Detection of *F. circinatum* by isolation and morphological identification

Characterization of the performance criteria according to PM 7/98

Véronique Wilson
ANSES - Plant Health Laboratory - Mycology Unit

Workshop on the revision of PM 7/98 – Maisons-Alfort 2019-02-11/13

Why characterizing the performance of the method?

Fusarium circinatum Nirenberg & O'Donnell, is the causal agent of pitch canker disease on *Pinus* spp.


In underbark tissue


In seedlings

In 2017, revision of the method for the Detection of *Fusarium circinatum* on vegetative tissue by mycological isolation and morphological caracterisation:

- ✓ Consideration of new species of *Fusarium* found on pines and morphologically close to F. circinatum (presence of coiled sterile hyphae)
- ✓ Consideration of strains of *F. circinatum* no longer making coiled sterile hyphae (Mullett 2017).

➢ Characterization of the method's performances according to the standard PM_7/98 (2) (appendix 5 et 6)

A risk analysis on the method

The risk analysis showed 2 sensitive points:
- Isolation of the fungi
- Morphological identification

Isolation of the fungi

The following performance criteria have to be characterised

- ✓ Analytical sensitivity
- ✓ Repeatability
- ✓ Reproducibility

2 parameters were considered as not applicable

- ✓ Analytical specificity
- ✓ Selectivity

This choice was based on PM 7/98 (2) appendix 5:
Isolation of target fungi from a sample is per definition non-specific and non-selective

A risk analysis on the method

Morphological identification

It is based on:

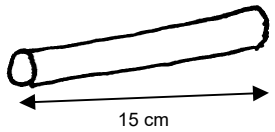
- ✓ Expertise in morphology
- ✓ The use of available documentation
  - original morphological descriptions
  - Identification keys
  - reference strains
  - …

As described in PM 7/98 (2)

Preparation of artificially infected samples

Twigs of *Pinus nigra* free from *F. circinatum*

Superficial disinfection with alcohol



Plug of mycellium under the bark (from Fc reference strain LSV 219)

Healthy tissue
(used for the preparation of the samples)

Infected tissue
Twig and plug of *F. circinatum*

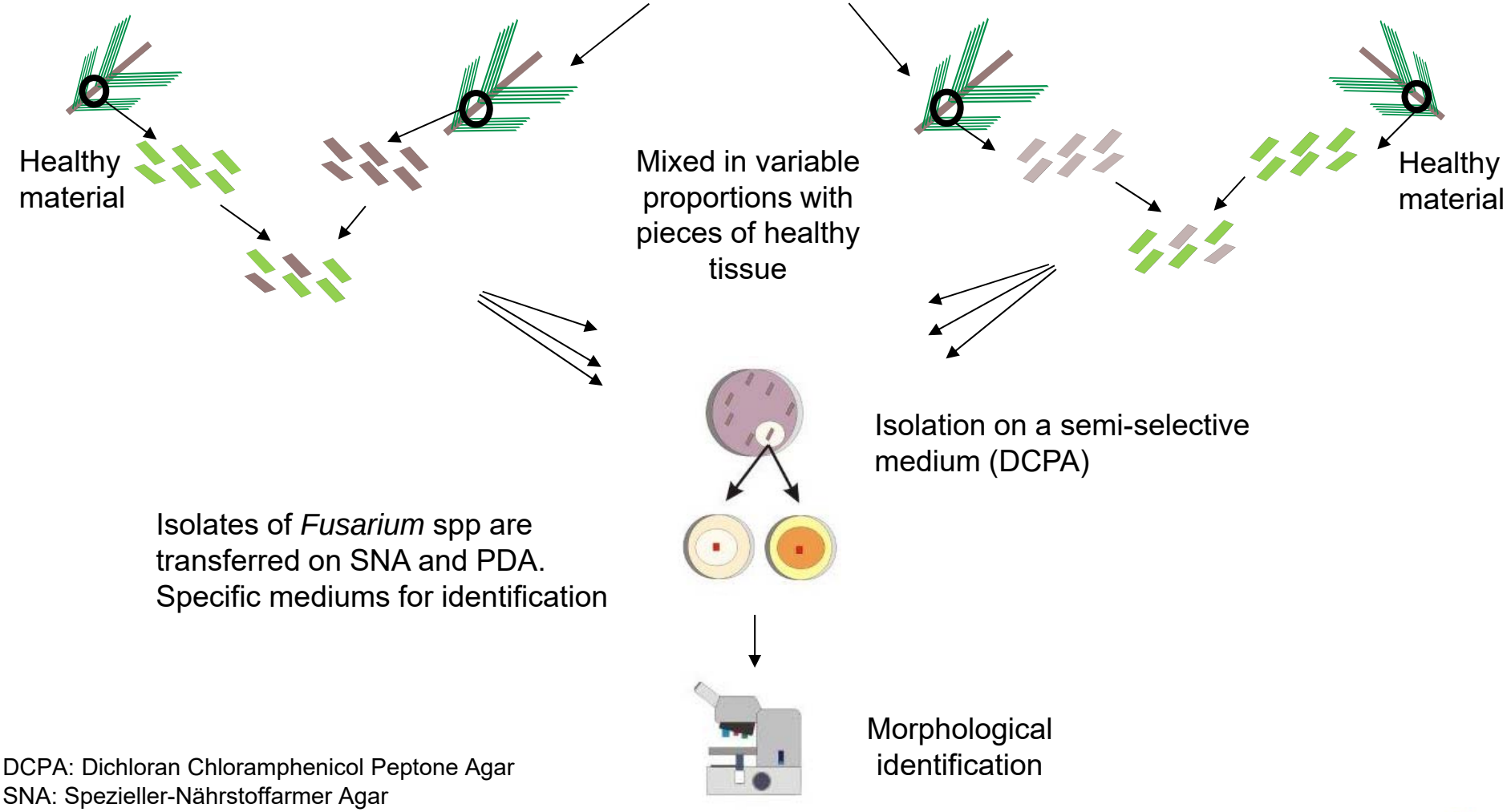
Negative controls
Twig and plug of sterile PDA

The ends of the twigs are protected with a plastic film to reduce dehydration.

Incubation 18 days at 22° C +/- 6° C

Analytical sensitivity

Wood chips are removed under the bark on the edge of the necrosis on infected tissues and negative controls



DCPA: Dichloran Chloramphenicol Peptone Agar
SNA: Spezieller-Nährstoffarmer Agar
PDA: Potato Dextrose Agar

Analytical sensitivity

Samples spiked with *F. circinatum*

	Pieces of potentially infected tissue	Pieces of healthy tissue
1	1	29
5	5	25
10	10	20

	Number of isolates of *F. circinatum*	%
1	0	0
5	4	80
10	6	60

Negative controls

	Pieces of negative controls	Pieces of healthy tissue
1	1	29
5	5	25
10	10	20

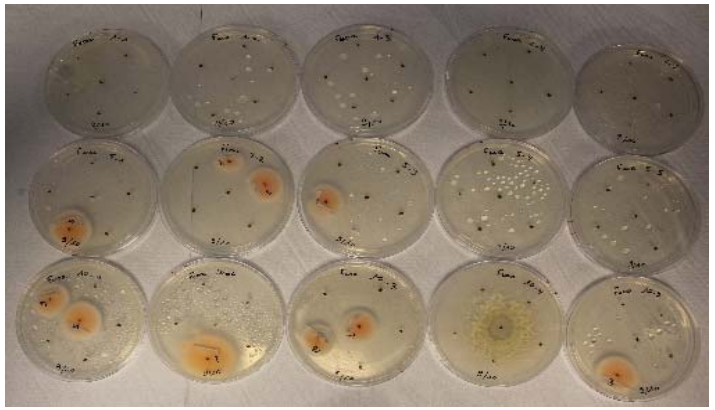
	Number of isolates of *F. circinatum*	%
1	0	0
5	0	0
10	0	0

Pieces of **potentially** infected tissue
=> Max = 80 %

1

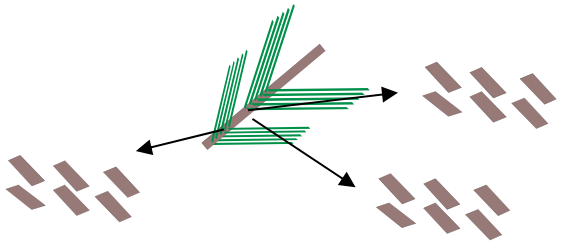
5

10



Repeatability

3 isolations from 1 inoculated pine tissue
3 isolations from 1 negative control
1 operator



Sample spiked with *F. circinatum*

Isolation	Pieces of potentially infected tissue	Pieces of healthy tissue
1	1	29
2	1	29
3	1	29

Isolation	Number of isolates of *F. circinatum*	%
1	1	100
2	1	100
3	1	100

Negative controls

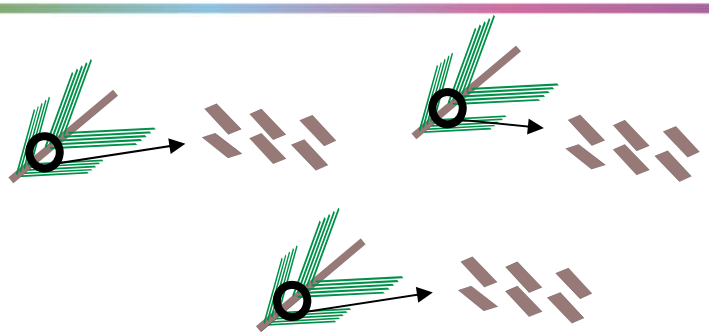
Isolation	Pieces of negative controls	Pieces of healthy tissue
1	1	29
2	1	29
3	1	29

Isolation	Number of isolates of *F. circinatum*	%
1	1	100
2	1	100
3	1	100

Repeatability = 100 % **1 piece among 30 pieces**

Reproducibility

3 isolations from 3 inoculated pine tissue
3 isolations from 3 negative controls
3 operators
3 differents days



Operator	Number of pieces	
	Fc inoculated tissue	Negative controls
1	30	30
2	30	30
3	30	30

Operator	Detection of *F. circinatum*	
	Fc inoculated tissue	Negative controls
1	Fc Detected (30/30)	Non-Detected
2	Fc Detected (30/30)	Non-Detected
3	Fc Detected (30/30)	Non-Detected

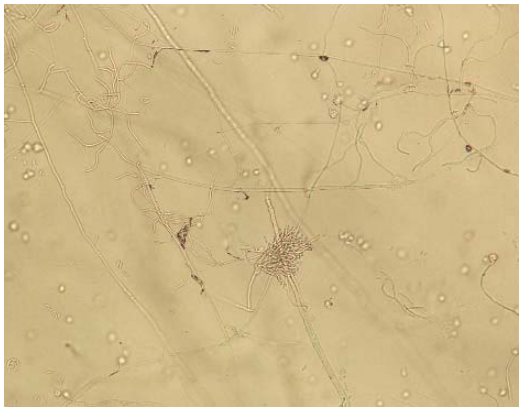
Reproducibility = 100 %

Morphological identification

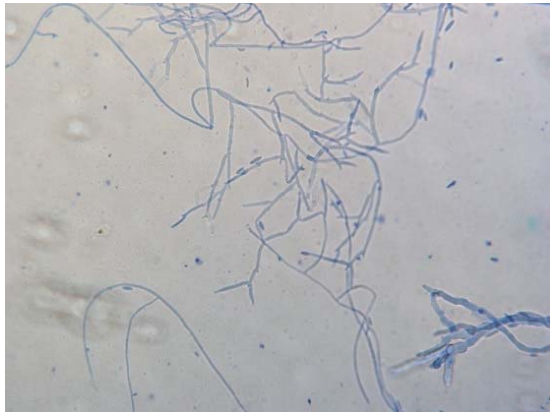
Morphological identification is an expertise based on the use of reference documentation.

The criteria for identifying *Fusarium circinatum* and closely related species are described:

- ✓ In peer-reviewed international publications that describe new species (Nirenberg *et al*., 1998, Herron *et al*., 2015)
- ✓ Identification keys of *Fusarium* spp. (Nelson *et al*., 1983, Leslie *et al*., 2006).



F. circinatum: coiled sterile hyphae



F. circinatum: Microconidia and conidiogenous cells.

Morphological identification

Morphological characteristics of *Fusarium* species encountered on pine (on SNA)

Fusarium species	Microconidia	Conidiogenous cells	Coiled sterile hyphae	Chlamydospores	Sources
F. circinatum	**Only in false heads**	**Monophialides and polyphialides**	**Presents**	**Absents**	**(1)**
F. subglutinans	Only in false heads	Monophialides and polyphialides	Absents	Absents	(1)
F. verticillioides	Chaînette et fausse tête	Uniquement monophialides	Absents	Absents	(1)
F. oxysporum	Uniquement en fausse tête sur un conidiophore très court (parfois invisible)	Only short monophialides	Absents	Présents	(1)
F. solani	Uniquement en fausse tête sur un long conidiophore	Only monophialides often quite long	Absents	Présents	(1)
F. pseudocircinatum	Fausse tête et courte chaînette	Monophialides and sometimes polyphialides	Presents	Absents	(1)
F. fracticaudum	Only in false heads	Monophialides and polyphialides	Absents	Absents	(2)
F. marasasianum	**Only in false heads**	**Monophialides and polyphialides**	**Presents**	**Absents**	**(2)**
F. parvisorum	**Only in false heads**	**Monophialides and polyphialides**	**Presents**	**Absents**	**(2)**
F. pininemorale	Only in false heads	Monophialides and polyphialides	Absents	Absents	(2)
F. sororula	Only in false heads	Monophialides and polyphialides	Absents	Absents	(2)

(1) From Leslie et Summerell (2006),
(2) From Herron *et al* (2015)

In the new version of our method, the result of the analysis is: suspicion of presence of *F. circinatum* or absence of *F. circinatum*

Thank you for your attention