Oligosaccharide induced resistance in grapevine as affected by cuticle permeability

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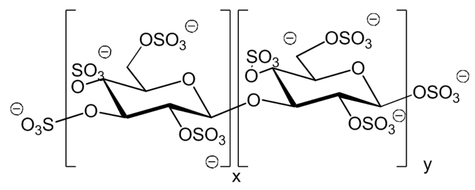
I. Context

Grapevine & downy mildew

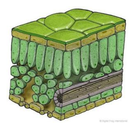
- Grapevine is susceptible to many diseases such as downy mildew, caused by the oomycete *Plasmopara viticola* (*Pv*).
- Elicitor induced resistance is an interesting strategy to help control this disease.

Sulfated ß-1,3-glucan



- Sulfated laminarin PS3 is obtained by chemical sulfation of laminarin, a storage oligosaccharide (OS) from the brown algae *Laminaria digitata.*
- PS3 has been shown to elicit plant defense reactions and to induce resistance against *Pv* in grapevine (1).

Penetration & resistance induction



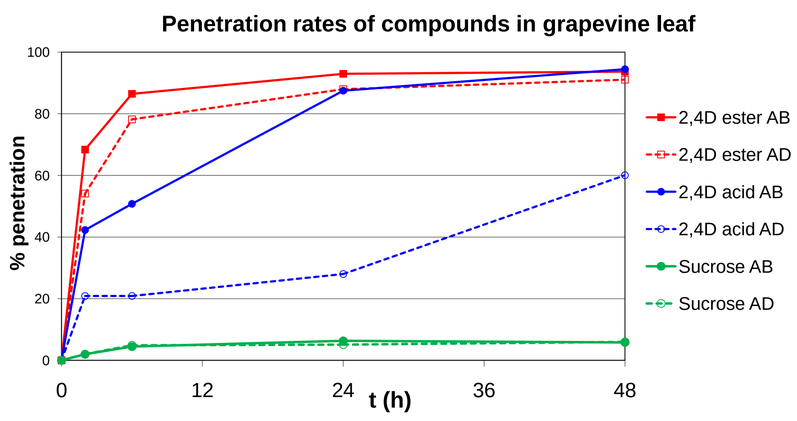
- OS in general suffer inconsistent disease control levels.
- A hypothesis is that hydrophilic elicitors of high MW (> 1000 Da) do not easily cross the hydrophobic cuticular barrier to reach internal receptors.

Aim

- To understand the penetration of OS in the leaf and to correlate it with the resistance induced against *Pv*.
- For this purpose, we studied the uptake of different labeled molecules in grapevine leaf, the effect of different adjuvants on penetration and PS3 efficacy in *Pv* resistance tests.

II. Grapevine leaf is nearly impermeable to sucrose unless an appropriate adjuvant is used

These studies were realized with ^{14}C labeled sucrose, 2,4-D-acid and ester. Measure of penetration used a wash-off and tissue combustion method after application of these radioactive compounds to the adaxial (AD) or abaxial (AB) leaf surface (2).



2,4-D-ester (hydrophobic) penetrates the leaf through both sides while the penetration of 2,4-D-acid (less hydrophobic) is severely reduced in the adaxial surface.
In contrast, only a very small amount of sucrose (hydrophilic) can get through the cuticle.

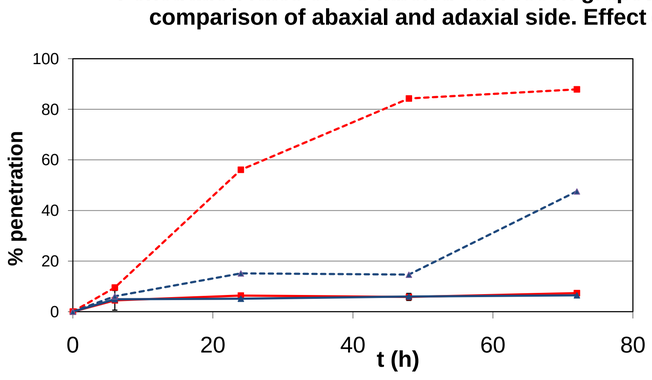
The hydrophobic cuticular barrier seems to prevent the penetration of hydrophilic compounds whereas hydrophobic ones of the same size easily get through.
Is it possible to increase their penetration, and possibly their efficacy, with an appropriate adjuvant ?

Adjuvant	HLB[1]	EO[2]	% penetration of sucrose in AB side: 6h	24h	72h
None			5	5	8
SLP4 (0,1%)	9,7	*4*	8	10	14
SilwetL77 (0,1%)		*8*	5	10	22
Tween 20 (0,1%)	16,7	*20*	5	42	68
DehscofixCO125 (0,1%)	13	*35*	10,5	43,3	83,1

Some adjuvants are actually able to enhance the penetration of sucrose through the abaxial side of grapevine leaf. Adjuvants with the highest ethoxylation increase it further.

(1) **HLB** = **H**ydrophilic **L**ipophilic **B**alance of the adjuvant determining if it is rather hydrophobic (low HLB) or hydrophilic (high HLB) on a scale ranging from 0 to 20.
(2) **EO** = **E**thylene **O**xide residues in a molecule (degree of ethoxylation).

Dehscofix CO125 (De) increases tenfold the penetration rate of an hydrophilic compound through the abaxial side of the leaf. This adjuvant will be used for further experiments.

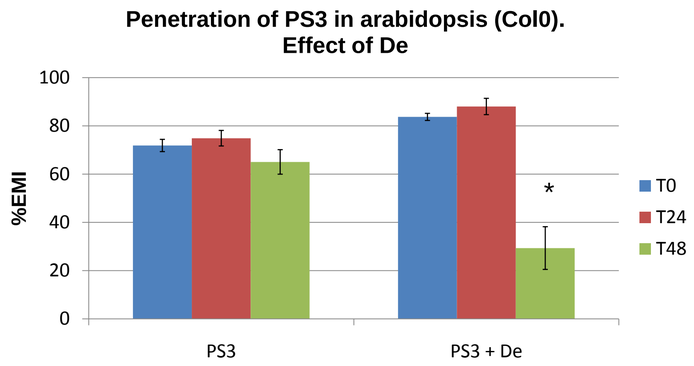


With Dehscofix CO125, sucrose is effectively able to cross the abaxial cuticle and in lesser amount, the adaxial one.

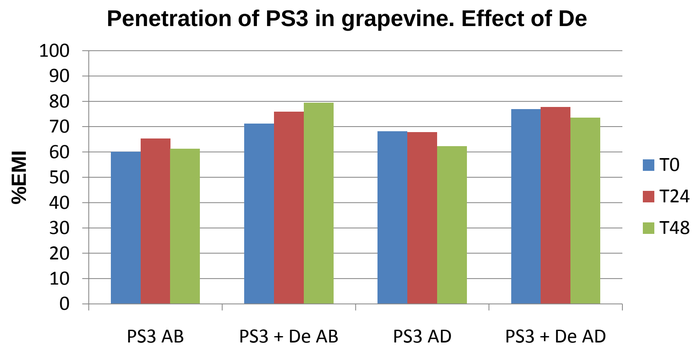
This difference of penetration between both sides of grapevine leaf could be explained by a difference in thickness, structure and composition of cuticular waxes and by the presence of stomata on the abaxial surface.
The adjuvant probably enhances leaf penetration by solubilisation of these cuticular waxes.

III. Comparison of PS3 penetration into arabidopsis and grapevine leaf

While ^{14}C labeled sucrose is commercially available, ^{14}C PS3 is not. Also, its synthesis would lead to too many radioactive wastes and was not considered . The following experiments make use of fluoresceine labeled PS3. Treatments are deposited on the leaf, washed off and quantified via spectrofluorimetric measures (3).



Addition of De (0,1%) significantly enhances the penetration of PS3 through arabidopsis leaf 48 h post treatment.

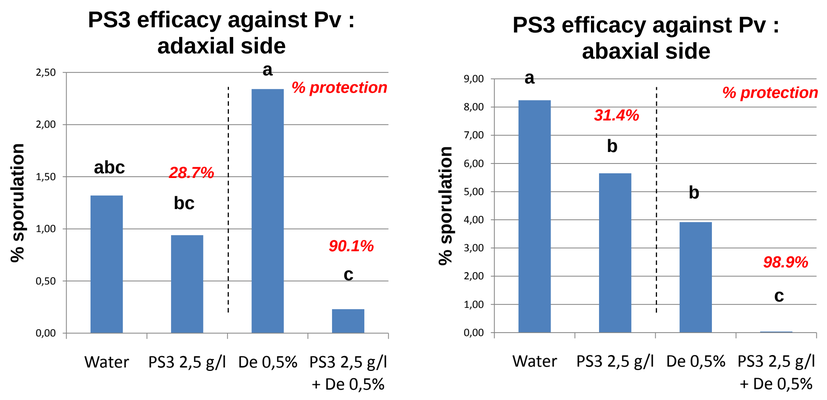


However, addition of De (0,5%) seems to have no detectable impact on the penetration of PS3 into grapevine leaf.

%EMI = % of fluorescence obtained after wash off.

While significant penetration rates can be observed after the addition of De in arabidopsis leaves, this is not the case with grapevine. The spectrofluorimetry method with fluoresceine labeled PS3 does not seem sensitive enough to reveal low penetration rates. However, a certain amount of PS3 should penetrate into grape leaf since it is effective as resistance inducer.

IV. PS3 induced resistance is enhanced by the use of an adjuvant when applied on the abaxial surface



The efficacy of PS3 against Pv is significantly increased when applied with De on the abaxial side of grapevine leaves rather than on the adaxial side.

On the adaxial side, treatments provide similar results (low efficacy). On the other hand, on the abaxial side, PS3 significantly induces resistance while the addition of De enhances it even more. This suggests that penetration of PS3 into the leaf is similar to that of sucrose.

V. Take home message

- Penetration rate of hydrophilic compounds is higher through the ***abaxial surface*** than through the adaxial one, leading to practical consequences for treatment application.
- The addition of appropriate ***adjuvants*** increases the ***efficacy*** of oligosaccharide elicitors when sprayed onto the leaf.
- Biodisponibility and formulation are major factors to consider when developing such a treatment.

Literature cited

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