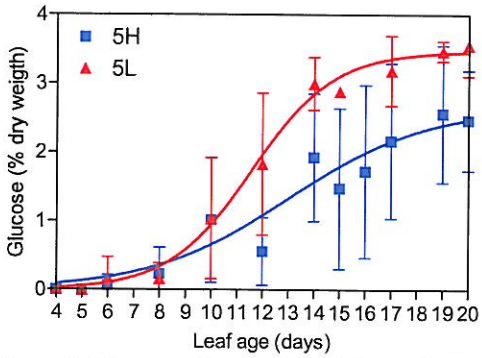

Figure 3: Glucose cell content in relation to leaf age and associated vigour level (5H: high vigour level, 5L: low vigour level). Each point corresponds to an average of 6 repetitions, and bars indicate standard errors.

Acknowledgements

This project was supported by the "Agence Nationale de la Recherche", programme Systerra-Archidémio ANR-08-STRA-04, and the Conseil Régional d'Aquitaine.

Literature Cited

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

Cartolaro P, Steva H. 1990. Maîtrise de l'oïdium au laboratoire. *Phytoma*, 419, 37-40.

Deytieux-Belleau C, Geny L, Roudet J, Mayet V, Donèche B, Fermaud M. 2009. Grape berry skin features related to ontogenic resistance to *Botrytis cinerea*. *European Journal of Plant Pathology*, 125, 551-563.

Doster MA, Schnathorst WC. 1985. Effects of leaf maturity and cultivar resistance on development of the powdery mildew fungus on grapevines. *Phytopathology*, 75, 318-321.

Ficke A, Gadoury DM, Seem RC. 2002. Ontogenic resistance and plant disease management: A case study of grape powdery mildew. *Phytopathology*, 92, 671-675.

Rumbolz J, Kassemeyer HH, Steinmetz V, Deising HB, Mendgen K, Mathys D, Wirtz S, Guggenheim R. 2000. Differentiation of infection structures of the powdery mildew fungus *Uncinula necator* and adhesion to the host cuticle. *Canadian Journal of Botany*, 78, 409-421.

Schnathorst WC. 1959. Resistance in lettuce to powdery mildew related to osmotic value. *Phytopathology*, 49, 562-571.

Schnee S, Jolivet J, Calonnec A. 2010. Consideration of dynamical plant-pathogen interactions for an improved management of powdery mildew epidemics in grapevine. *IOBC wprs Bulletin*, under press.

Tennenhaus M. 1999. L'approche PLS. *Revue de statistique appliquée*, 47 (2), 5-40.

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.