Investigating the mode of action of essential oils against the wheat pathogen *Zymoseptoria tritici*

Thomas Garçon[1], Clément Burgeon[1], Jérôme Muchembled[2], Magali Deleu[3], Sébastien Massart[1], M. Haïssam Jijakli[1], Ali Siah[2]

[1] *University of Liège, UMRt BioEcoAgro 1158-INRAE, Plant Secondary Metabolites, TERRA Teaching and Research centre, Gembloux Agro-BioTech, Gembloux, B-5030, Belgium*
[2] *JUNIA, UMRt BioEcoAgro 1158-INRAE, Plant Specialized Plant Metabolites, Lille, F-59000 France*
[3] *University of Liège, UMRt BioEcoAgro 1158-INRAE, Microbial Secondary Metabolites, TERRA Teaching and Research centre, Gembloux Agro-BioTech, Gembloux, B-5030, Belgium*

Introduction

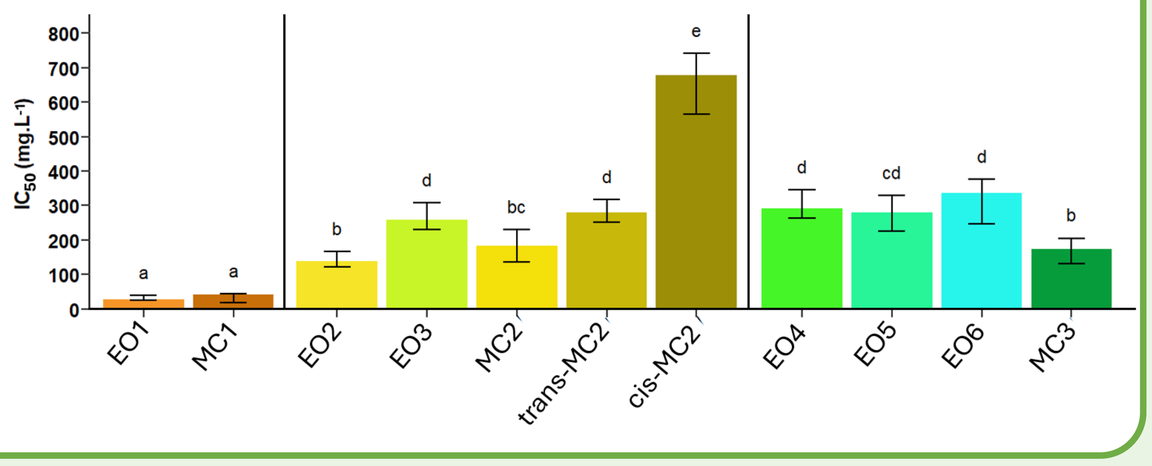
Wheat production faces numerous **threats**, from **abiotic stressors to biotic stressors**. Among them, ***Zymoseptoria tritici*** poses a major threat as it is the **most damaging pathogenic fungi**, provoking up to **50% yield losses in Europe[1].**

Management of this disease mostly relies on **synthetic fungicides.** However, current **agroecological practices** favor the development of eco-friendly alternatives. **Plant secondary metabolites** have been investigated for their potential use as **bioprotection products**. **Essentials oils (EOs)** are gaining an interest for their **fungicidal properties[2].**

Six promising EOs and their major compounds (MCs) were identified in the lab and their antifungal activities were determined against *Z. tritici in vitro*.

➢ **In this study,** we aim to investigate the mode of action of **six essential oils and their major compounds** for the **biocontol of *Z. tritici*.**

IC_{50} of the studied essential oils and their major compounds

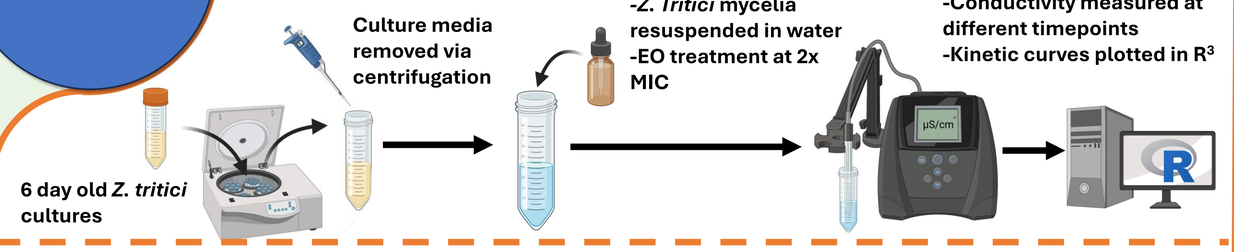


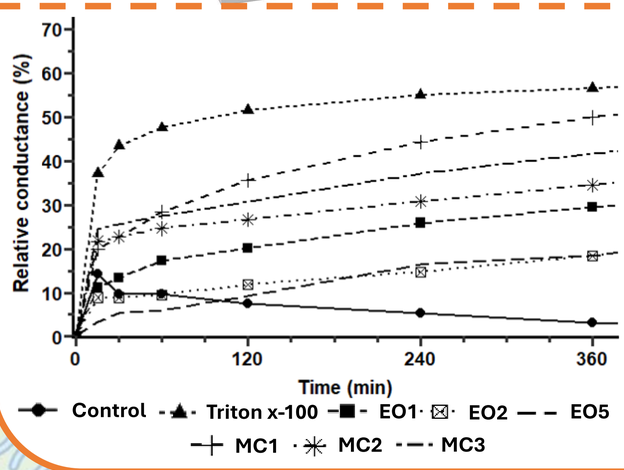
Methodology and Results

In vitro experiments

Conductivity assessment of *Z. tritici* treated with EOs and MCs

- **Methodology**





- **Results**

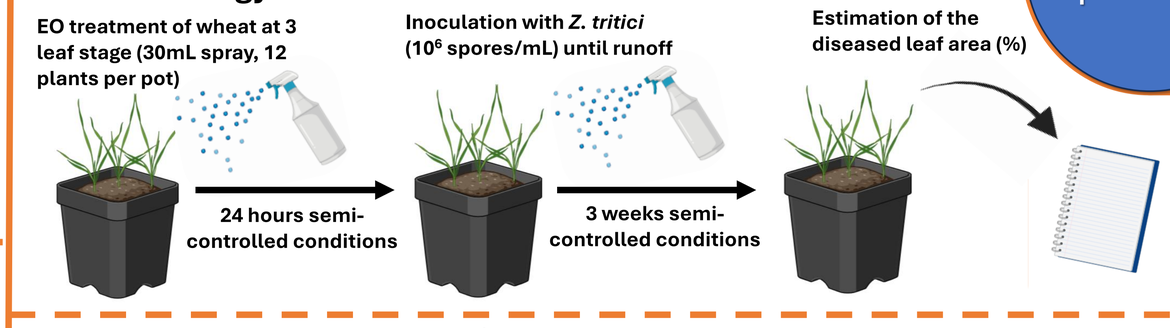
EOs and MCs provoked an **increase in relative conductance** over the course of 6 hours. Relative to EOs, MCs saw a higher increase in relative conductance.

→ **EOs and MCs cause the permeation of the fungal membrane.**

In planta experiments

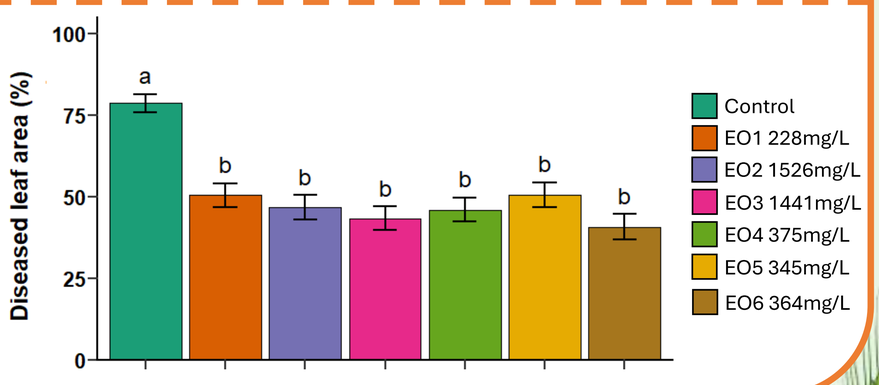
In planta protection assessment against *Z. tritici*

- **Methodology**

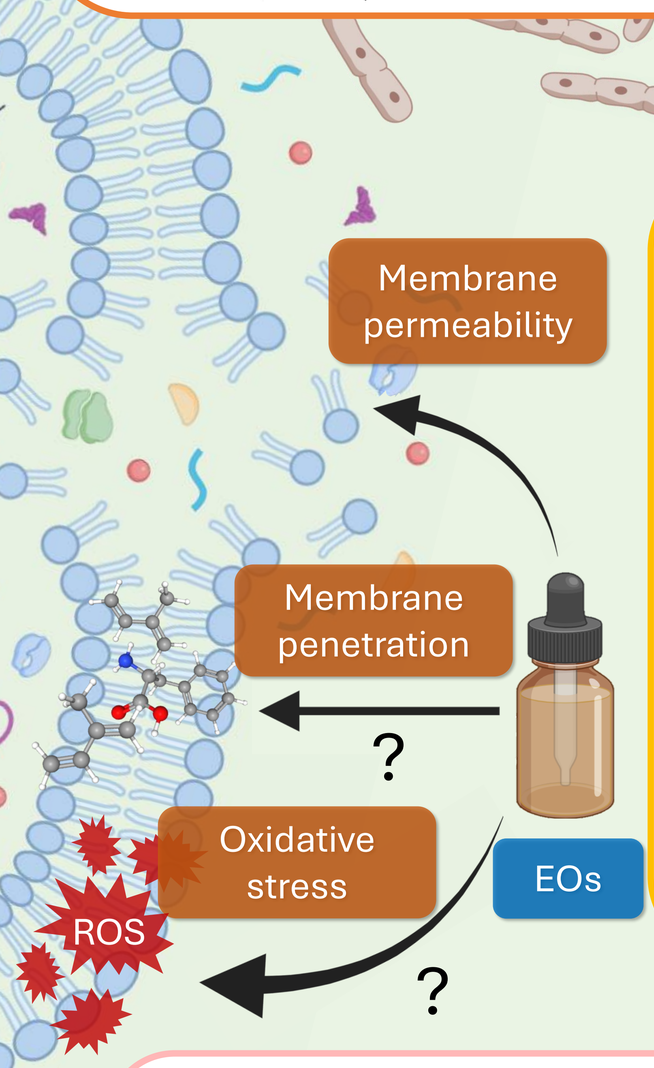


- **Results**

→ At the **phytotoxic threshold**, the selected essential oils **significantly reduced disease intensity.**



Mode of action



➢Direct mode of action

The direct mode of action on membrane permeability has been investigated with **conductivity measurements.** However, other fungal targets have yet to be investigated

- **Membrane penetration**

Due to their **lipophilic nature**, EOs can **penetrate the membrane** causing lipid alterations. Evidence of this mechanism can be investigated through **lipid monolayer and bilayer studies *in vitro*** and ***in silico*[4]**.

- **Oxidative stress**

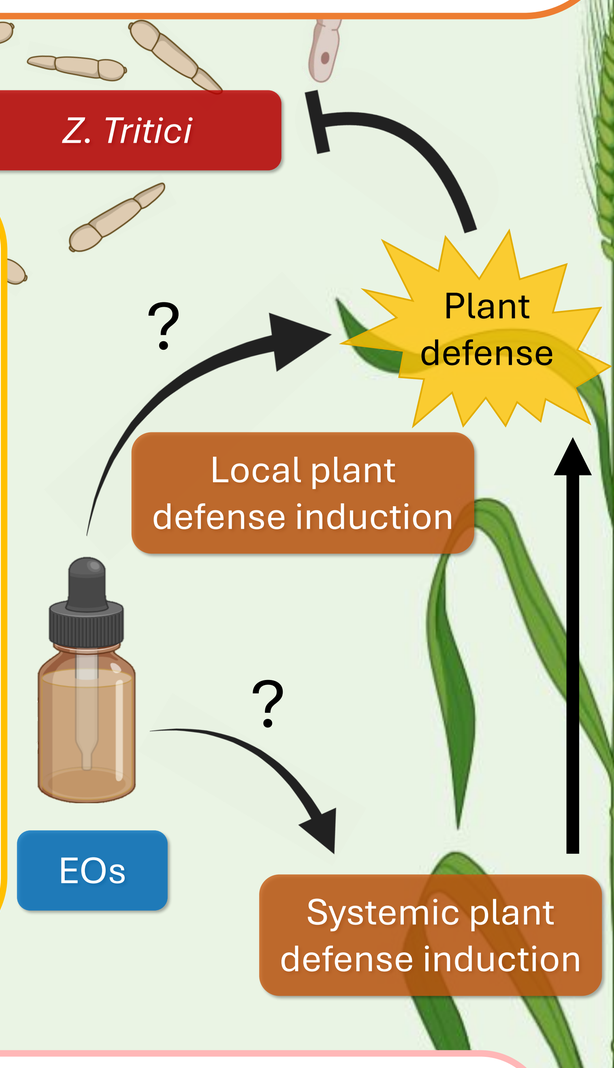
Fungi produce **ROS** in response to stress which can be analyzed using **fluorescent dyes** such as **DCFH-DA.**

➢Indirect mode of action

The bioprotection of EOs against *Z. tritici* was effective with a pretreatment **24 hours before infection**. However, due to this short delay, we speculate that most of the **protection** was achieved through **direct action**, and the **indirect effect** through **elicitation of plant defense** has yet to be determined.

- **Plant defense induction**

To identify the **induction of plant defense with EOs, transcriptomic anlyses** can demonstrate the plant's response to EOs.



Conclusion and perspectives

All of the **six essential oils** have been **proven effective at a similar level** for the **bioprotection of *Z. tritici*** with a **preventive treatment 24 hours before infection**, further experiments on the **formulation of the EOs** are in progress to allow for earlier preventive applications. **In the conductivity assessment MCs** provoked the **highest increase in relative conductance**, followed by **EO1, EO2 and EO3**. Further experiments on the direct mode of action using **fluorescent dye and colorimetric assays** are planned. Moreover, experiments on **lipid monolayers and bilayers** are being discussed in order to understand the **effect of essential oils** on the **fungal membrane**.

References

[1] Fones H, Gurr S. The impact of Septoria tritici Blotch disease on wheat: An EU perspective. **Fungal Genet Biol.** 2015 Jun;79:3-7.
[2] Raveau, R.; Fontaine, J.; Lounès-Hadj Sahraoui, A. Essential Oils as Potential Alternative Biocontrol Products against Plant Pathogens and Weeds: A Review. **Foods** 2020, 9, 365.
[3] R Core Team (2026). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
[4] Lins, L.; Dal Maso, S.; Foncoux, B.; Kamili, A.; Laurin, Y.; Genva, M.; Jijakli, M.H.; De Clerck, C.; Fauconnier, M.L.; Deleu, M. Insights into the Relationships Between Herbicide Activities, Molecular Structure and Membrane Interaction of Cinnamon and Citronella Essential Oils Components. *Int. J. Mol. Sci.* **2019**, *20*, 4007. https://doi.org/10.3390/ijms20164007