Interreg
France-Wallonie-Vlaanderen

UNION EUROPÉENNE
EUROPESE UNIE

PATHOFLAX

AVEC LE SOUTIEN DU FONDS EUROPÉEN DE DÉVELOPPEMENT RÉGIONAL
MET STEUN VAN HET EUROPEES FONDS VOOR REGIONALE ONTWIKKELING

PATHOFLAX

Development of sustainable control strategies for Verticillium on flax

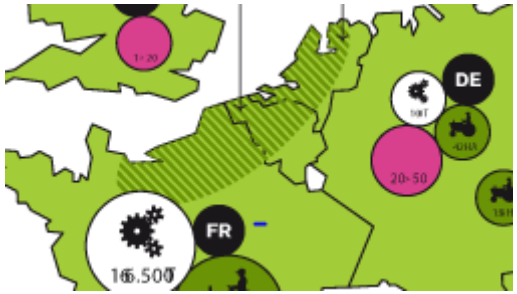
23-4-19

Flax – *Linum usitatissimum*

- 2 types of flax are cultivated
  - fibre production (fibre flax)
  - oil production extracted from the seed (linseed)

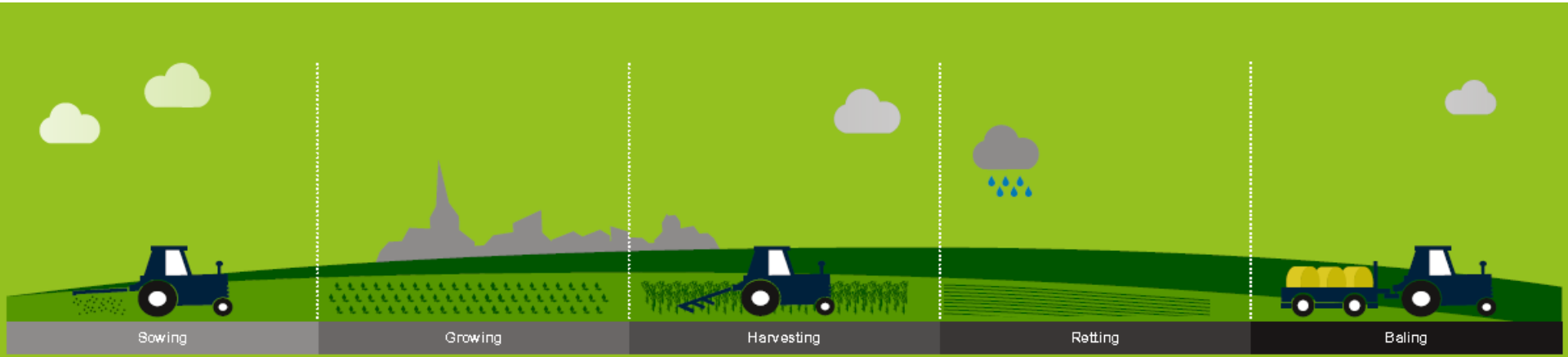
> 80% of the world's production of scutched flax fibers are originated from NW-Europe

France: ± 96 000 ha
België: ± 15 500 ha
Nederland: ± 2 250 ha



Verticillium in flax

- *Verticillium dahliae*
- Frequency is increasing
- Early infection (microsclerotia in the soil infect the roots of young flax plants)
- Symptoms at the end of the growing season
- Significant yield losses
- No control strategies



Verticillium in flax

Verticillium dahliae

Symptoms

- Discoloration and drying out of the stems at the end of the growing season
- Fragile stems during retting with a blue-gray color

Significant yield losses

Interreg
France-Wallonie-Vlaanderen

UNION EUROPÉENNE
EUROPESE UNIE

www.interreg-fwvl.eu
@InterregFWVL

AVEC LE SOUTIEN DU FONDS EUROPÉEN DE DÉVELOPPEMENT RÉGIONAL
MET STEUN VAN HET EUROPEES FONDS VOOR REGIONALE ONTWIKKELING

Avec le soutien de la Wallonie

west-vlaanderen
de gedreven provincie

PATHOFLAX

Development of sustainable control strategies for Verticillium on flax (1/1/2019- 31/12/2022)

Main objectives:

- conducting an epidemiological study of the fungus in the entire area where fiber flax is grown
- research and implementation of sustainable control strategies based on the stimulation of the plant's natural defenses by means of non-pathogenic antagonistic strains or natural elicitors
- use of the natural biodiversity of flax to identify resistant varieties to this fungus

Interreg
France-Wallonie-Vlaanderen
UNION EUROPÉENNE
EUROPESE UNIE

PATHOFLAX

Collaboration of 11 partners in France, Flanders and Wallonia, each with their expertise

Project Leader = inagro
ONDERZOEK & ADVIES IN LAND- & TUINBOUW

UNIVERSITÉ de Picardie Jules Verne

ARVALIS Institut du végétal

Wallonie recherche CRA-W

ILVO Instituut voor Landbouw-, Visserij- en Voedingsonderzoek

UNIVERSITEIT GENT

Terre de Lin

Linéa Semences de lin

UNIVERSITÉ DE NAMUR

fytofend
Pioneering Biological Alternatives

Algemeen Belgisch Vlasverbond v.z.w.

5 Work Packages

1. Project Management (Inagro)
2. Communication (Arvalis)
3. Monitoring (ILVO)
4. Disease Management (UPJV)
5. Field trials (FytoFend)

Interreg
France-Wallonie-Vlaanderen
UNION EUROPÉENNE
EUROPESE UNIE
PATHOFLAX

WP3: Monitoring

- ILVO, CRA-W, Arvalis, Inagro & UGent

- Activity 1: Evaluation/validation of the diagnostic tests
- Activity 2: Monitoring of *V. dahliae* in the soil
- Activity 3: Dose-respons
- Activity 4: Detection of *V. dahliae* in the seed
- Activity 5: Isolation and characterisation of *V. dahliae* isolates

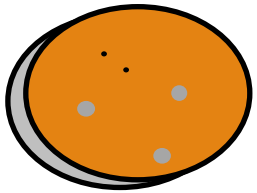
Interreg
France-Wallonie-Vlaanderen
UNION EUROPÉENNE
EUROPESE UNIE
PATHOFLAX

1. Evaluation/validation of the diagnostic tests

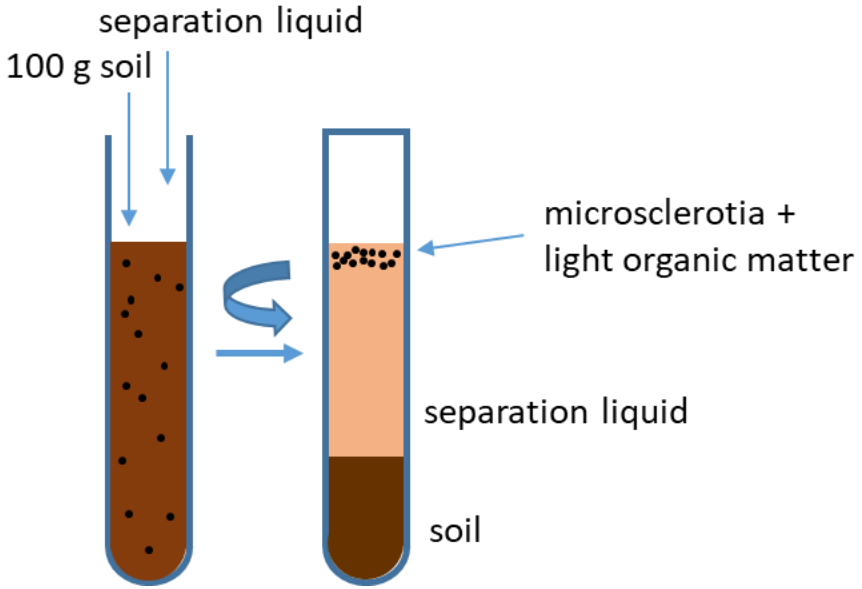
- Compare 3 diagnostic techniques for *Verticillium dahliae* detection in soil
- Have at least one validated technique available for the growers in each region

Classical plating (Harris et al., 1993)

Wet sieving 12.5g of soil + plating on semi-selective medium



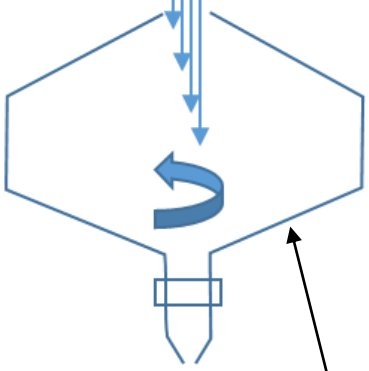
Density flotation + qPCR (Debode et al., 2011)



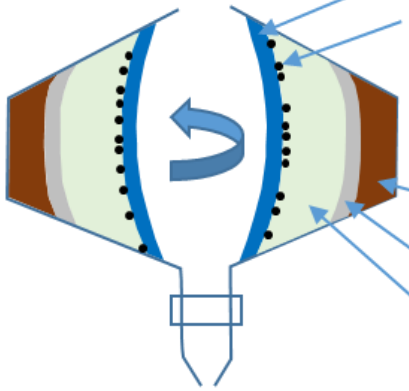
Zonal centrifugation + qPCR

Loading

1. separation liquid
2. water
3. 100 g soil + water
4. kaolin

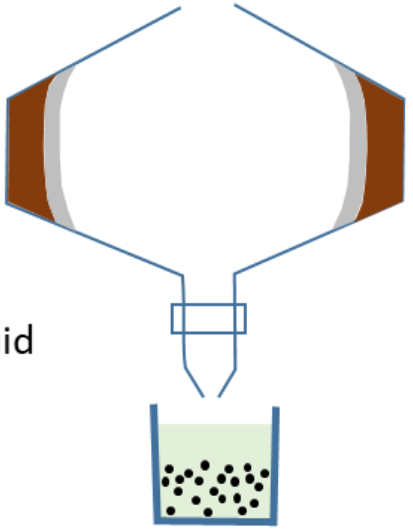


Separation



Excess water
+ light organic matter

Harvesting microsclerotia in supernatans



2. Monitoring

- 50 fields/region/year: sampling & analysis + survey

- Knowledge about the amount of *Verticillium dahliae* in the soils of flax fields in the different regions -> will be presented on a map

- Based on the surveys: regression analyses to determine the links between flax quality or quantity (= response variables) and *V. dahliae* amount, rotation, soil management, cultivar, etc. (= predictor variables)

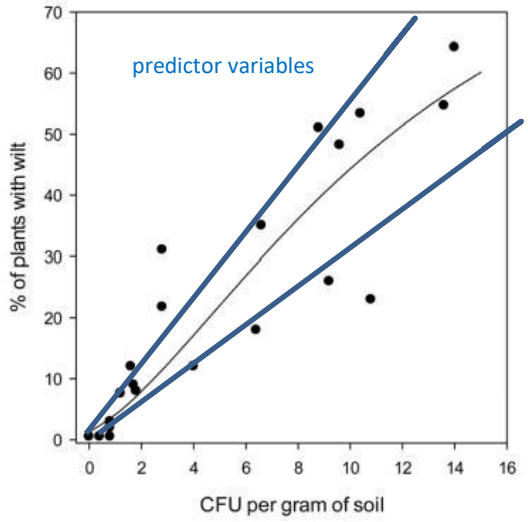
- Use this data as a tool to increase awareness among growers

Interreg
France-Wallonie-Vlaanderen
UNION EUROPÉENNE
EUROPESE UNIE
PATHOFLAX

3. Dose response

- 1 miniplot/field: soil analysis and scoring of the symptoms (incl. microsclerotia after retting)

- Establish a dose-response curve between the amount of *Verticillium* and the amount of symptoms



Wei et al. (2015) Phytopathology 105:220-229.

Blum et al. (2018) Phytopathology 102:2421-2429.

Interreg
France-Wallonie-Vlaanderen
UNION EUROPÉENNE
EUROPESE UNIE
PATHOFLAX

4. Detection of *Verticillium dahliae* in the seed

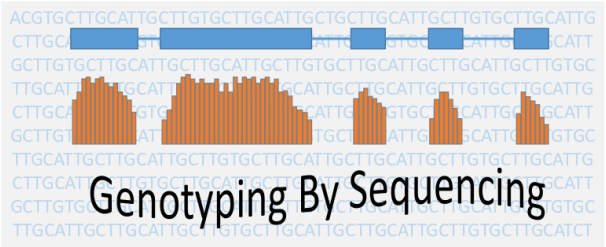
- Establish whether *Verticillium* can be detected in the seed from infected plants source of spread of Verticillium
- If so, establish whether it is present in or on the seed

Note: a preliminary analysis in 2018 showed the presence in a single batch of commercial seed (based on N_2 grinding of subsamples, DNA extraction, qPCR). It is not known yet whether this is due to internal or external presence.

5. Isolation & characterisation of *Verticillium* isolates

- What is genetic diversity among *V. dahliae* isolates from flax fields & from flax?

  Within field
  Within the same region
  Between regions



➢ What is the pathogenic diversity among *V. dahliae* isolates from flax?

  Differences in virulence (pathotypes)?
  Differences in aggressiveness?

Blum et al. (2018) Phytopathology 102:2421-2429.

Interreg
France-Wallonie-Vlaanderen
UNION EUROPÉENNE
EUROPESE UNIE
PATHOFLAX

WP4: Disease management

- Activity 1: Verticillium/flax bioassay
- Activity 2: Screening of flax varieties for Verticillium sensitivity and defense stimulation
- Activity 3: Screening of BCP-activity against Verticillium in flax
- Activity 4: Characterization of the working mechanism of BCPs

1. Verticillium/flax bioassay

- LINEA, Terre de lin, UGent, UPJV
- Tool to test:
  - tolerant flax varieties
  - biocontrol products (BCPs)

2. Screening of flax varieties

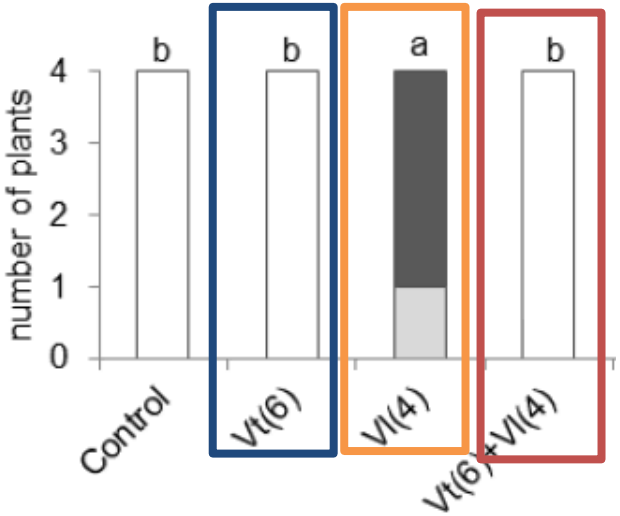
- Currently no flax variety is resistant to Verticillium
- Screening of 50 flax varieties for Verticillium sensitivity and defense stimulation (Terre de Lin, LINEA, Fytofend, UNamur)
- Selecting of most tolerant varieties and varieties with best response on elicitors (activity 3)

Interreg
France-Wallonie-Vlaanderen
UNION EUROPÉENNE
EUROPESE UNIE
PATHOFLAX

3. Screening of BCP-activity against Verticillium in flax

- Currently no Plant Protection Product (PPP) available to control Verticillium in flax
- Screening of:
  - **Antagonistic strains (UGent)**

a. *V. isaacii* is able to control *V. longisporum* in cauliflower



Tyvaert et al. (2014) *Journal of Applied Microbiology* 116, 1563 - 1571

b. Cyclic lipopeptide (CLP)-producing Pseudomonas strains

3. Screening of BCP-activity against Verticillium in flax

- Currently no Plant Protection Product (PPP) available to control Verticillium in flax
- Screening of:
  - Antagonistic strains (UGent): Verticillium endophytes and Pseudomonas strains
  - **Elicitors (Fytofend, UNamur)**: biopesticides FytoSave® and FytoSol®
    - stimulates plant innate immunity: **COS** – **OGA**

COS = chitooligosaccharides => chitosan

OGA = oligogalacturonides => pectin

mimics plant-pathogen interaction

    - against a range of diseases among which powdery mildew on various crops (grape, strawberry,…), downy mildew on grape, …

Interreg
France-Wallonie-Vlaanderen
UNION EUROPÉENNE
EUROPESE UNIE
PATHOFLAX

3. Screening of BCP-activity against Verticillium in flax

- Currently no Plant Protection Product (PPP) available to control Verticillium in flax
- Screening of:
  - Antagonistic strains (UGent): Verticillium endophytes and Pseudomonas strains
  - Elicitors (Fytofend, UNamur): biopesticides FytoSave® and FytoSol®
- Evaluation of combinations of different flax varieties and BCPs (UPJV)

4. Characterization of the working mechanisms of BCPs

- Minimal characterization of the working mechanisms of BCPs is necessary (EPPO directive 1/296) for registration of BCPs in flax (UGent, UNamur)

WP5: Field trials

- Field trials in fibre flax to define the best method for the integrated control of Verticillium
  - Varieties
  - Bio Control Products (BCPs)
  - Demonstration trials
- Coördinator: FytoFend
- Field trials: Arvalis, Inagro, ILVO, Linea, Terre de Lin

Interreg
France-Wallonie-Vlaanderen
UNION EUROPÉENNE
EUROPESE UNIE
PATHOFLAX

Varieties

- Currently no flax variety is resistant to Verticillium

- 2 types of field trials will be conducted:
  - Trials with commercially available varieties
    - to be able to correctly assess the differences in Verticillium tolerance of the different varieties
  - Trials with new breeding lines and/or varieties of the INRA collection
    - selecting varieties that are more resistant than the varieties that are now available for the Belgian and French flax growers

Interreg
France-Wallonie-Vlaanderen
UNION EUROPÉENNE
EUROPESE UNIE
PATHOFLAX

Bio Control Products (BCPs)

- Testing BCPs againt V. dahliae in flax field trials
  - Elicitors (for example FytoSave® from FytoFend)
  - Antagonistic strains (provided by Ugent, selected after lab tests)

- Test factors:
  - Different BCPs
  - Time of the treatment (BBCH-stage)
  - Number of treatments
  - Effect of varieties

Define the most effective control strategy for Verticillium in flax

Interreg France-Wallonie-Vlaanderen UNION EUROPÉENNE EUROPESE UNIE

PATHOFLAX

Demonstration trials

- Last year of the project (2022)
- Validation of the control strategy and demonstration trials:
  - A limited number of varieties for which a positive response to BCP was found
  - Optimum doses of the BCPs
  - Optimum number of applications
  - At the most relevant stage of the crop

Demonstration of sustainable control strategies for Verticillium on flax

Interreg
France-Wallonie-Vlaanderen
UNION EUROPÉENNE
EUROPESE UNIE

PATHOFLAX

Interreg
France-Wallonie-Vlaanderen

UNION EUROPÉENNE
EUROPESE UNIE

PATHOFLAX

AVEC LE SOUTIEN DU FONDS EUROPÉEN DE DÉVELOPPEMENT RÉGIONAL
MET STEUN VAN HET EUROPEES FONDS VOOR REGIONALE ONTWIKKELING

PATHOFLAX

Development of sustainable control strategies for Verticillium on flax

23-4-19