Stilbene content in leaf discs of different grapevine genotypes treated with downy mildew (Plasmopara viticola) or UV-C

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Introduction

Stilbenes are considered as the main phytoalexins in grapevine. They are known to contribute to the protection against various pathogens among which downy mildew. The major molecules involved in this phenomenon are resveratrol and its derivatives: pterostilbene, piceid and viniferins. The grapevine defence response induced by UV-C irradiation has been reported to be correlated with the resistance level of the genotype (*1, 2*).

This work aims to test the hypothesis of a relation between the synthesis of stilbenes and the resistance to downy mildew derived from *Muscadinia rotundifolia*

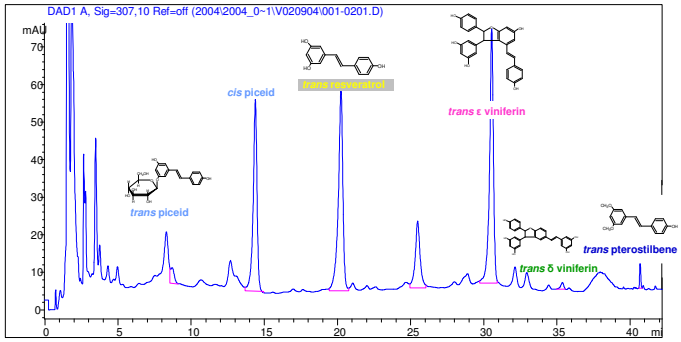
Materials and methods

Three genotypes were studied:
- *Vitis vinifera* Syrah (susceptible to *P. viticola*),
- a hybrid called RV1 (partially resistant, BC1 between *M. rotundifolia* and *V. vinifera*)
- *M. rotundifolia* (totally resistant).

Leaf discs (1 cm diameter) were taken from the 5th and 6th leaf of 10 to 12 leaves greenhouse plants and placed into Petri dishes.

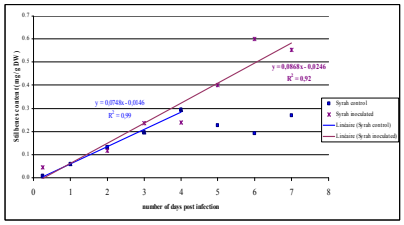
The abaxial leaf disc surfaces were then sprayed with a suspension of *P. viticola* (10^5 sporangia/ml) or irradiated by UV-C (254 nm, 10 mn). Twelve leaf discs were extracted with methanol (45 mn, 60°C). Three replicates were performed.

Time course of production of the *trans*- and *cis*-isomers of resveratrol, piceid, ε and δ viniferin and pterostilbene was studied with HPLC-DAD.

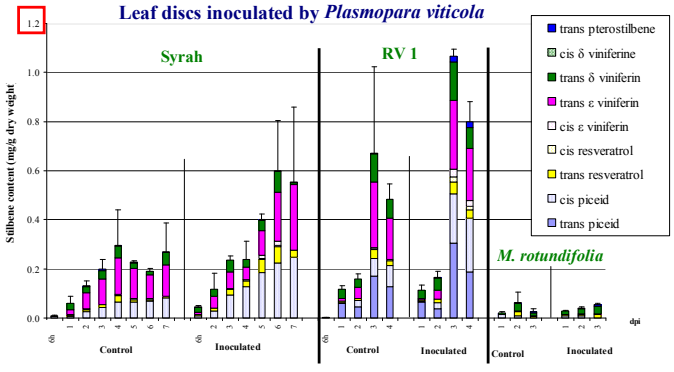


Chromatogram 307 nm : RV1 96h UV

Results



- Making leaf discs (control) provoked a wound reaction inducing a linear synthesis of stilbene. The linear phase of the production was different depending on the genotype (i.e. 4 dpi for Syrah R2=0.99).



- In leaf discs inoculated with P. viticola, the stilbene content increased similarly to the control but the linearity lasted longer (i.e. 7 dpi for Syrah, R^2=0.92).

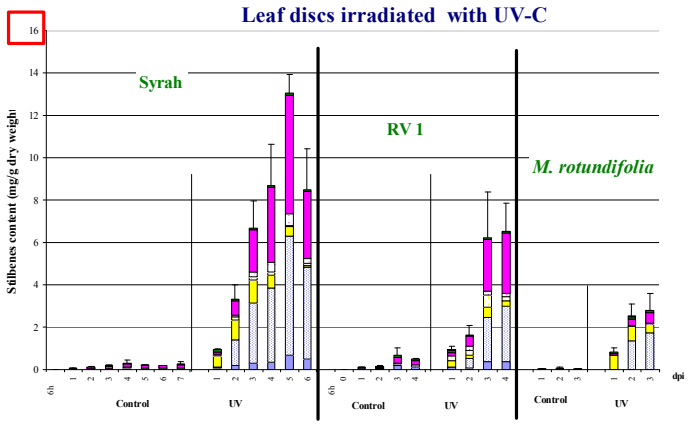
- UV treatment caused a linear increase of stilbene content (5 dpi Syrah, R^2=0.99; 4 dpi RV1 R^2=0.87; 3 dpi *M. rotundifolia* R^2=0.86).

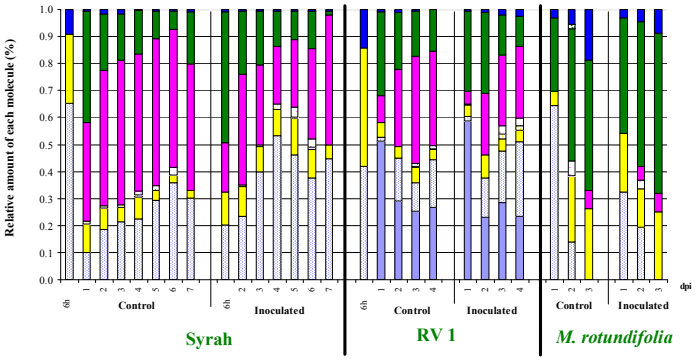
- Induction of stilbene production by UV-C was 8 to 48 times higher than by inoculation with *P. viticola*

- Induction of stilbene production by inoculation with *P. viticola* was faster in the partially resistant genotype (RV1) than in the susceptible (Syrah).

- *M. rotundifolia* produced very low stilbene compared to RV1 and Syrah but a molecule inducible; looking close to a flavonoid (data not shown) is in course of determination.

- Ratio between stilbenes varied depending on the way of induction.





Conclusions

In this first experiment we don't find a link between the level of resistance to *P. viticola* and the stilbene content in leaf discs of the genotypes studied.

Different hypothesis could be put forward:

- **Mechanisms involved in the resistance of *M. rotundifolia* are independent of the synthesis of stilbene but numerous flavonoids are present in that species.**
- **Assays performed on large surface of leaves (cm^2) are not representative of the spatial repartition of these molecules: microscopic observations show very local distribution of the defense reactions (μm^2). Other approaches are in progress.**

References

1.Dercks, W., and Creasy, L. L. (1989) *Physiological and Molecular Plant Pathology 34*, 189-202.
2.Douillet-Breuil, A. C., Jeandet, P., Adrian, M., and Bessis, R. (1999) *Journal of Agricultural and Food Chemistry 47*, 4456-4461.