

Monitoring sensitivity to **CAA**, **Qil** and **Fluazinam**
among populations of *Phytophthora infestans*
collected from French potato producing areas
in 2016 & 2018:
New methodology and First results

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CONIPHY

CJH Scientific Expertise

AIMS OF THE STUDY

- Characterize the sensitivity of natural populations of late blight to several modes of action :
 - **CAA** : *dimethomorph, mandipropamid...*
 - **Qil** : *cyazofamid, amisulbrom*
 - **Fluazinam**
- Identify the possible presence of resistant phenotypes in commercial fields
- Connect these phenomena with practices of treatment or regional specificities

THE SAMPLES : OBJECTIVES

Number and Origin

- Sampling in a minimum of 8 to 10 fields for every area of Potato production = to obtain a representativeness of the situation related to the cultural practices
- Choice of the fields for sampling:
 - Random
 - Presence of late blight
 - Knowledge of the program of fungicide treatment realized during the season
 - Representative of the practices recommended in the geographical zone
- Expected : **60 fields per year**

Transplanting on untreated potato leaves: Multiplication and homogenization of the inoculum

From infected potato leaves, technicians have:

- Cut by discs from zones with young and sporulating symptoms
- Placed the discs in Petri dishes with filter paper
- Washed the discs in order to eliminate the old sporulations and the residues of fungicide (surface products)
- Dried then incubated the discs during 12 hours in 18°C

The next day, young sporulations are collected with a cotton tip and inoculated on fresh untreated leaves.

All the leaves are incubated during 1 week in 18°C and in the light (12/12h of day/night).

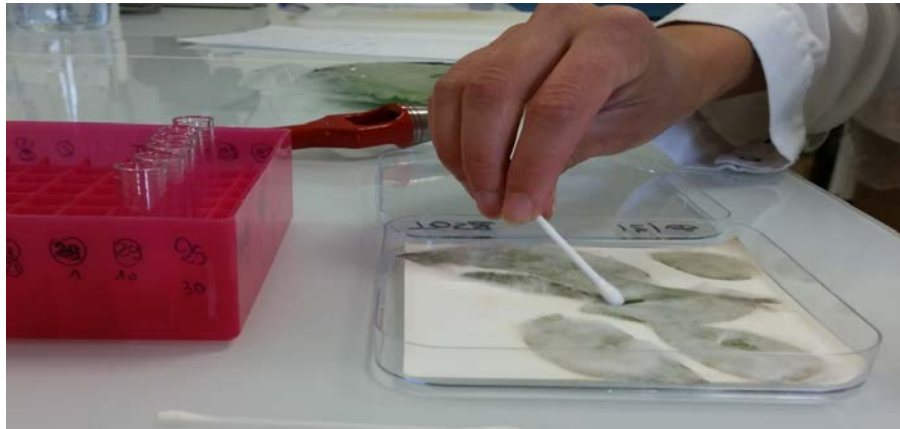


Tests on Potato leaf discs:

Preparation of the sporangial suspension

At the end of 7 days of incubation, a suspension of spores is prepared and titled to approximately 10^5 UFC/mL

- Placed at 4°C during 2 hours in order to promote the released of the sporocysts



Tests on Potato leaf discs:

Products and mode of application

Focus on 3 modes of action used on Potato late blight

- **CAA** : Carboxylic Acyl Amides
- **Qil** : Quinones Inside Inhibitors
- **Dinitro-Anilines** : fluazinam

For each mode of action, one reference active ingredient and several concentrations :

- **CAA** : dimethomorph 0,1 - 0,3 - 3 - 10 mg/L
- **Qil** : cyazofamid 0,01 - 0,1 - 1 - 10 mg/L (only 1 mg/L in 2018)
- **Dinitro-Anilines** : fluazinam 0,1 - 1 - 10 - 30 mg/L

Tests on Potato leaf discs :

Inoculation and incubation

Mixing the sporocyst suspension with fungicide solution (or water)

Deposit of a drop of 10 microliters on each disc



Drops are left during the night in 18°C and are dried the next day

All the dishes are put in climatic chamber in 18°C and in the light of day during 7 days (12h/12h)

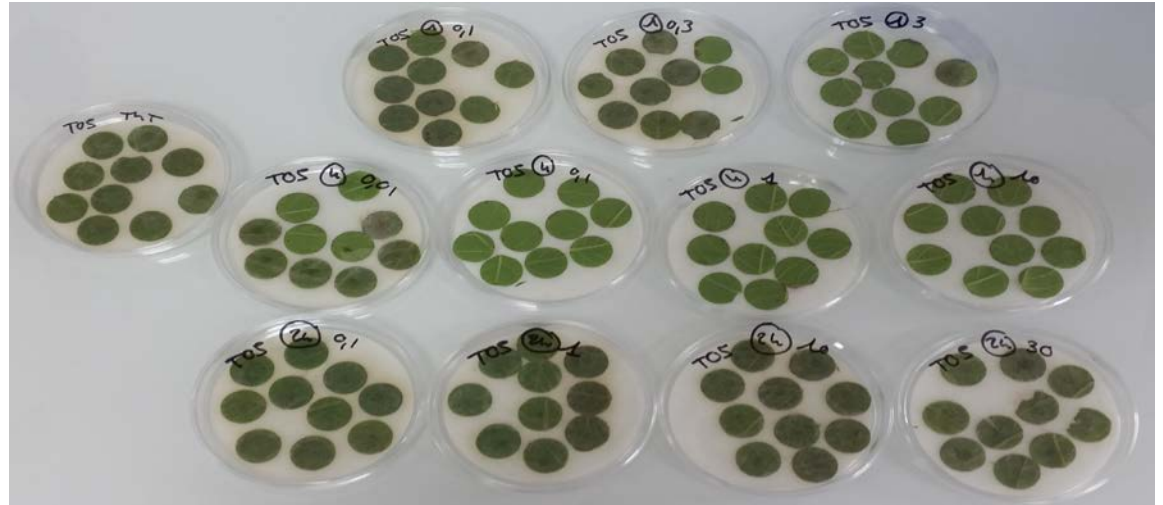
Analysis on a very large number of sporocysts for each sample (field) : deposit of 10 droplets of 10 microliters with suspension 100.000 so study of **10 000 sporocysts !**

Tests on Potato leaf discs: Notation of the symptoms : Efficacy

Individual observation of each disc, evaluation of the symptoms

Scale of notation

- 0 – No necrotic area
- 1 – 25% of necrotic area
- 2 – 50% of necrotic area
- 3 – 75% of necrotic area
- 4 – 100% of necrotic are



Calculation of efficacy for each concentration of fungicide compared to the control (water)

Tests on Potato leaf discs: Results

Presentation of the results : Examples 2016 and 2018

Area	Dept.	Location	Sampling date	Control	Diméthomorphe (CAA)					MIC	Control	Cyazofamide (Qil)					MIC	Control	Fluazinam				MIC
					0,1	0,3	3	10	MIC			0,01	0,1	1	10	MIC			0,1	1	10	30	
Champagne	51	Livry Louvercy	05/07/2016	4	30	80	100	100	0,3-3	3,2	50	100	100	100	0,01-0,1	4	40	70	30	90	>30		
Champagne	51	Montépreux	04/07/2016	1,6	75	100	100	100	0,1-0,3	1,6	100	100	100	100	<0,01	1,2	33	67	100	100	1-10		
Champagne	51	Breuvry-sur-Coole	04/07/2016	2,4	100	100	100	100	<0,1	2	100	100	100	100	<0,01	2	83	100	100	100	0,1-1		
Champagne	51	Breuvry-sur-Bole	11/07/2016	2,8	43	86	100	100	0,3-3	3,2	63	100	100	100	0,01-0,1	3,2	12	0	37	87	>30		

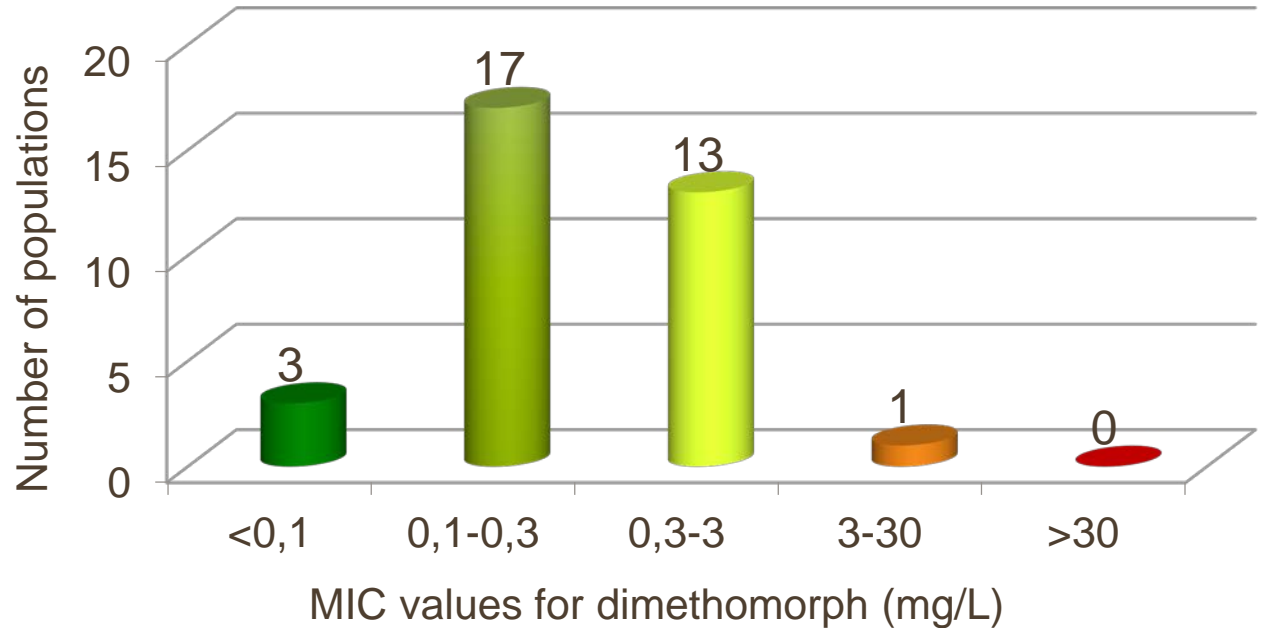
Area	Dept.	Location	Sampling date	Control	Diméthomorphe (CAA)				MIC	Control	Cyazofamide	Control	Fluazinam				MIC
					0.1	0.3	3	30					mg/l	1	0.1	1	
Champagne	10	Maizières	18/07/2018	2.4	0	67	100	100	0,3-3	1.6	100	2.8	100	100	100	100	<0,1
Champagne	51	Auve	10/09/2018	4	0	100	100	100	0,1-0,3	4	100	4	20	0	50	60	>30
Champagne	51	Auve	10/09/2018	4	50	100	100	100	0,1-0,3	4	100	4	10	0	50	80	>30
Champagne	51	Valmy	25/09/2018	4	0	70	100	100	0,3-3	4	100	4	0	0	30	20	>30

- Average rate of disease on the control (scale from 0 to 4)
- Efficacy of each rate of fungicide
- Evaluation of MIC (Minimal Inhibitory Concentration)
- Distribution of the populations (samples) according to the MIC values

Results : Sensitivity of the populations to CAA

Distribution of the populations according to MIC

France – 2016 – 34 populations



Discriminatory rate
of 3 mg/L

Normal situation

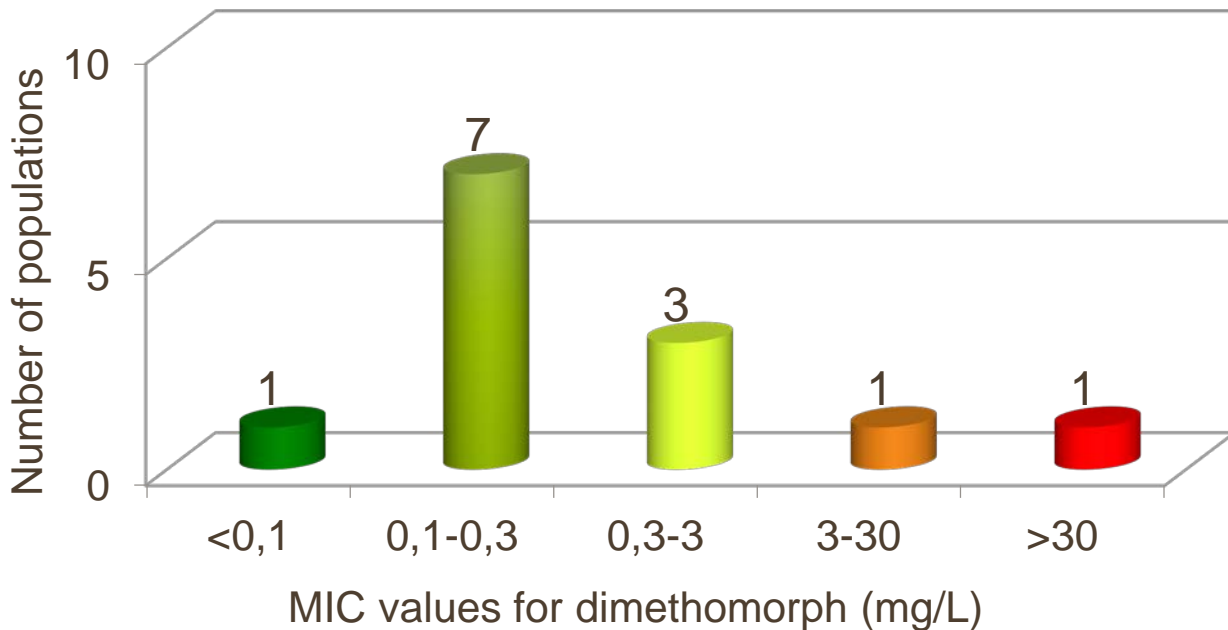
One population less
sensitive ?

Strains isolated have
a very low fitness !

Results : Sensitivity of the populations to CAA

Distribution of the populations according to MIC

France – 2018 – 13 populations



Discriminatory rate
of 3 mg/L

Normal situation

Two populations less
sensitive ?

FRAC group often
used : to be
monitored.

Results : Sensitivity of the populations to Qil

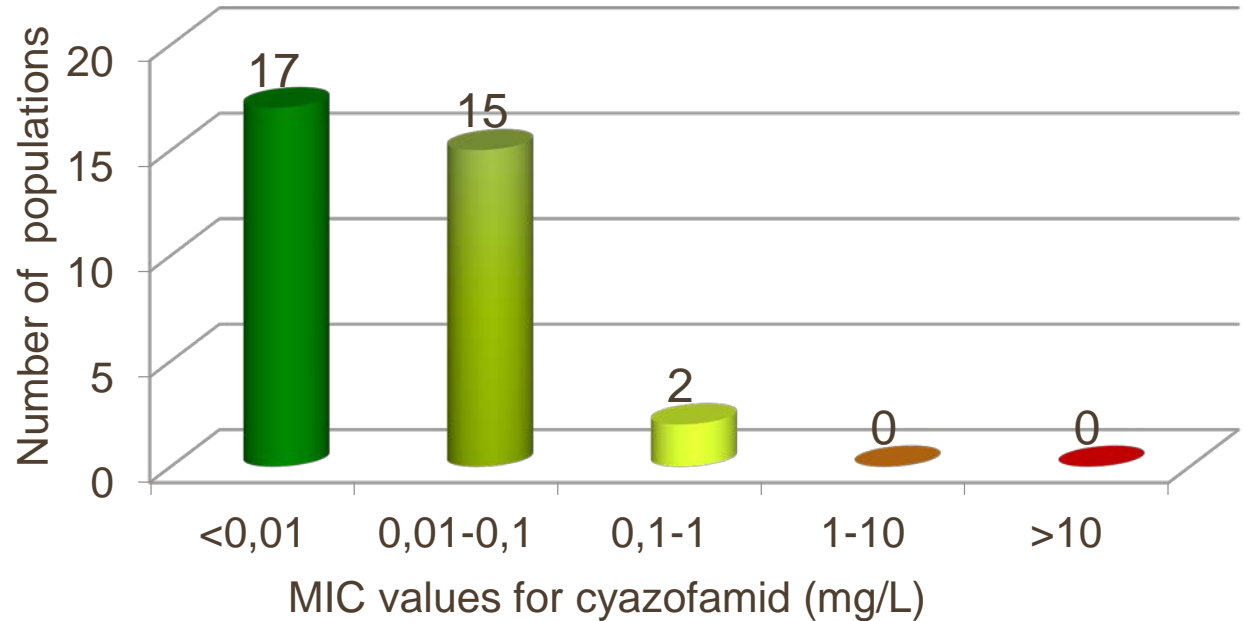
Distribution of the populations according to MIC

France – 2016 – 34 populations

Discriminatory rate of
1 mg/L

Normal situation

No detection of
specific resistance to
Qil or non specific
resistance AOX



Results : Sensitivity of the populations to Qil

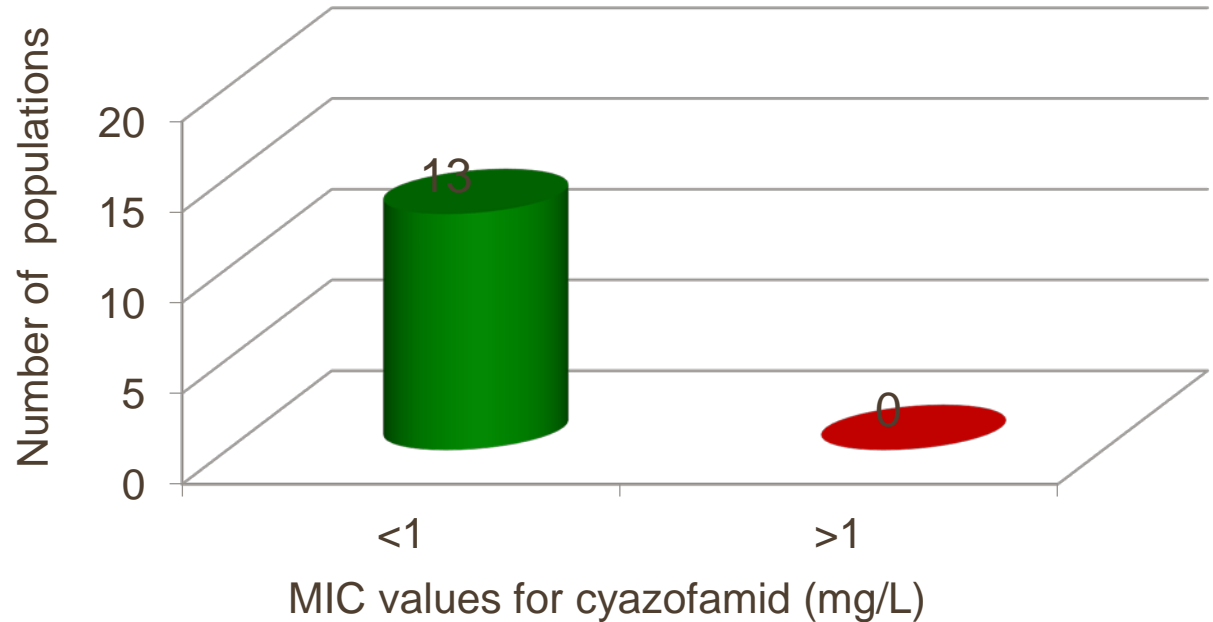
Distribution of the populations according to MIC

France – 2018 – 13 populations

Discriminatory rate of
1 mg/L

Normal situation

No detection of
specific resistance to
Qil or non specific
resistance AOX



Results : Sensitivity of the populations to fluazinanam

Distribution of the populations according to MIC

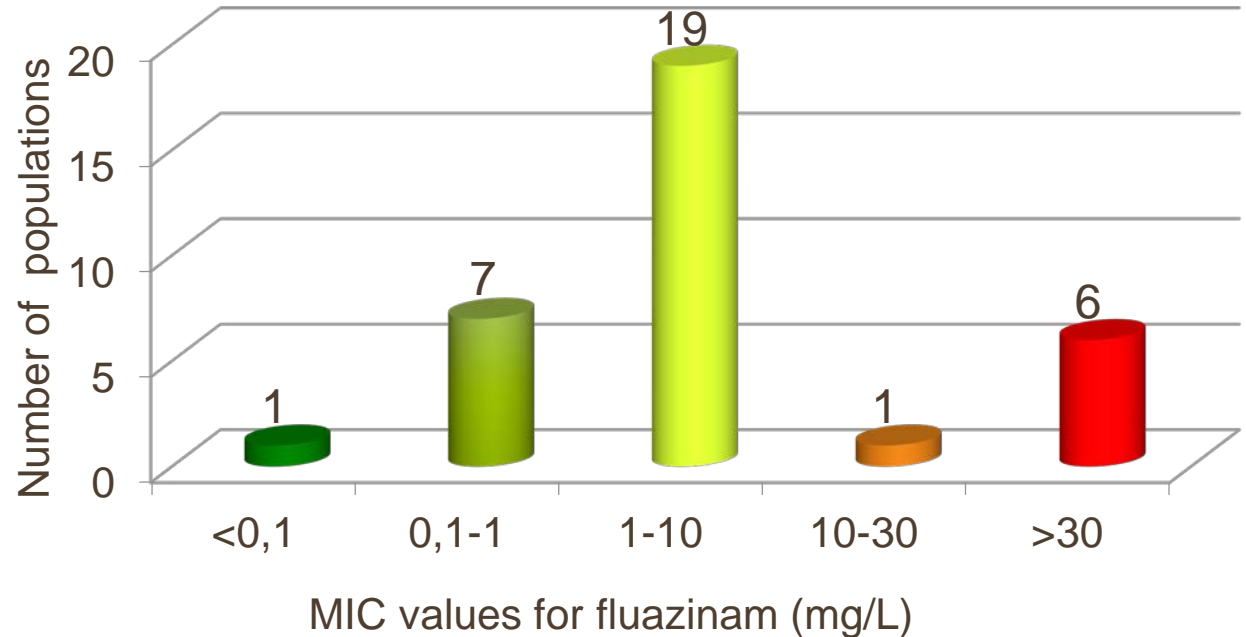
France – 2016 – 34 populations

Discriminatory rate of
10 mg/L

Abnormal situation

6 to 7 populations with
resistant phenotypes

Isolation of many
resistant strains with
RF >100 and good
fitness



Results : Sensitivity of the populations to fluazinam

Distribution of the populations according to MIC

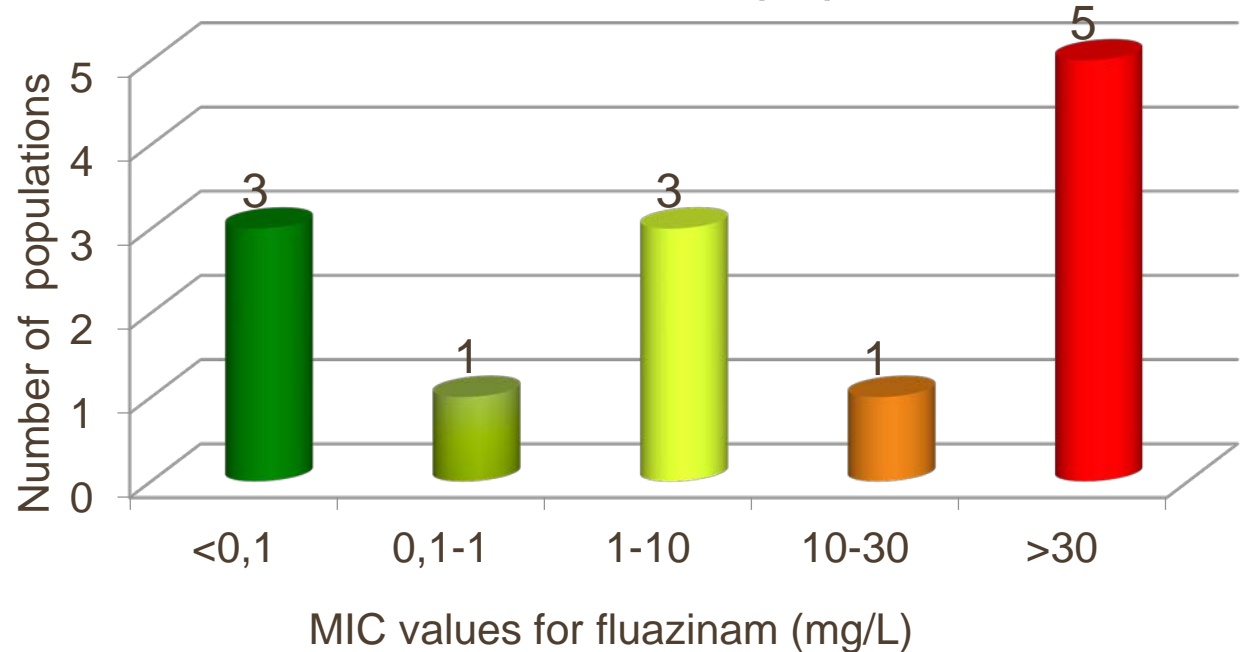
France – 2018 – 13 populations

Discriminatory rate of
10 mg/L

Abnormal situation

5 to 6 populations with
resistant phenotypes

Genotypes in the
fields: mostly 37_A2
but not only (one
13_A2).



CONCLUSIONS :

Preliminary results on French resistance status

Synthesis on **47 populations**: low number -> Caution in the interpretation of the results and conclusions

- **CAA**

- Normal situation except three populations non controlled by 3 mg/L
- Low fitness for these less sensitive strains in 2016.

- **Qil**

- All the populations controlled by 1 mg/L
- **Normal situation**

CONCLUSIONS :

Preliminary results on French resistance status

Synthesis on **47 populations**: low number -> Caution in the interpretation of the results and conclusions

- **Fluazinam**

- Detection of **13 populations non controlled by 10 mg/L including 11 populations non controlled by 30 mg/L.**
- **Frequency seems to be increasing.**
- Validation of this character of resistance (monosporangial strains)
- In 2018: mostly 37_A2 genotype but not only (in fields)
- Resistance widespread (in all the Potato growing areas)
- **Watch this mode of action!**

THANK YOU FOR YOUR ATTENTION !

