Biostimulation of grapevine and wheat : mode of action and possible agronomic uses

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Evidence for foliar uptake of sulfated laminarin into grapevine depending on surfactant use and leaf surface.

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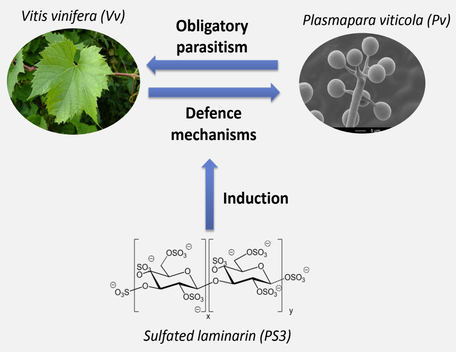
Context

THE PATHOSYSTEM

- **GRAPEVINE :** Perennial crop with major **economic and cultural value**, but susceptible to many cryptogamic diseases such as downy mildew.
- **DOWNY MILDEW :** Disease caused by the biotrophic oomycete *Plasmopara viticola* (Pv), responsible for **considerable damages** in worldwide vineyards : loads of **fungicides** are required.

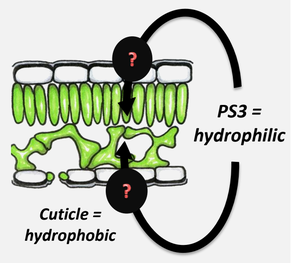


ELICIORS : A PROMISING CONTROL STRATEGY ....

- **ELICITORS :** Compounds able to trigger plant defences, resulting in some cases, in an enhanced **resistance to bioagressors**. Some oligosaccharides (OS) like ß-glucans are elicitors (Delaunois *et al.*, 2014).
- **SULFATED LAMINARIN (PS3) :** Obtained by chemical sulfation of laminarin, a ß-(1,3)glucan from the brown algae *Laminaria digitata*. **PS3 was shown to elicit plant defences** and to induce resistance against Pv in controlled conditions (Trouvelot *et al.*, 2008).

...UNFORTUNATELY ENCOUNTERING MANY BARRIERS.

- Disease control by OS is often inconsistent. Elicitors' efficacy depend on many factors (Walters, 2013), including their **bioavailiability.**
- One hypothesis is that hydrophilic elicitors with high MW (>1000Da), such as PS3, have to cross **the hydrophobic cuticular barrier,** to reach internal receptors.



AIMS

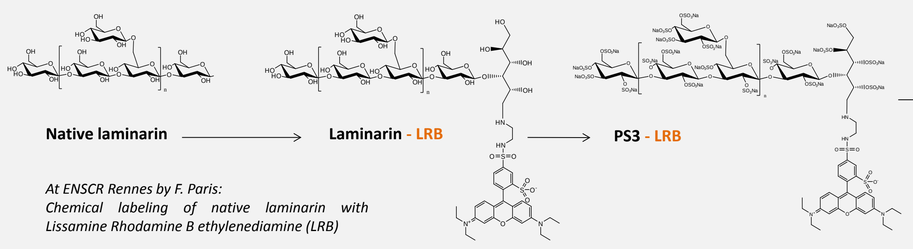
Understanding the uptake of PS3 in grapevine leaves, to improve its protection rate against Pv by:

(1) Developping a method to vizualize its penetration *in planta*

(2) Studying the effect of an ethoxylated surfactant use and influence of leaf surface.

Developping the method

1 ***Labeling of laminarin***



At ENSCR Rennes by F. Paris:
Chemical labeling of native laminarin with Lissamine Rhodamine B ethylenediamine (LRB)

2 ***Sulfation of labeled laminarin***

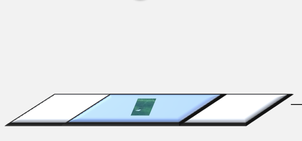
3 ***PS3-LRB application***

1 µL droplet deposit

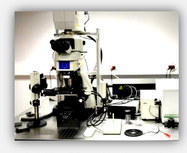
5 droplets per leaf

- *With or without 0,5% Dehscofix CO125® (DE) as the surfactant*
- *On the adaxial or abaxial surface*
- *Different duration of contact: 1, 2, 3 days*

4 ***Sample preparation***

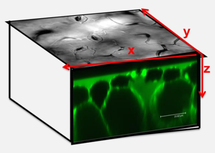


- *Removing the unabsorbed PS3-LRB by washing the droplet deposit area with 1 mL of 0,1% Tween20.*
- *Mounting in distilled water*



Nikon A1-MP x60 objective, DimaCell Platform

5 ***Two-Photon Laser Scanning Laser Microscopy (TPLSM) vizualisations***



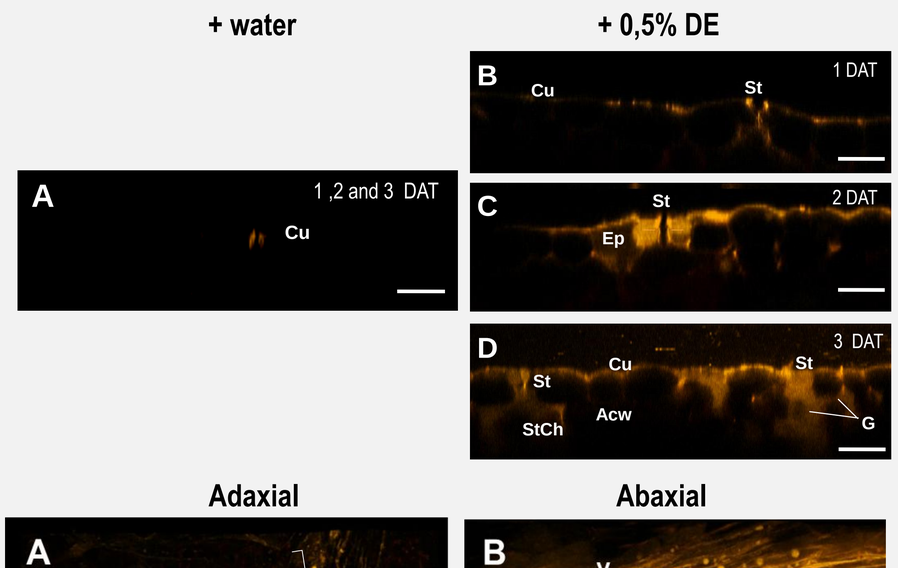
- *Laser excitation : 840 nm*
- *Collecting the fluorescence emission : on orange [563-588] nm and red channels [601-657] nm.*
- *Cross-sections : 50+/- 10 µm depth, every 1 µm of Z-axis*
- *5 acquisitions per droplet deposit*

RESULTS

- Observation 1 -

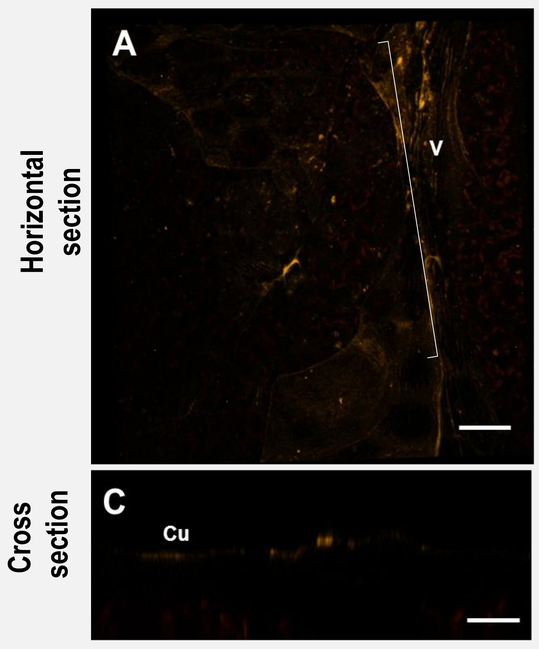
Better uptake of PS3-LRB when formulated in an adequate surfactant than in water.

DE = Dehscofix CO 125 ®
DAT = Days after treatment
Cu = Cuticle
St = Stomata
St = Substomatal chamber
Ep = Epidermal cells
V = Vein
G= Intercellular gaps
Acw =Anticlinal cell wall
Bar = 20µm



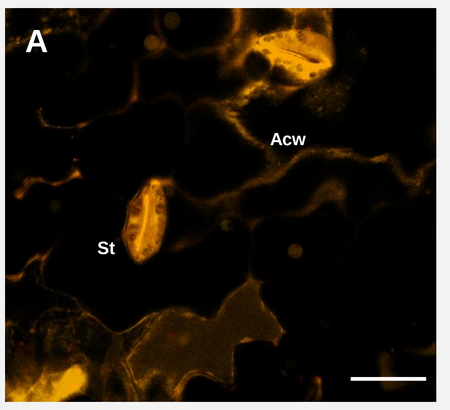
- Observation 2 -

Better uptake of PS3-LRB with DE into the abaxial foliar tissue than into the adaxial one.



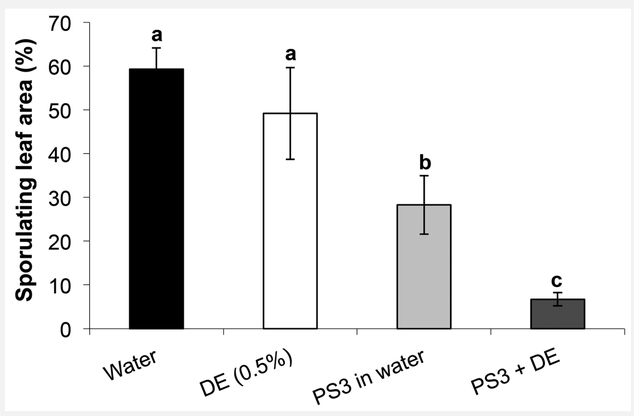
- Observation 3 -

Preferential uptake of PS3-LRB along the anticlinal cell walls and especially through stomata.



- Observation 4 -

In bioassays, PS3 in DE reduces the sporulation of Pv by 20 to 30%, compared to PS3 in water.



DISCUSSION

In the literature, surfactants were shown :

1. ***to promote the uptake** of a range of hydrophilic compounds such as glucose derivatives (Forster, 2005).*
2. *to increase the water content of cuticle, and consequently to **improve leaf permeability** to water-soluble compounds (Ramsey, 2005).*

This could be due:

1. *to the fact that grapevine possesses **stomata on the abaxial** surface only*
2. *to the difference in **waxes and cuticular structure and composition** between its leaf surfaces*

➔ *Therefore, further studies should focus in analyzing differences in the thickness and composition between the two leaf surfaces*.*

*Hydrophilic compounds can enter the leaf via **polar pathways** in the cuticle. They consist of **polysaccharide fibers** embedded in the cuticle.*

*These polar zones were shown to occur preferentially in the cuticle above **anticlinal cell walls, around the stomata and at the bases of trichomes** (Schönherr 2006).*

The beneficial effect of DE on PS3 uptake into grapevine leaf is backed by increased PS3-induced resistance in these biological tests.

➔ *This supports our hypothesis that **insufficient bioavailability of OS elicitors can limit their efficacy.***

Take-home messages

1. **The development of this microscopic observation method** enabled us to show that an OS elicitor such as PS3 does not readily enter the grapevine leaf without an *ad hoc* formulation.
2. **Spray application of OS elicitors preferentially targeting the abaxial surface** of the grapevine leaves may improve the effectiveness of such treatments.

*** Want to know more?** See our online publication: Paris et al. (2015) An ethoxylated surfactant enhances the penetration of the sulfated laminarin through leaf cuticle and stomata, leading to increased induced resistance against grapevine downy mildew. *Physiologia plantarum.* doi: 10/1111/ppl.12394

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