# RHAMNOLIPID SOLUTIONS WITCH ANTIFUNGAL AND ELICITING PROPERTIES TO PROTECT RAPESEED AGAINST LEPTOSPHAERIA MACULANS

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# Rhamnolipid solutions with antifungal and eliciting properties to protect rapeseed against *Leptosphaeria maculans*.

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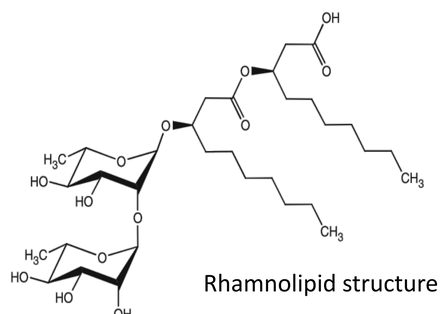
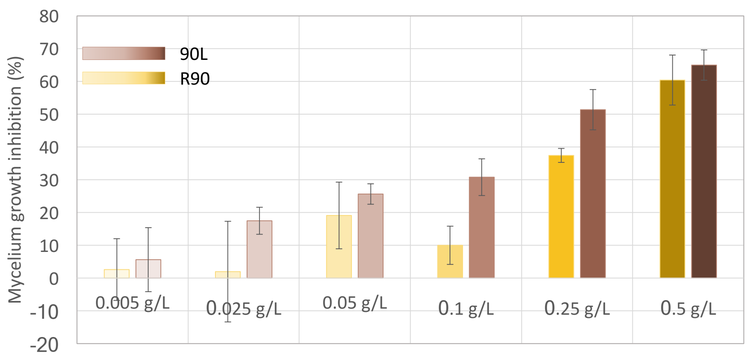
The rapeseed crop has to cope with fungal diseases that impact significantly the yield. In the present context of reducing fongicides used in agriculture, it is essential to develop innovative and sustainable products to protect rapeseed crops. Currently, farmers do not have cost-effective and eco-friendly products to replace phytopharmaceuticals. Natural glycolipids, especially rhamnolipids from bacterial origin, were shown to have an elicitor effect (stimulation of defenses) in Arabidopsis, rapeseed and grapevine and antimicrobial properties on different pathogenic microorganisms[1,2,3,4]. The present project aims to develop and optimize the properties of these compounds against the fungal pathogen *Leptosphaeria maculans*, the causal agent of blackleg disease on rapeseed.



Rhamnolipid structure

## *In vitro* experiments

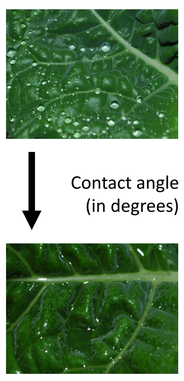
### Growth inhibition of *L. maculans* mycelium

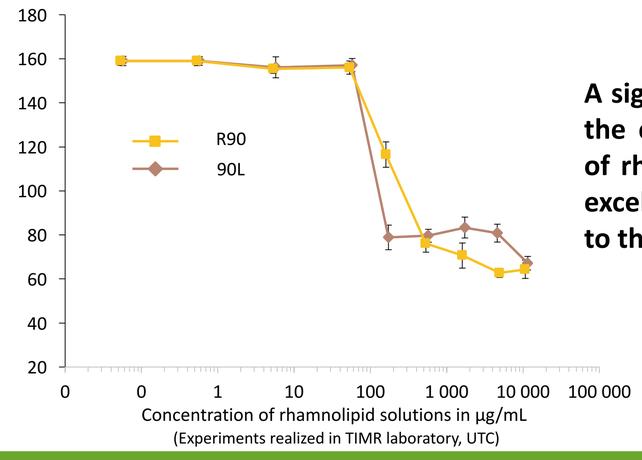



Percentage of *M. maculans* mycelium growth inhibition observed 4 days post inoculation

**Both R90 and 90L rhamnolipid solutions induce a significant growth inhibition of the fungus**

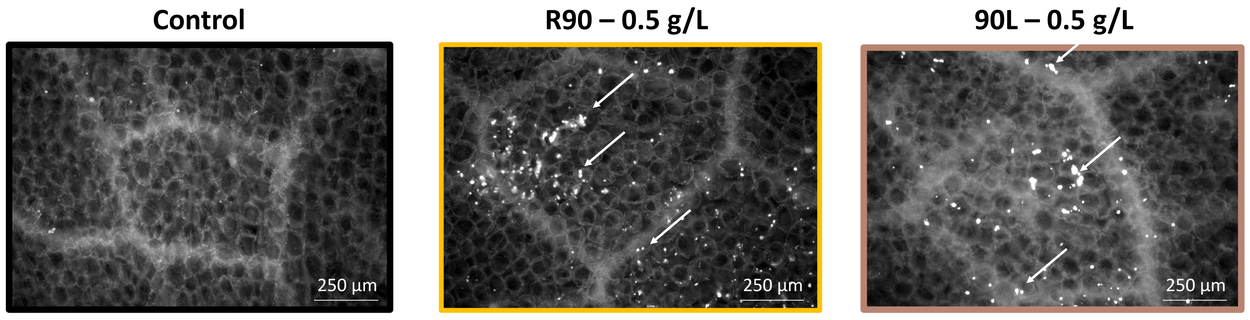
### Adherence to rapeseed leaf

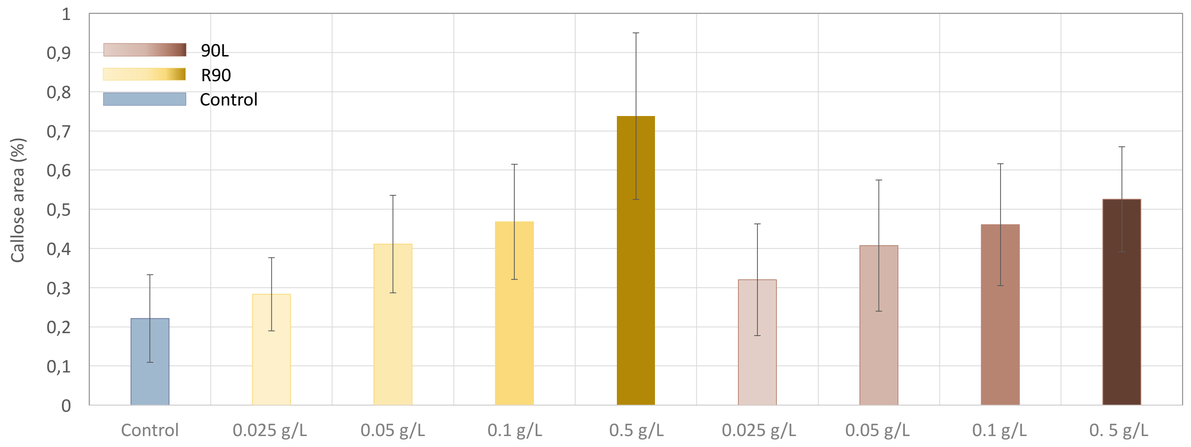



**A significant reduction is observed in the contact angle above 0.1 mg/mL of rhamnolipid solutions resulting in excellent adherence of the molecules to the leaves.**

### Callose deposits on rapeseed



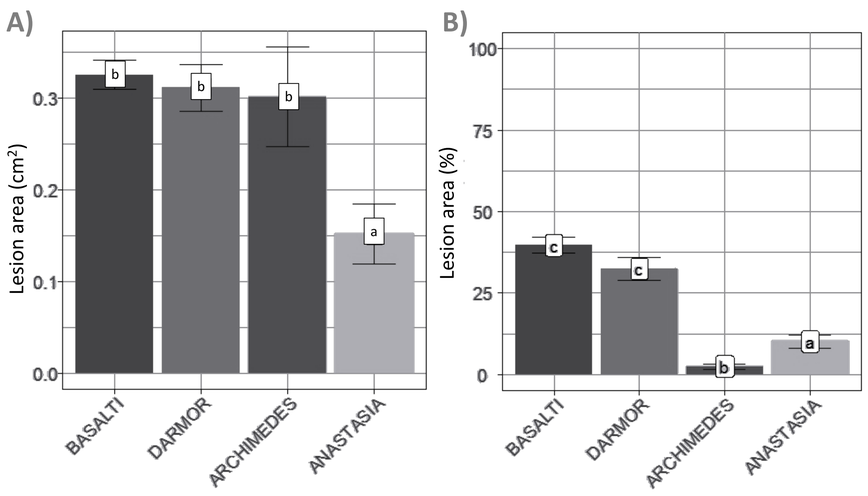




Percentage of area corresponding to callose deposits. The error bars represent the standard error.

**Callose deposits were observed on Basalti rapeseed cotyledons sprayed with both rhamnolipid solutions**

## *In vivo* experiments in controlled conditions

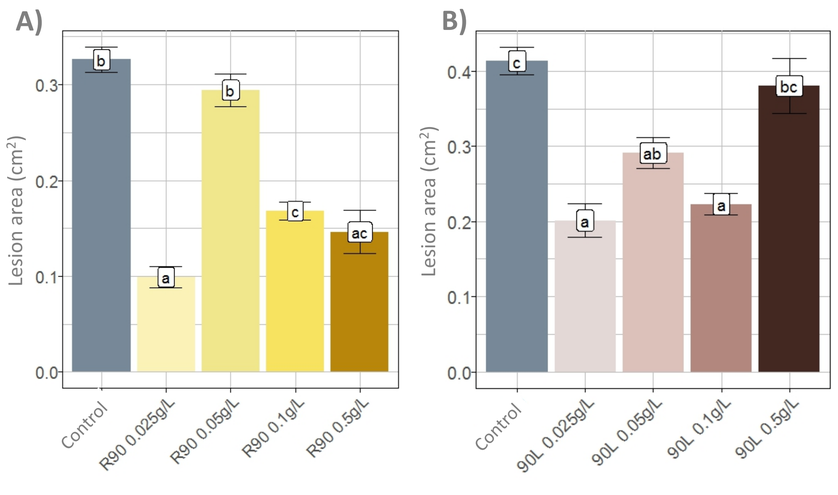
### Variability of cultivar resistance




Lesion areas observed on 29-day-old rapeseed cotyledons. 12-day-old cotyledons are inoculated with *L. maculans* at $10^6$ spores/mL. Error bars represent the standard errors. (**A**) Lesion areas on untreated cotyledons (**B**) Percentage of lesion areas on cotyledons treated 2 days before inoculation with R90 at 0.1 g / L. Percentage is calculated from the untreated control of the corresponding cultivar set at 100%.

***L. maculans* sensitivity is cultivar-dependent. The variability is found when the plants are treated with the R90 solution before inoculation, illustrating a cultivar effect.**

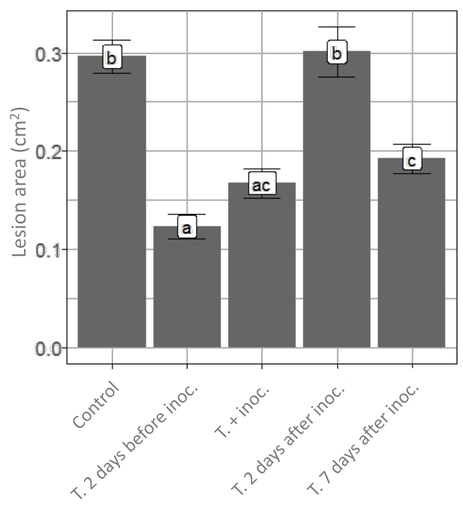
### Protective effect of rhamnolipids




Lesion areas on 27-day-old cotyledons of Basalti cultivar, untreated (control) or treated with R90 (A) or 90L (B) solutions at different concentrations. Treatment is realized 2 days before inoculation and 12-day-old cotyledons are inoculated with *L. maculans* at $10^6$ spores/mL. Error bars represent the standard errors.

**The preventive application of both rhamnolipid solutions triggers a plant protection with concentration-dependent intensity. Even at low concentration (0.025 g/L) the solutions are still very effective.**

### Effect of treatment timing on protection




Lesion areas on detached leaves from 35-day-old Basalti cultivar, untreated (control) or treated with R90 0.1 g/L. Treatment 2 days before inoculation; treatment and inoculation at the same time (T. + inoc); treatment 2 days after inoculation; treatment 7 days after inoculation. Twelve-day-old cotyledons are inoculated with *L. maculans* at $10^6$ spores/mL. Error bars represent standard errors.

**The R90 rhamnolipid solution treatment triggers a protection of rapeseed cotyledons against *L. maculans* under controlled conditions when the treatment occurs 2 days before inoculation (preventive), in co-inoculation (direct antimycelium effect) and 7 days post-inoculation (curative effect).**

## Conclusions

**Both rhamnolipid solutions show antifungal activity, eliciting properties and good adherence to rapeseed leaf. These properties lead to an effective protection against *L. maculans*, including concentrations where the antifungal activity is not significant. These results suggest a major role of the eliciting properties of these compounds in the rapeseed protection. Rhamnolipids therefore appear to be potentially cost-effective biocontrol products to fight fungal diseases of rapeseed.**

References : [1] Varnier *et al.*, *Plant. Cell Environ.*, 2009; [2] Vatsa *et al.* Int J Mol Sci. 2010; [3] Sanchez *et al.*, *Plant Physiol.*, 2012; [4] Monnier *et al.*, *Front. Plant Sci.*, 2018

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