Influence of grapevine cultivation practices on berry PR-protein accumulation and botrytis cinerea infection

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INFLUENCE OF GRAPEVINE CULTIVATION PRACTICES ON BERRY PR-PROTEIN ACCUMULATION AND *BOTRYTIS CINEREA* INFECTION

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INTRODUCTION

***Botrytis cinerea*, the fungus responsible for gray mold symptoms on ripe berries at harvest and severe taste and smell defects in the wines of Champagne, is one of the most important grape pathogene in the Champagne vineyards of France. In order to protect themselves against this fungus, grapes express several defense substances which include secondary metabolites (a stilbene named resveratrol) and PR-proteins such as chitinases and thaumatin-like proteins. These later proteins even seem to play a crucial role during the interaction process. Two of them, a thaumatin-like protein (GO) and a chitinase (CHV5) are actually the 2 most abundant polypeptides expressed in a ripe berry. Moreover, they have been shown to strongly inhibit *B. cinerea* conidia germination in vitro, an effect which is potentiated by the high glucose content of ripe grapes. Interestingly, it has been shown that PR-protein content in grapes is not constant through their development. Their concentration indeed rises sharply from veraison to harvest. However, no study has yet aimed to evaluate the influence of cultural practices on this physiologically regulated increase in PR-protein accumulation. In the Champagne vineyards, grapevine is cultivated under very different cultivation practices and conditions. We have thus sampled healthy and ripe berries on various plots of grounds. We've then estimated CHV5 and GO content in each of these samples by enzymatic activity measurements and western blotting to see whether their correlate to any given cultivation practice or *Botrytis* pressure in their original plot of ground.**

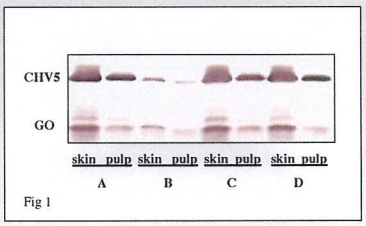

Fig 1

CHV5 and GO are equally affected by cultivation practices.

Fig 1: Crude grape pulp and skin protein extracts have been separated by SDS-PAGE and later analysed by western blotting using a combination of 2 antibodies : anti-CHV5 and anti-GO. The position of CHV5 and GO is indicated on the left-hand side of the figure. The results obtained after analysing 4 samples (A-D) is shown but similar results have been obtained on the 140 other samples which have been analysed in this study. In later experiments, we have thus used chitinase activity measurements to estimate PR-protein content.

Chitinase activity in grape skin and pulp are equally affected by cultivation practices.

Fig 2 : The chitinase activity of grape samples from 140 plots of grounds (includes 3 cultivars) has been estimated on their skin and pulp using chitin-azure as a substrate.

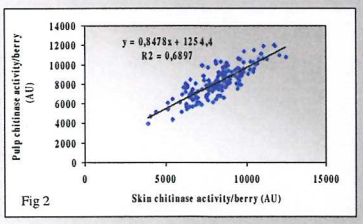

Fig 2

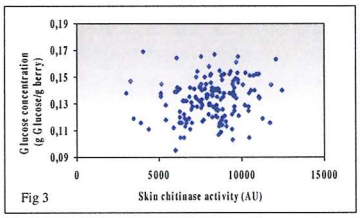

Fig 3

Reducing sugars and PR-proteins accumulation are not equally affected by cultivation practices.

Fig 3 : Reducing sugars of grapes samples on 140 plots of grounds (includes 3 cultivars) has been estimated by spectrophometer ATAGO and their skin chitinase activity has been estimated using chitin-azure as a substrate.

Ripe and healthy grapes differ greatly in their PR-protein content.

Fig 4 : The chitinase activity of grapes (*Vitis vinifera* L. cv. Pinot noir) sampled on 130 plots of grounds has been estimated on their skin using chitin-azure as a substrate. Chitinase activity measurements have a level of accuracy of about 5% which includes sample and assay errors.

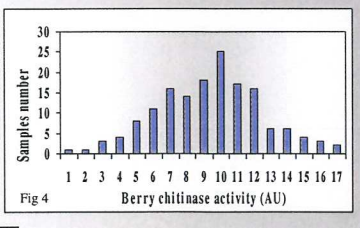

Fig 4

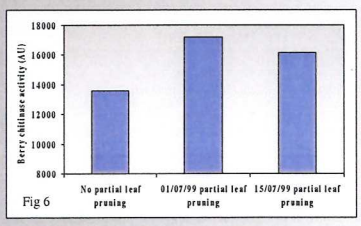

Fig 6

Partial leaf pruning effect on PR-protein amount.

Fig 6 : The chitinase activity of ripe grapes samples originating from the same plot of ground but which had suffered partial leaf pruning on different dates has been estimated on their skin using chitin-azure as a substrate. These chitinase activities inversely correlate with fungal attack.

Grape cultivars accumulate different amounts of PR-proteins.

Fig 5 : Crude grape pulp and skin protein extracts have been separated by SDS-PAGE and later analysed by western blotting using a combination of 2 antibodies : anti-CHV5 and anti-GO. The position of CHV5 and GO is indicated on the left-hand side of the figure . The results obtained after analysing 3 samples (Chardonnay : CH, Pinot meunier : MN and Pinot noir : PN) is shown. 3 Chardonnay cultivars samples as well as 130 samples of Pinot noir and 6 of Pinot meunier have been tested. Statistical analysis shows that, on average, less PR-protein is found in Chardonnay grapes compared to their Pinot counterparts which accumulate about equal amounts of PR-proteins. These results are in good agreements with the lower resistance of Chardonnay to *Botrytis*.

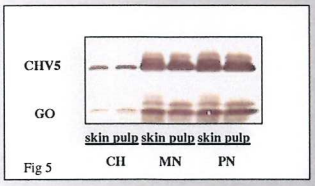

Fig 5

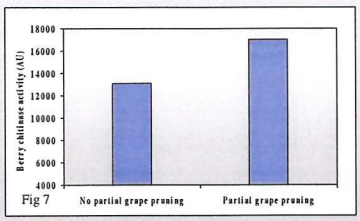

Fig 7

Partial grape pruning effect on PR-protein amount.

Fig 7 : The chitinase activity of ripe grapes samples originating from the same plot of ground but which had received (or not received) a partial grape pruning has been estimated on their skin using chitin-azure as a substrate. These chitinase activities are correlate with fungal attack.

Correlation between gray mold sensitivity of grapes and their PR-protein content.

Fig 8 : On the different plots of ground Which have been studed, visual estimates of gray mold frequency of attack have been realised. Each frequency estimate is compared to the chitinase activity of the grape skin (*Vitis vinifera* L. cv. Pinot noir). No global and strict correlation appears to exist between PR-protein content and resistance to *Botrytis*. Still under a certain threshold in chitinase activity, frequency index increase with lowering PR-protein contents with all samples suffering from a large disease pressure (grey triangle below dotted line).

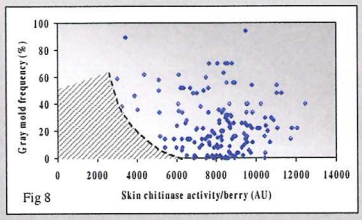

Fig 8

DISCUSSION

- **PR-protein accumulation is highly sensitive to grapevine growing conditions and differs greatly among various plots of ground. The origin of this variability is unknown and is greater than the one affecting glucose content.**
- **Eventhough glucose and PR-proteins accumulations have been shown to correlate in one given berry through its ripening, both parameters display no obvious correlation when comparing different plots of ground and thus may not share the same sensitivity to different environmental factors.**
- **Our data suggest that PR-proteins constitute only one out of many defense substances used by grapes to protect themselves against *Botrytis*. Under a certain threshold, they seem to render the grapes more sensitive to fungal attack while above this same threshold adding more does not yield higher resistance as *Botrytis* has ways to cope with them and as other defense factors may now be under their own threshold of activity to allow the fungus to overcome the plant defense machinery.**

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