Induced resistance as a strategy for vineyard protection

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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, H_2O_2 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; Lesnievska *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 ß-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 H_2O_2 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 H_2O_2 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 ß-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 bioagressors 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 (3^{rd} fully expended leaf under the apex of 6 expended leaf plantlets) compared to that of "young" leaves (1^{st} and 2^{nd} fully expended leaves). We have also shown that these differences were correlated to a higher induced H_2O_2 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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References

- Adrian M, Jeandet P, Veneau J, Weston LA, Bessis R (1997) Biological activity of resveratrol, a stilbenic compound from grapevines, against *Botrytis cinerea*, the causal agent for gray mold. J Chem Ecol 23:1689-1702
- Adrian M., De Rosso M., Bavaresco L., Poinssot B., Héloir MC. (2012). Resveratrol from vine to wine. In *Resveratrol in health and disease*. D. Delmas (ed.).
- Albersheim P, Darvill A (1985) Oligosaccharins. Sci Amer 253:44-50
- Aziz A, Poinssot B, Daire X, Adrian M, Bézier A, Lambert B, Joubert J, Pugin A (2003) Laminarin elicits defense responses in grapevine and induces protection against *Botrytis cinerea* and *Plasmopara viticola*. Mol Plant-Microbe Interact 16:1118-1128
- Bari R, Jones J (2009) Role of plant hormones in plant defence responses. Plant Mol Biol 69: 473-488
- Belhadj A, Saigne C, Telef N, Cluzet S, Bouscaut J, Corio-Costet MF, Mérillon JM (2006) Methyl jasmonate induces defense responses in grapevine and triggers protection against *Erysiphe necator*. J Agric Food Chem 54:9119-9125
- Biondi E, Bini F, Anaclerio F, Bazzi C (2009) Effect of bioagents and resistance inducers on grapevine crown gall. Phytopathologia Mediterranea 48(3):379
- Boller T, Felix G (2009) A Renaissance of Elicitors: Perception of Microbe-Associated Molecular Patterns and Danger Signals by Pattern-Recognition Receptors. Annu Rev Plant Biol 60:379-406
- Bolton MD (2009) Primary metabolism and plant defense - fuel for the fire. Mol Plant Microbe Interact. 22(5):487-97
- Boudart G, Charpentier M, Lafitte C, Martinez Y, Jauneau A, Gaulin E, Esquerré-Tugayé MT, Dumas B (2003) Elicitor activity of a fungal endopolygalacturonase in tobacco requires a functional catalytic site and cell wall localization. Plant Physiol 131:93-101
- Conrath U, Beckers GJ, Flors V, Garcia-Agustin P, Jakab G, Mauch F, Newman MA, Pieterse CM, Poinssot B, Pozo MJ, Pugin A, Schaffrath U, Ton J, Wendehenne D, Zimmerli L, Mauch-Mani B (2006) Priming: getting ready for battle. Mol Plant-Microbe Interact 19:1062-1071
- Côté F, Hahn M (1994) Oligosaccharins: structures and signal transduction. Plant Mol Biol 26:1379-1411
- Dai GH, Andary C, Mondolot-Cosson L, Boubals D (1995) Histochemical studies on the interaction between three species of grapevine, *Vitis vinifera*, *V. rupestris* and *V. rotundifolia*. Physiol Mol Plant Pathol 46:177-188
- Ebel J, Cosio EG (1994) Elicitors of plant defense responses. Int Rev Cytol 148:1-36
- Garcia-Brugger A, Lamotte O, Vandelle E, Bourque S, Lecourieux D, Poinssot B, Wendehenne D, Pugin A (2006) Early signaling events induced by elicitors of plant defenses. Mol Plant-Microbe Interact 19:711-724
- Gindro K, Pezet R, Viret O (2003) Histological study of the responses of two *Vitis vinifera* cultivars (resistant and susceptible) to *Plasmopara viticola* infections. Plant Physiol Biochem 41:846-853
- Iriti M, Rosoni M, Borgo M, Faoro F (2004) Benzothiadiazole enhances resveratrol and anthocyanin biosynthesis in grapevine, meanwhile improving resistance to *Botrytis cinerea*, J Agric Food Chem 2004, 52(14):4406-4413.
- Lesniewska E, Adrian M, Klinger A, Pugin A (2004) Cell wall modification in grapevine cells in response to UV stress investigated by atomic force microscopy. Ultramicroscopy 100:171-178
- Malacarne G, Vrhovsek U, Zulini L, Cestaro A, Stefanini M, Mattivi F, Delledonne M, Velasco R, Moser C (2011) Resistance to Plasmopara viticola in a grapevine segregating population is associated with stilbenoid accumulation and with specific host transcriptional responses. BMC Plant Biol. 11:114.
- Ménard R, Alban S, de Ruffray P, Jamois F, Franz G, Fritig B, Yvin JC, Kauffmann S (2004) Beta-1,3 glucan sulfate, but not beta-1,3 glucan, induces the salicylic acid signaling pathway in tobacco and Arabidopsis. Plant Cell. 16:3020-3032
- Ménard R, de Ruffray P, Fritig B, Yvin JC, Kauffmann S (2005) Defense and resistance inducing activities in

tobacco of the sulfated beta-1,3 glucan PS3 and its synergistic activities with the unsulfated molecule. Plant Cell Physiol 46:1964-1972

- Nürnberger T, Brunner F, Kemmerling B, Piater L (2004) Innate immunity in plants and animals. Striking similarities and obvious differences. Immunol Rev 198:249-266
- Pezet R, Gindro K, Viret O, Spring JL (2004) Glycosylation and oxidative dimerization of resveratrol are respectively associated to sensitivity and resistance of grapevine cultivars to downy mildew. Physiol Mol Plant Pathol 65:297-303
- Poinssot B, Vandelle E, Bentéjac M, Adrian M, Levis C, Brygoo Y, Garin J, Sicilia F, Coutos-Thévenot P, Pugin A (2003) The endopolygalacturonase 1 from *Botrytis cinerea* activates grapevine defense reactions unrelated to its enzymatic activity. Mol Plant-Microbe Interact 16:553-564
- Reglinski T, Elmer PAG, Taylor JT, Parry FJ, Marsden R, Wood PN (2005)□Suppression of Botrytis bunch rot in Chardonnay grapevines by induction of host resistance and fungal antagonism Aus. Plant Pathol. 34 :481-488□
- Reuveni M, Zahavi T, Cohen Y (2001) Controlling downy mildew (*Plasmopara viticola*) in field-grown grapevine with beta-aminobutyric acid (BABA). Phytoparasitica 29:125-133
- Steimetz E, Trouvelot S, Gindro K, Bordier A, Poinssot B, Adrian M, Daire X (2012) Influence of leaf age on induced resistance in grapevine against *Plasmopara viticola*. Physiol Mol Plant Pathol 79:89-96.
- Tally A, Oostendorp M, Lawton K, Staub T, Bassy B (1999) Commercial development of elicitors of induced resistance to pathogens. In: Agrawal AA, Tuzun S, Bent E (eds) Inducible Plant Defenses Against Pathogens and Herbivores: Biochemistry, Ecology, and Agriculture. Amer Phytopathol Soc Press, St Paul
- Thordal-Christensen H (2003) Fresh insights into processes of non host resistance. Curr Opin Plant Biol 6: 351-357
- Trouvelot S, Varnier A, Allegre M, Mercier L, Baillieul F, Arnould C, Gianinazzi-Pearson V, Klarzynski O, Joubert J, Pugin A, Daire X (2008) A beta-1,3 glucan sulfate induces resistance in grapevine against *Plasmopara viticola* through priming of defense responses, including HRlike cell death. Mol Plant-Microbe Interact 21:232-243
- Vandelle E, Poinssot B, Wendehenne D, Bentejac M, Pugin A (2006) Integrated signaling network involving calcium, nitric oxide, and active oxygen species but not mitogen-activated protein kinases in BcPG1-elicited grapevine defenses. Mol Plant-Microbe Interact 19:429-440
- Vidal S, Eriksson ARB, Montesano M, Denecke J, Palva ET (1998) Cell Wall-Degrading Enzymes from *Erwinia carotovora* Cooperate in the Salicylic Acid-Independent Induction of a Plant Defense Response. Mol Plant-Microbe Interact 11:23-32

Table 1 : Mean efficiency of oligosaccharidic elicitor treatments against downy and powdery midews. 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

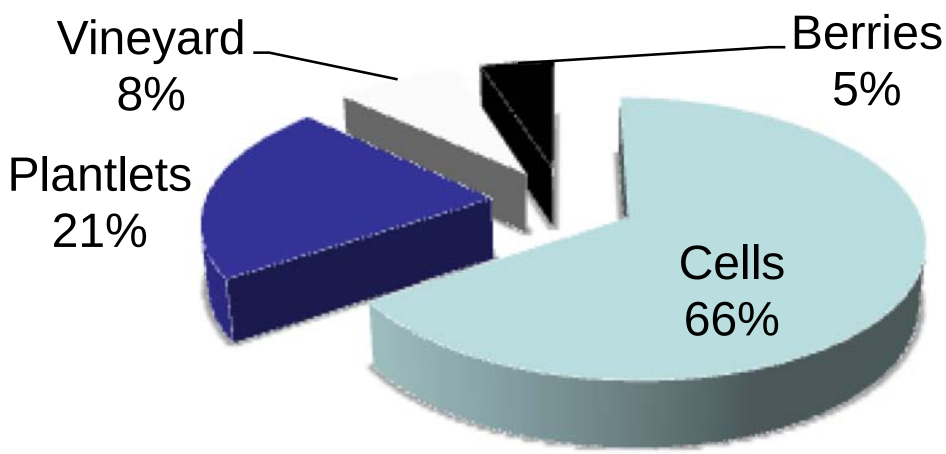

Figure 1

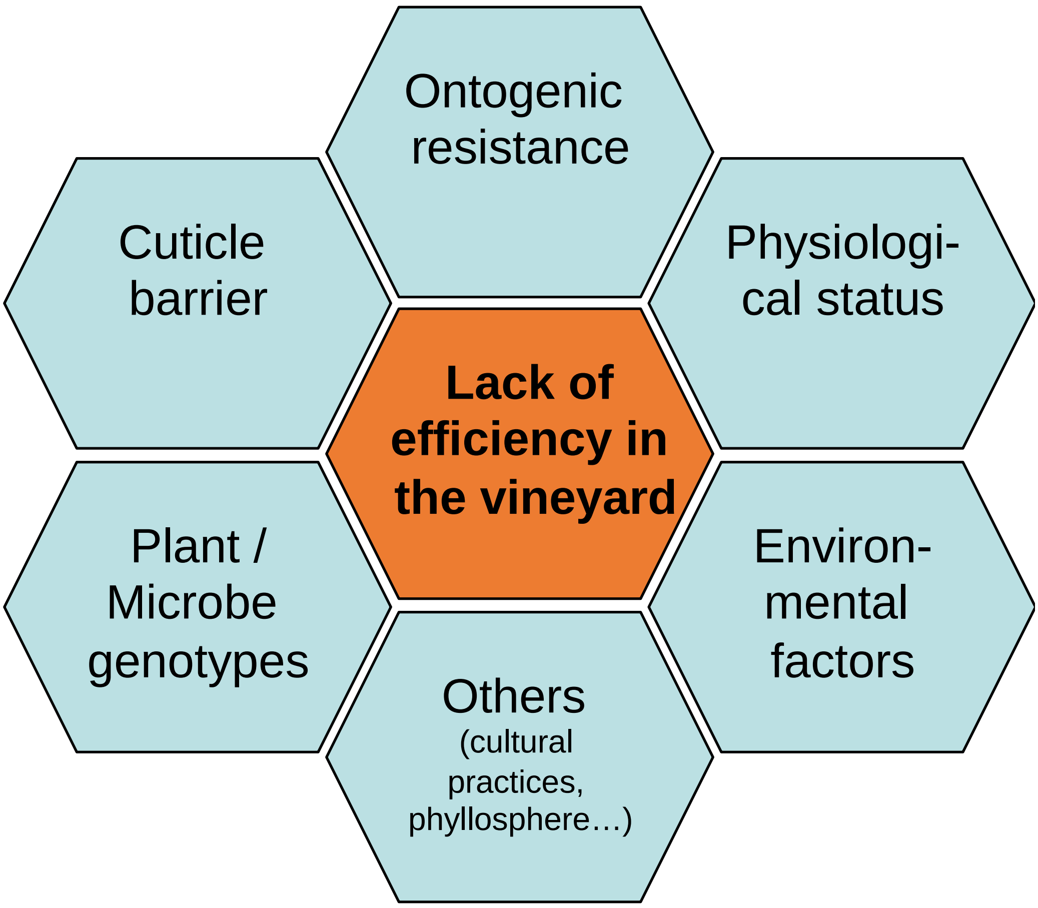

Figure 2