Ontogenic resistance to Plasmopara viticola

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ONTOGENIC RESISTANCE TO *PLASMOPARA VITICOLA*

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Introduction

Different factors are known to be implicated in the resistance of grapevine to downy mildew *(Plasmopara viticola*, Oomycetes). The most obvious one is the presence of resistance genes (1-4). The **physiological stages** of plants and leaves are also determining factors (5-6). Understanding the mechanisms underlying ontogenic resistance will contribute to a better management of downy mildew infections in experimental trials and potentially in vineyards (trimming). Here, we focused on the **hypothetical implication of secondary metabolites, especially stilbenes and flavonoids, in ontogenic resistance**.

Results

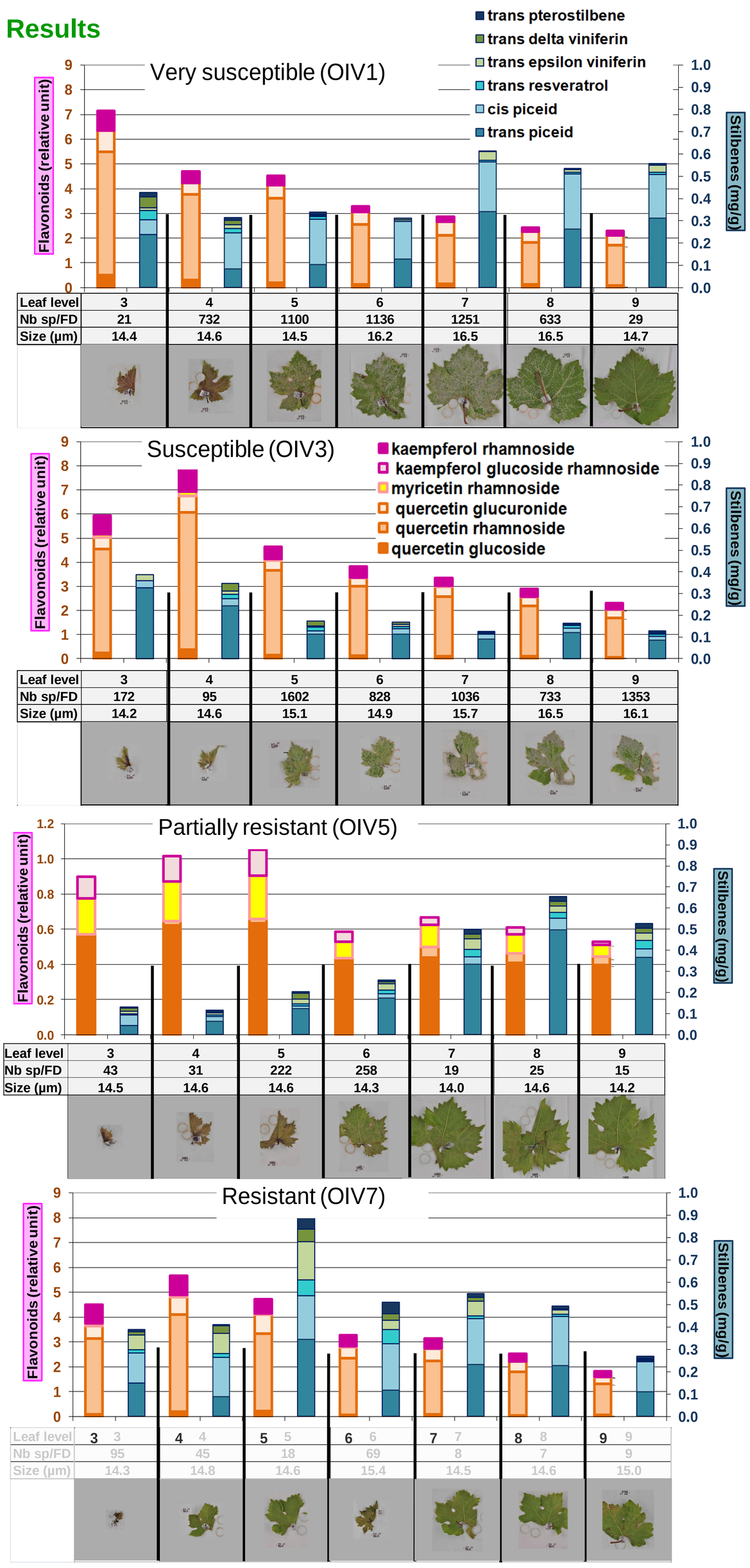

Fig 1: Histogram: flavonoid (left scale) and stilbene (right scale) contents for 4 genotypes and 7 leaf levels on the stem
Table: Number of sporangia per foliar disc (Nb sp/FD) and size of sporangia. Mean over 10 one-cm-diameter foliar discs 7 days post inoculation (dpi)
Pictures of the corresponding leaf at 7 dpi

Number of sporangia depended on the leaf level, generally increasing from leaf level 3 to 6 in the most resistant genotypes and from leaf level 3 to 7 or 9 in susceptible genotypes. Flavonoid content decreased linearly from leaf levels 3-4 to 9 (Fig. 1) and was equal in inoculated and control leaves while stilbene not present in control, increased after inoculation (data not shown).

Genotype	OIV	Molecules	Leaf level	Linear regression Function	R^2
Very susceptible	1	Flavonoids	3 to 7	y = -0.0032x + 7.205	**0.92**
Very susceptible	1	Stilbenes	3 to 6	y = -9E-05x + 0.4169	**0.79**
Susceptible	3	Flavonoids	3 to 7	y = -0.0021x + 6.6604	**0.51**
Susceptible	3	Stilbenes	3 to 7	y = -0.0002x + 0.3591	**0.71**

Table 1: Linear regressions between flavonoid content and the number of sporangia per foliar disc, and between stilbene content and the number of sporangia per foliar disc

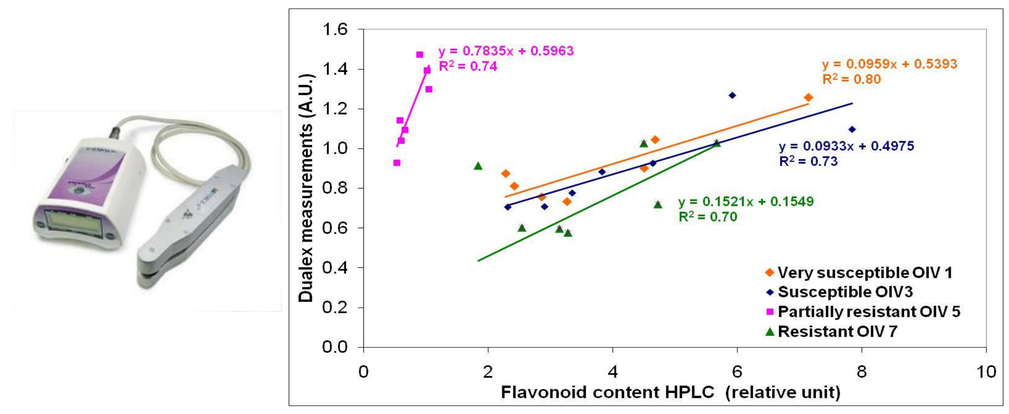

Fig 2: Relation between the mean of 3 Dualex measurements on the abaxial side and 3 on the adaxial side of the leaf and the flavonoid content (HPLC)

From leaf level 3 to 6 or 7, negative linear relations were found between the number of sporangia per foliar disc and flavonoid content as well as stilbene content in susceptible genotypes (Table 1).
Flavonoid contents measured by HPLC-DAD and Dualex (portable leaf-clip (7)) were always highly correlated, but the slope depended on the genotype (Fig. 2).

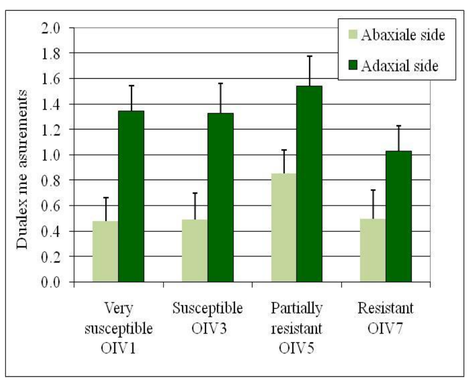

Fig 3: Mean of 3 Dualex measurements on leaf 3 to 9 on both sides of the leaf

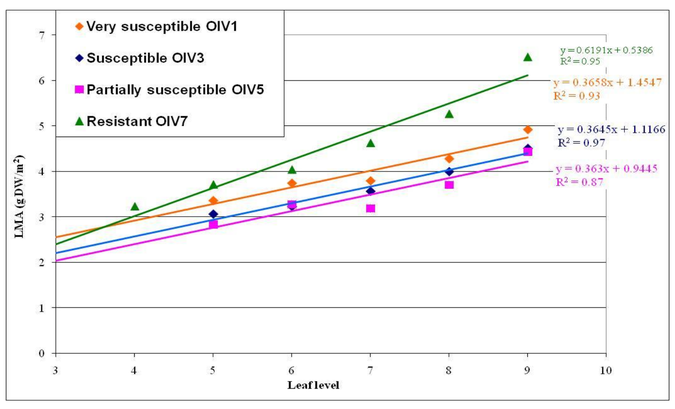

Fig 4: Relation between dry leaf mass per area (LMA) and leaf level for the 4 genotypes

Dualex measurements show that the abaxial to adaxial distribution of the flavonoid was genotype dependant, but the flavonoid content was 2 to 3 times higher on the adaxial side (Fig 3). Leaf mass per area increased linearly from leaf level 3 to leaf level 9 (Fig 4).

Conclusion

This preliminary study has shown that:

- **each leaf on the same stem reacts differently to the same *P. viticola* infection**
- there is **an increase in sporulation from leaf level 3-4 to 6-7**
- there is a **negative correlation between the sporulation from leaf levels 3 to 6-7 in susceptible genotypes and the flavonoid and stilbene contents**

These results led us to consider the **hypothesis of two kinds of ontogenic resistance**: the first one linked to very young leaves and the second one taking place in older leaves (after leaf level 7). Phenolic compounds, flavonoids and stilbenes, could increase the resistance of young leaves of susceptible genotypes while another factor, perhaps in relation with the leaf mass per area, could protect older leaves.

Materials and methods

The influence of the age of the leaf on the resistance to *Plasmopara viticola* was studied in four genotypes presenting varying levels of resistance.
The plants were cultivated in open field. Two stems were studied per plant. On the first one, seven leaves from level 3 to 9 (level one corresponded to the first developed leaf at the top of the stem) were harvested, rinsed then inoculated for 5 hours, by flotation in a *P. viticola* inoculum solution at 10^4 sporangia/ml. On the second stem, two leaves were used: levels 5 and 6. Level 6 was inoculated whereas level 5 was not (control).
Sporangia were numbered on foliar discs with a particle counter at 7 days post inoculation (dpi). Dry leaf mass per area (LMA) were measured. Metabolites were extracted from leaf with MeOH at 60°C. HPLC analyses were performed 3 dpi. Stilbene (piceid, resveratrol, viniferins, pterostilbene) and flavonoid (myricetin-3-O-rhamnisode, quercetin-3-O-galactoside, quercetin-3-O glucoside, quercetin-3-O-rhamnoside, kaempferol-glucose-rhamnose, kaempferol-3-O-rhamnoside) contents were analyzed with HPLC-DAD in non inoculated and inoculated extracts. Leaf flavonoid contents were also measured with a Dualex (portable leaf-clip (7)).

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