VERTICILLIUM WILT ON FLAX : *IN VITRO* INFECTION, BIOCONTROL AND SCREENING

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Introduction

- Flax (*Linum usitatissimum*) cultivation in **Europe**, counts for **80% of worldwide flax production**. This place is known for **appropriate climate conditions** to grow flax properly and **ancestral skills** in terms of cultural practices. Moreover, France and Belgium produce the **best quality fibers**.
- For the last 15 years, the observation of *Verticillium* wilt has increased in French and Belgian fields, which leads to significant **economic losses** because of the **alteration** of **bast fibers** by the pathogenic fungus.
- *Verticillium* wilt of flax is caused by a **vascular fungal pathogen**: *Verticillium dahliae*. Long-lasting surviving structures of this fungus (**microsclerotia**) are present in natural soils. **Root exudates** of potential host plants stimulate the **germination** of microsclerotia. Resulting **hyphae** can enter the **root tissue** in the **intercellular** space (Fig. 1). Then, *V. dahliae* reaches the **xylem vessels** and **spreads systemically**.

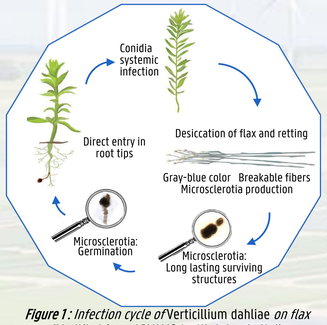

***Figure 1**: Infection cycle of* Verticillium dahliae *on flax*
(Modified from ARVALIS-Institut du végétal)

PATHOFLAX project

This **INTERREG** project brings together **11 partners** from Hauts-de-France, Wallonia and Flanders regions, involved either in **study** or **control** of **fungal plant diseases** or **study** of **flax**. Launched in **January 2019** for **4 years**, its aims are to:

- Realize a **state of progress** of the disease on flax in the studied area
- Test **biological control strategies** to control the disease
- **Map** *Verticillium occurrence* in fields soils
- Assess the **efficiency** of **elicitors** and **antagonistic** strains
- Select suitable **cultivars** and crop **management techniques**
- Organize a **laboratories network** to help farmers facing *Verticillium* wilt

Interreg France-Wallonie-Vlaanderen
PATHOFLAX

PhD thesis work: Studying the pathosystem and finding a potential biocontrol product

INFECTION: Setting up a **protocol** of **infection** of flax (cultivar Lisette, Wiersum Plantbreeding BV) with *Verticillium dahliae* microsclerotia to be **as close as possible to what happens in soil in the field**:

- Produce
- Harvest
- Test the viability of
- Mix with soil

} microsclerotia (MS)

Flax seeds are sown in a mix of soil + MS powder.

V. dahliae : ID card

Verticillium dahliae isolated in Amiens (France) (unpublished)

Pathogenic strain from **symptomatic flax plants** from the field (Oise, France)

Causal agent of *Verticillium* wilt of flax

SCREENING: Searching for **new markers** of the **disease** in a **phenotyping robot** (Fig. 2):

- Automatization of watering and measures
- Fluorescence camera
- Visible top and side cameras (Fig. 3)
- Hyperspectral visible and near infrared (350 to 900 nm) and short-wave infrared (900 to 1700 nm) top cameras

***Figure 2**: Insight of the phenotyping robot in CRRBM of Amiens*

***Figure 3**: Side visible camera pictures*
Left : Control flax plants
Right : V. dahliae *Infected flax plants*

BIOCONTROL: Testing a potential biocontrol strain: ***V. isaacii* (Vt305)** to decrease the impact of *Verticillium* wilt on flax

- **Protocol**: *V. isaacii* isolate **Vt305 MS** (powder) + clean flax seeds (250 MS / seed)
  With *V. dahliae* microsclerotia in the soil or not
- Measuring **heights** (30 plants / condition) : (Fig. 4) : the significant **reduction in plant height** due to ***V. dahliae*** infection is counteracted in the presence of ***V. isaacii***.
- **Scoring** plants for the intensity of their symptoms : (Fig. 5)
  The addition of *V. isaacii* seems to **decrease** the **symptoms** of infected flax plants.

V. isaacii / Vt305: ID card

Verticillium isaacii isolated in UGent (Tyvaert *et al*, 2014)

Endophyte from **suppressive soil** in **cauliflower fields**

Shows abilities **to control** *Verticillium* wilt on **cauliflower** (Deketelaere *et al*, 2020)

MOLECULAR QUANTIFICATION: qPCR assays have been done on **infected** and **inoculated** or **not** with ***V. isaacii* plants** to follow the **amount** of *V. dahliae* DNA *in planta*
Early results showed a **decrease** of *V. dahliae* DNA amount *in planta* when ***V. isaacii*** is present (data not shown)

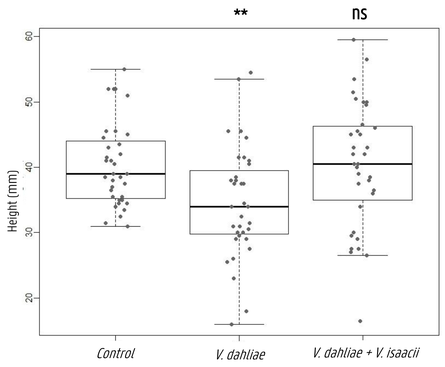

***Figure 4**: Heights of flax plants per condition*
Every dot is a flax plant
ns : no significant difference
** : 0,001 < p-value < 0,01 (Student's t-test)

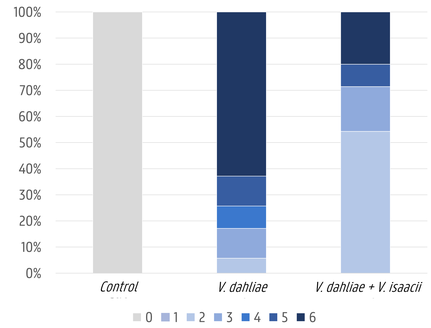

***Figure 5**: Disease scores of plant per condition*
V. isaacii alone on flax plants did not show any symptoms
0 = no symptoms
3 = a **third** of the plant presents chlorosis/necrosis
6= the **entire** plant shows chlorosis/necrosis

METABOLOMICS: **Xylem sap** of **infected** and **control** plants have been harvested. *Verticillium* wilt seems to have an impact on xylem sap flow and composition (unpublished)

- **GC-MS** analysis has been performed, looking for polar **metabolites**
- **Searching** for potential **differences** in the amounts of those metabolites between **infected** and **control plants** (Fig. 6): **white bars** mean that metabolites are **less present** in **infected** plants and **blue bars** mean that metabolites are **more present** in **infected** plants

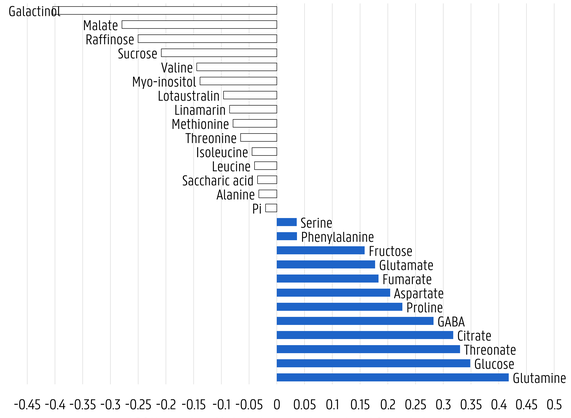

***Figure 6**: Metabolite accumulation in flax xylem sap after infection with* V. dahliae
Differences between **area ratios** of **control** and **infected plants** for each analyzed metabolite
(6 control samples and 8 infected samples)

Perspectives

- Testing ***V. isaacii*** efficiency in the field: → **Plants** are currently growing
- Testing ***V. isaacii*** efficiency on other cultivars : → **Plants** are growing in controlled conditions
- **Carry on** metabolomics: → **Confirm** results; **Other** cultivars, **biocontrol** conditions

References:

Deketelaere, S., Spiessens, K., Pollet, S., Tyvaert, L., Rooster, L. D., Callens, D., França, S. C., & Höfte, M. (2020). Towards Practical Application of Verticillium isaacii *Vt305 to Control* Verticillium *Wilt of Cauliflower : Exploring Complementary Biocontrol Strategies. Plants, 9(11), 1469. https://doi.org/10.3390/plants9111469*

Tyvaert, L., França, S. C., Debode, J., and Höfte, M. (2014). The endophyte Verticillium *Vt305 protects cauliflower against* Verticillium *wilt. J. Appl. Microbiol. 116, 1563–1571. doi: 10.1111/jam.12481*