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
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

Development of sustainable control strategies for Verticillium on flax (1/1/2019- 31/12/2022)
Collaboration of 11 partners in France, Flanders and Wallonia, each with their expertise

**Project Leader = inagro**
5 Work Packages

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

- Separation liquid
- 100 g soil
- Microsclerotia + light organic matter
- Soil
Zonal centrifugation + qPCR

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

**Separation**
- soil
- kaolin
- separation liquid
- water
- microsclerotia

**Harvesting microsclerotia in supernatans**

Excess water + light organic matter
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
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


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\textsubscript{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?

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)
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®

  o stimulates plant innate immunity: COS – OGA

  COS = chitooligosaccharides => chitosan

  OGA = oligogalacturonides => pectin

  mimics plant-pathogen interaction

  o against a range of diseases among which powdery mildew on various crops (grape, strawberry,...), downy mildew on grape, ...
3. Screening of BCP-activity against Verticillium in flax

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• 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
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
Bio Control Products (BCPs)

• Testing BCPs against 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
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
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