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INDUCED RESISTANCE AS A STRATEGY FOR VINEYARD PROTECTION

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Summary

As most grown grapevine *Vitis vinifera* varieties are susceptible to diseases such as downy and powdery mildews, numerous treatments are required to ensure a satisfactory yield and harvest quality. However, the use of phytochemical fungicides has serious drawbacks: some of them are potentially harmful for the environment and human health and contribute to the selection of resistant pathogen strains. Nowadays, in an objective of sustainable viticulture, there are increasing societal request, political incitation and winegrower's awareness to reduce the use of pesticides. For these reasons, alternative / complementary strategies of protection are under research. In our laboratory, we are studying the activation of the grapevine defense reactions by compounds called elicitors.

Regarding the elicitor-induced resistance of grapevine leaves against downy and powdery mildews, promising results have been obtained in greenhouse conditions but this strategy is still not controlled in the vineyard conditions. We propose to present the state of the art concerning induced resistance in grapevine: from concept to vineyard application.

Introduction

Grapevine is a major crop of high economical importance, with about eight millions hectares in the world. However, one major problem is that most of the grown grapevines are *V. vinifera* cultivars susceptible to cryptogamic diseases such as downy mildew, powdery mildew and gray mold, respectively caused by *Plasmopara viticola*, *Erysiphe necator* and *Botrytis cinerea*. Such diseases can cause important qualitative and quantitative losses and

huge amounts of fungicides are generally required to fight them and to ensure a satisfactory yield and the quality of the harvest. Despite fungicides are generally effective, some of them are potentially harmful for the environment and human health, and contribute to the selection of resistant pathogen strains. Nowadays, in an objective of sustainable viticulture, there is increasing social request, political incitation, and winegrower wishes to develop alternative or complementary strategies of protection allowing a reduced use of pesticides. Among strategies already developed or investigated are organic farming, biodynamics, and resistant hybrids. Another way is the induction of grapevine resistance to disease by the use of molecules able to stimulate its natural defenses. Such molecules are called elicitors (Ebel and Cosio, 1994).

Elicitors are natural or synthetic and belong to various biochemical families: carbohydrates, lipids, (glycol)peptides and (glycol)proteins. Some natural elicitors are released during plant microbe interactions. Those of plant origin are released from the cell walls and called DAMPS for Damage Associated Molecular Patterns (Boudart et al., 2003; Vidal et al., 1998). Those of microbial origin are secreted or released from the cell wall by hydrolytic enzymes; they are called PAMPs (Pathogen Associated Molecular Pattern) or MAMPs (Microbial Associated Molecular Pattern) (Albersheim and Darvill, 1985; Boller and Felix, 2009; Côté and Hahn, 1994; Nürnberger et al., 2004). Their perception by receptors located at the plant cell surface allows the activation of plant defense and lead to PAMP-triggered immunity (PTI) (Zipfel, 2008). Elicitors induce a signaling cascade involving ion fluxes, H₂O₂ and NO production, Mitogen Activated Protein Kinase activation (Garcia-Brügger *et al.* 2006). These events lead to the activation of the expression of defense genes encoding PR proteins, enzymes involved in phytoalexin production and cell-wall strengthening. Phytohormones, especially salicylic acid, jasmonic acid, ethylene, and abscisic acid are also involved in defense signaling and their role depends on plant/pathogen interactions (Bari and Jones, 2009). Some elicitors have a particular mode of action. They condition the plant and when the pathogen attempts to infect the plant, all these defenses are activated more rapidly and intensively; this is the so-called priming (Conrath *et al.* 2006).

We have investigated the grapevine response to elicitors at different scales: from cell suspensions to plantlets using BcPG1 (Poinssot *et al.* 2003; Lesniewska *et al.* 2004; Vandelle *et al.* 2006) or laminarin (Aziz-Aziz *et al.* 2003). We have studied in detail the mode of action of PS3 (Trouvelot *et al.* 2008). PS3 is a sulfated derivative of the β -1,3 glucan laminarin extracted from the brown algae *Laminaria digitata* (Ménard *et al.* 2004). We have observed that PS3 induces resistance against *P. viticola* and acts by priming. Among the events primed

by PS3 is H₂O₂ production and accumulation [revealed *in situ* by the occurrence of brown precipitates after DAB staining (Thordal-Christensen, 2003)] that contributes to restrict the mycelial development, as indicated by alterations of the mycelium at the sites of accumulation of this active oxygen species. Interestingly, a similar H₂O₂ accumulation was made in leaves of the downy mildew tolerant Solaris inoculated by *P. viticola* but without prior treatment by PS3. The stilbenes resveratrol and its derivatives are produced and accumulated in response to elicitor treatment (Adrian *et al.* 2012) and they are phytoalexins, as demonstrated by their antimicrobial effects (Adrian *et al.* 1997, Malacarne *et al.*, 2011, Pezet *et al.* 2004). Their accumulation, together with other phenolic compounds, is also primed by PS3 in Marselan leaves in response to *P. viticola* infection. A similar but more intensive response naturally occurs in the resistant *V. rupestris* inoculated by *P. viticola* infection, without prior PS3 treatment (Adrian *et al.* 2012). Scanning electron micrograph observations of the lower side of Marselan leaves treated by PS3 and infected by *P. viticola* present scarce and abnormal sporangiophores, as previously described by Dai *et al.* (1995) for *V. rupestris*. Moreover, some stomata were closed by callose, similarly to what was reported by Gindro *et al.* (2003) as a natural defense response of the hybrid Solaris. So we have demonstrated that PS3-induced resistance (IR) mimics, in some extent, the natural resistance of tolerant / resistant genotypes. These results thus validate the idea of a strategy based on elicitor-induced resistance.

So the question is « Is IR effective in the vineyard? ». In other words, does an elicitor application trigger grape defenses and prevent infection by a pathogen in field conditions? A limited number of papers have reported trials with elicitors in the vineyard (Figure 1), probably for two main reasons. First, for a long time, the interest was mainly focused upon the study of defense events induced by elicitors (and not IR) and the use of cell suspensions was generally preferred. Secondly, few molecules are active in field conditions.

Among the elicitors experimented in the vineyard are principally chemicals such as jasmonic acid and salicylic acid derivatives (BTH, Benzothiadiazoles), and β -aminobutyric acid (BABA) (Tally *et al.* 1999; Reuveni *et al.* 2001, Iriti *et al.* 2005; Reglinski *et al.* 2005, Belhadj *et al.* 2006, Biondi *et al.* 2009). We have studied IR using leaf discs, and plants grown in controlled and in field conditions. Despite the results obtained in controlled conditions were promising, those performed in the vineyard were not reproducible and often disappointing. Globally, one can note a decrease of efficiency from assays performed with leaf disc assays to assays with plantlets grown in greenhouses to assays in field conditions (Table 1). Many reasons could account for the lack of efficiency of IR in the vineyard (Figure

2). We started studies on three of them: the age-dependent organ responsiveness (or ontogenic resistance), the cuticle barrier in relation to the elicitor availability, and the physiological status of the plant.

The plant natural resistance to bioaggressors can be influenced by various factors including the age-related resistance that is the ability of whole plants or plant parts to resist or tolerate disease when they age and mature. Using plants grown in greenhouses, we have observed that the level of IR was also higher for “old” leaves (3rd fully expanded leaf under the apex of 6 expanded leaf plantlets) compared to that of “young” leaves (1st and 2nd fully expanded leaves). We have also shown that these differences were correlated to a higher induced H₂O₂ production, defense gene expression and phytoalexin production (Steimetz *et al.* 2012).

The role of the cuticle could be crucial regarding the availability of elicitors and the efficiency of IR. Once sprayed on the leaf surface, the elicitor has to go through the hydrophobic cuticular waxes and the cuticle to reach the plant cell to be perceived and to induce the defense reactions. We intend to identify the elicitors that possess the highest probability to go through this barrier (depending on their size, physical and chemical characteristics), and to determine which quantity of the elicitor actually reach the cell plasma membranes.

Unlike fungicides that directly targets the pathogens, elicitors use the plant to activate defenses. It induces a cell reprogramming that requires energy that the plant has to fuel (Bolton, 2009). So the plant responsiveness to induced resistance could depend on its physiological status. That is why we presently investigate what is the impact of IR on grapevine physiology and, conversely, how the physiological status of grapevine can impact the level of induced resistance. We also study if environmental factors affect the plant responsiveness to elicitor-IR, either directly or indirectly *via* their effect on plant physiology.

In conclusion, IR is a complex response. Despite intensive research is performed, a large area of investigation still remains to improve our knowledge of IR. As far as basic research is concerned, the mechanisms associated to IR and the factors able to modulate them remain partly decrypted. From an applied point of view, elicitors possess a potentially high interest for crop protection since they can not only elicit defenses in a broad spectrum of plants, but are also mostly deprived of toxicity and suitable for industrial production from abundant sources. However, the most efficient molecules have to be identified and the optimal conditions of application have to be determined. It is also clear that IR should not be considered as a unique strategy but as a part of an integrated strategy for vineyard protection.

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Table 1 : Mean efficiency of oligosaccharidic elicitor treatments against downy and powdery mildews. Biotests were performed in controlled and field conditions as follow :*: 24h floating on elicitor solution , ** : hand-held sprayer, run-off - *** : pneumatic sprayer (~200L/ha) (unpublished results).

Leaf discs*		Plantlets in greenhouse **		Vineyard***	
Downy mildew	Powdery mildew	Downy mildew	Powdery mildew	Downy mildew	Powdery mildew
100%	nt	65%	80%	<10%	>60%

Legends of the figures

Figure 1: Mean percentage of papers reporting elicitor studies conducted on cell suspensions, plantlets and berries in controlled conditions, and in the vineyard. (From 81 papers published since 1991 – elicitor AND grapevine & elicitor AND *Vitis* as keywords ; « Bibliovie » as database)

Figure 2: Factors that potentially affect plant responsiveness to elicitor-induced resistance

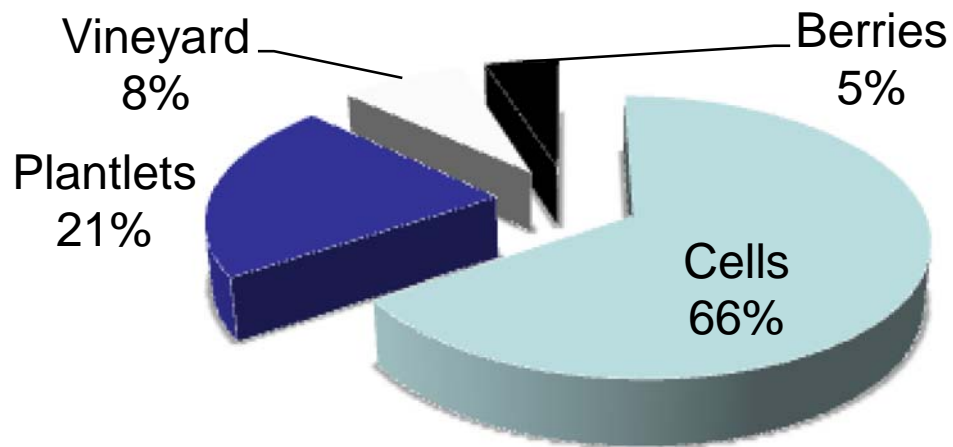


Figure 1

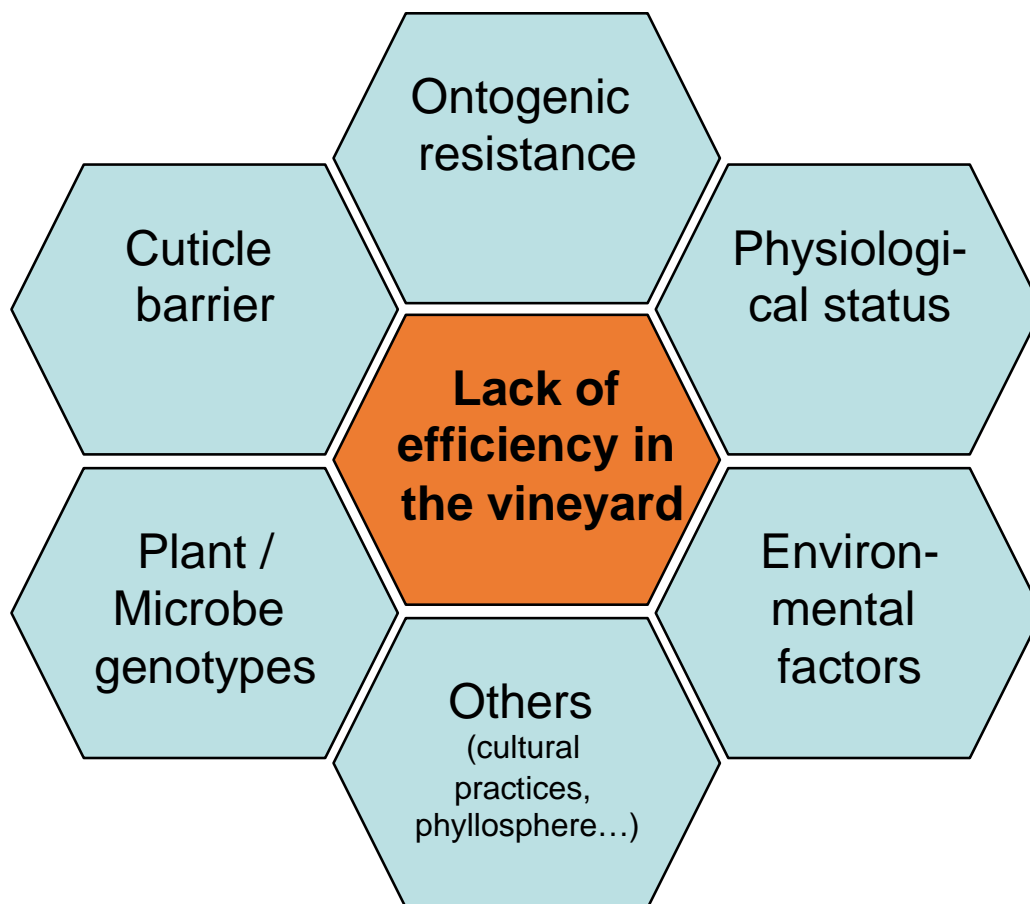


Figure 2