

Monitoring sensitivity to CAA, Qil and Fluazinam among populations of *Phytophthora infestans* collected from French potato producing areas in 2016 :

New methodology and Preliminary results

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CONIPHY

CJH Scientific Expertise

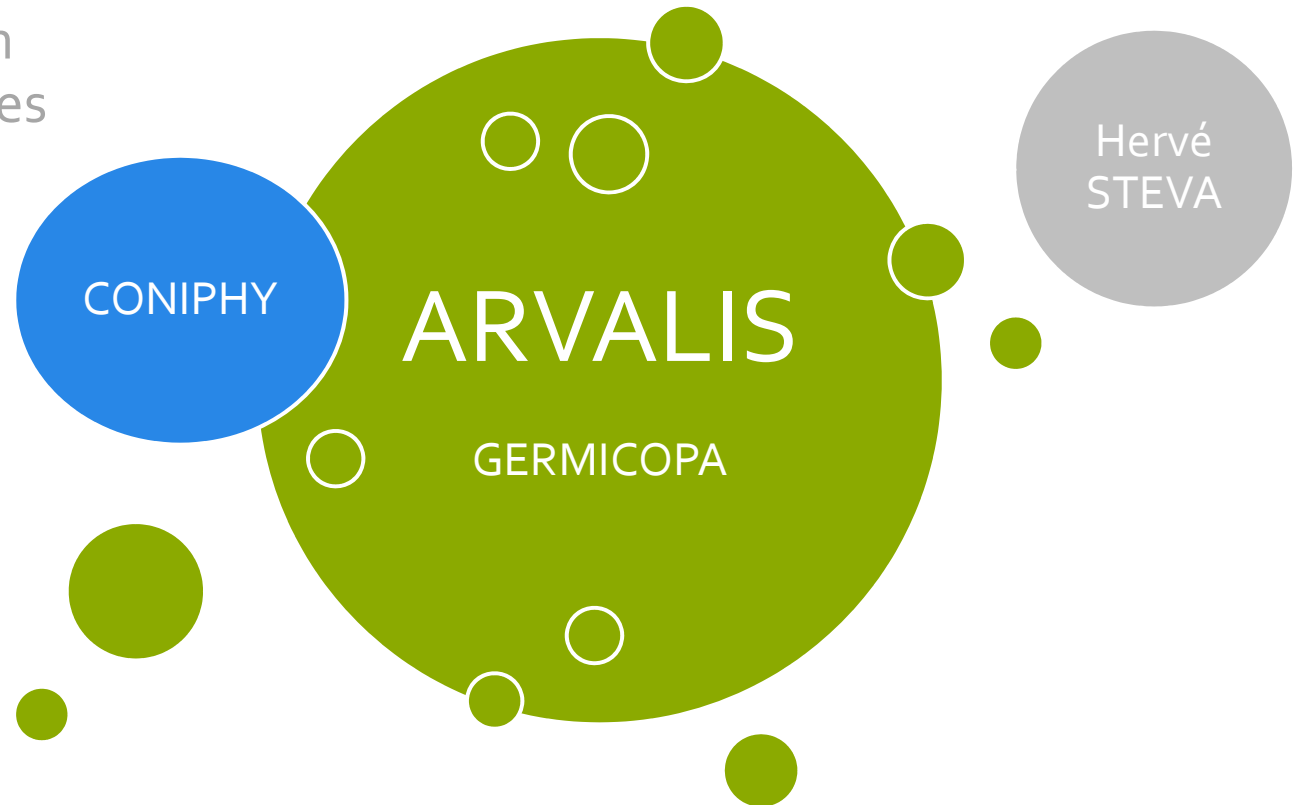
# AIM OF THE STUDY

- 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

# ORGANIZATION OF THE PROJECT



- Project promoter
- Network of samplings
- Laboratory for analysis
- Scientific Expertise on resistance to fungicides



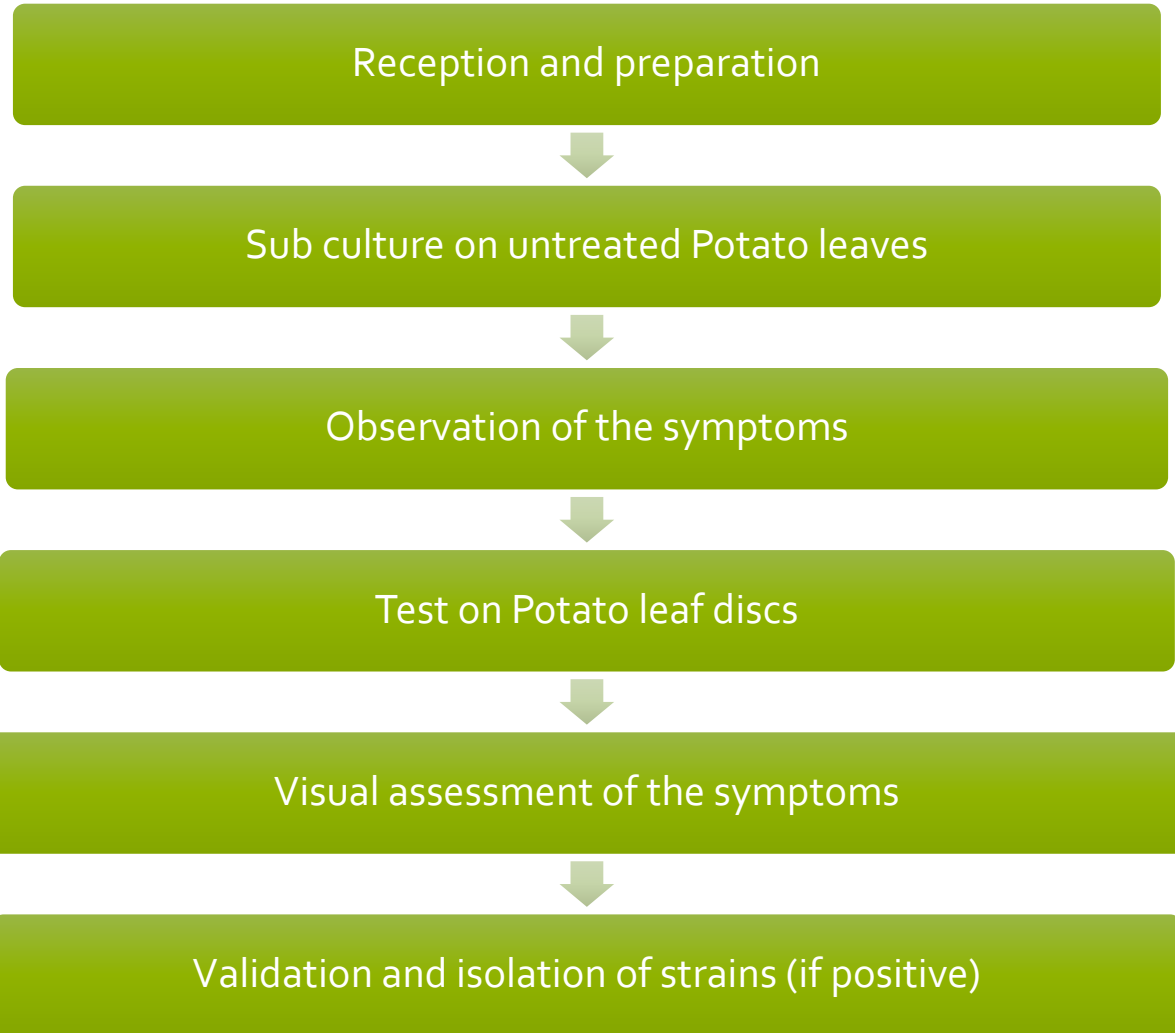
# THE SAMPLES : OBJECTIVES

## *Number and Origin*

- Sampling in a minimum of 8 to 10 land (farm) for every area of Potato production = to obtain a representativeness of the situation related to the cultural practices
- Choice of the plots for sampling:
  - Random
  - Presence of late blight
  - Knowledge of the program of fungicide treatment realized during the 2016 season
  - Representative of the practices recommended in the geographical zone
- Provisional : **60 plots or fields**

# PROCESS OF ANALYSIS : POPULATIONS

**Model developped in Grapevine**  
*Plasmopara viticola*



# Reception of the samples: Check of the quality and identification

Very different qualities!!!

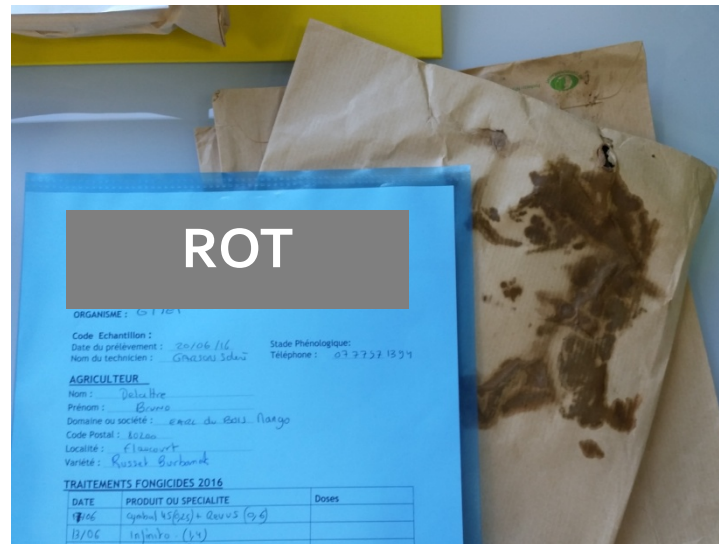
**NORMAL**



**DRY**



**ROT**



# 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: 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 :

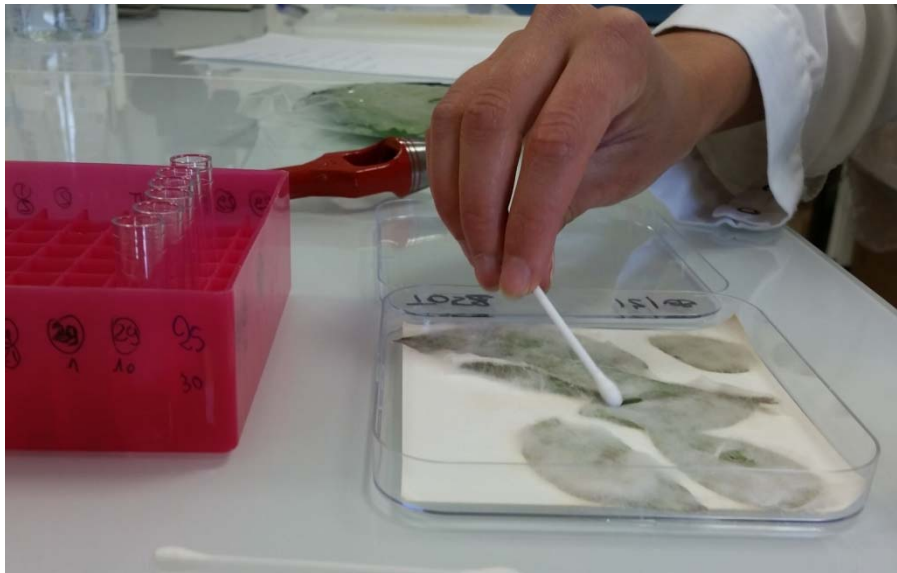
- **CAA** : dimethomorph                    0,1 - 0,3 - 3 - 10    mg/L
- **Qil** : cyazofamid                        0,01 - 0,1 - 1 - 10   mg/L
- **Dinitro-Anilines** : fluazinam        0,1 - 1 - 10 - 30    mg/L

All the active ingredients are applied by mixing the fungicide with the sporangial suspensions



# 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  $10^4$  or  $10^5$  UFC/mL
- Placed at  $4^{\circ}\text{C}$  during 2 hours in order to promote the released of the sporocystes



# Tests on Potato leaf discs : Inoculation and incubation

Mixing the sporocyste 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)

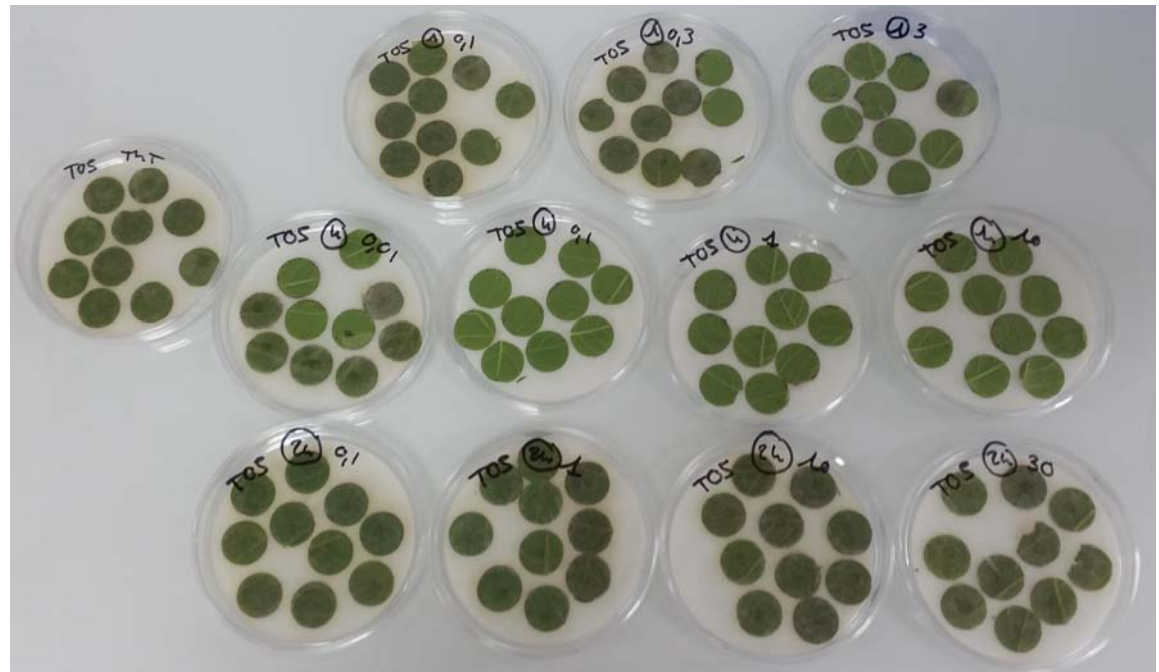
Analysis on a very large number of sporocystes for each sample (field)  
: deposit of 10 droplets of 10 microliters with suspension  $10^5$  so  
study of **10 000 sporocystes !**

# 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 in comparison with the control (water)

# Tests on Potato leaf discs: Results

## *Presentation of the results : Examples*

Area	Dept.	Location	Sampling date	Diméthomorphe (CAA)					Cyazofamide (Qil)					Fluazinam							
				Control	0,1	0,3	3	10	MIC	Control	0,01	0,1	1	10	MIC	Control	0,1	1	10	30	MIC
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-Cooles	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

- 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*

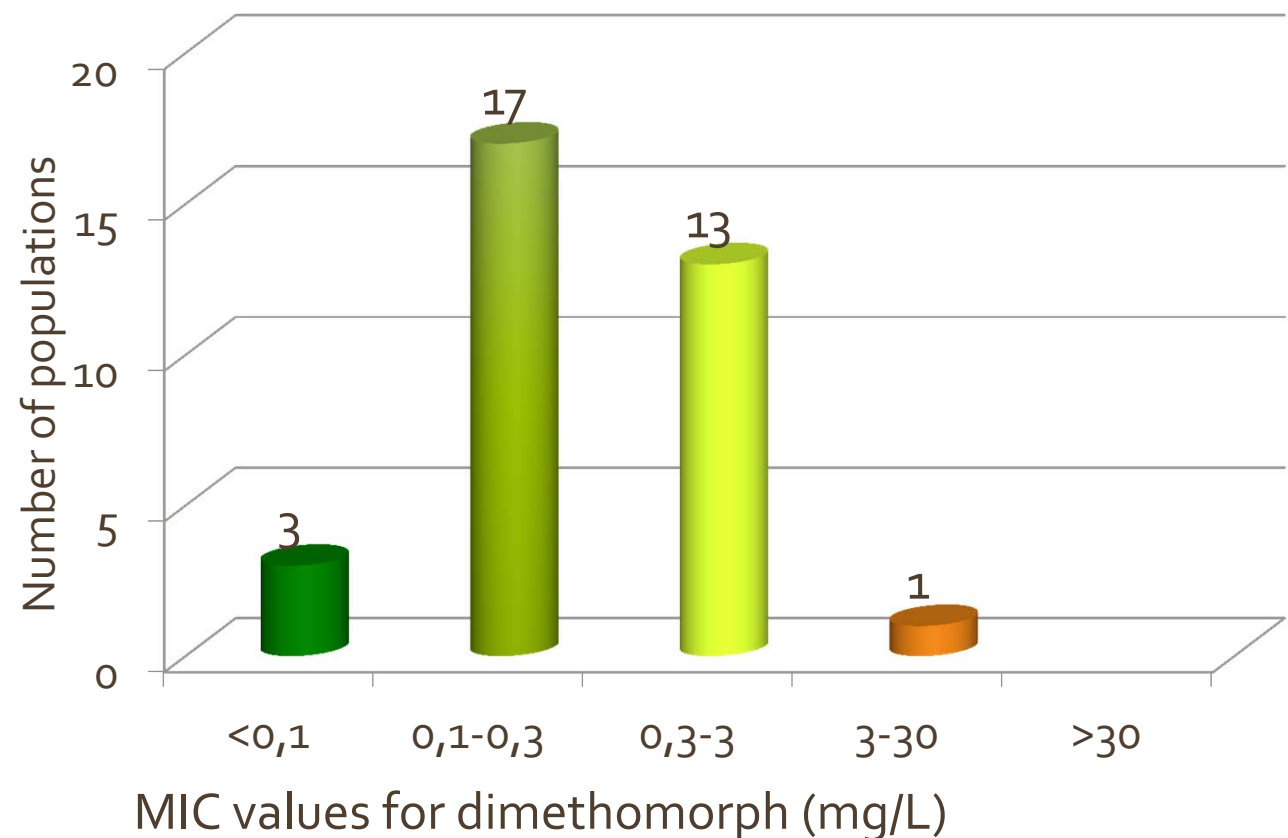
France – 2016 – 34 populations

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 Qil

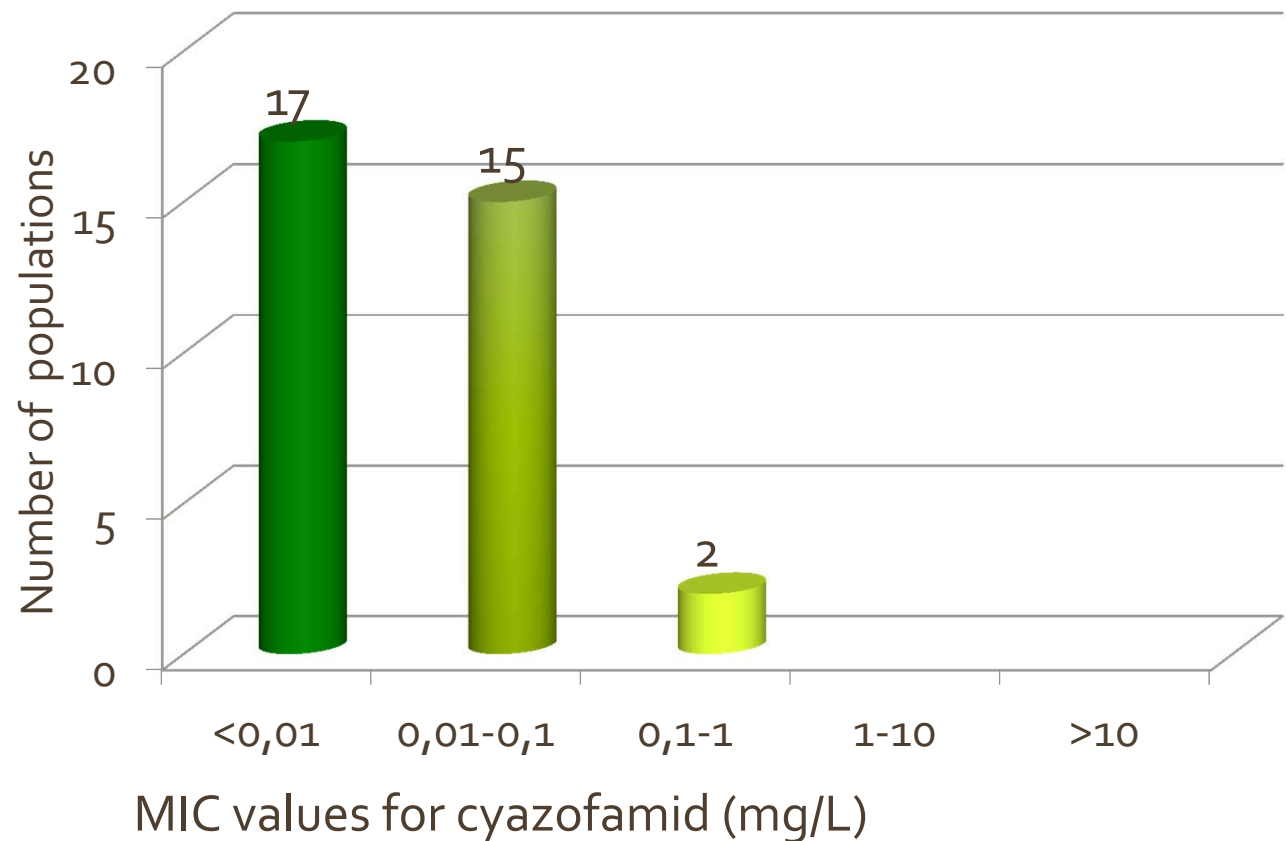
## *Distribution of the populations according to MIC*

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 fluazinam

## *Distribution of the populations according to MIC*

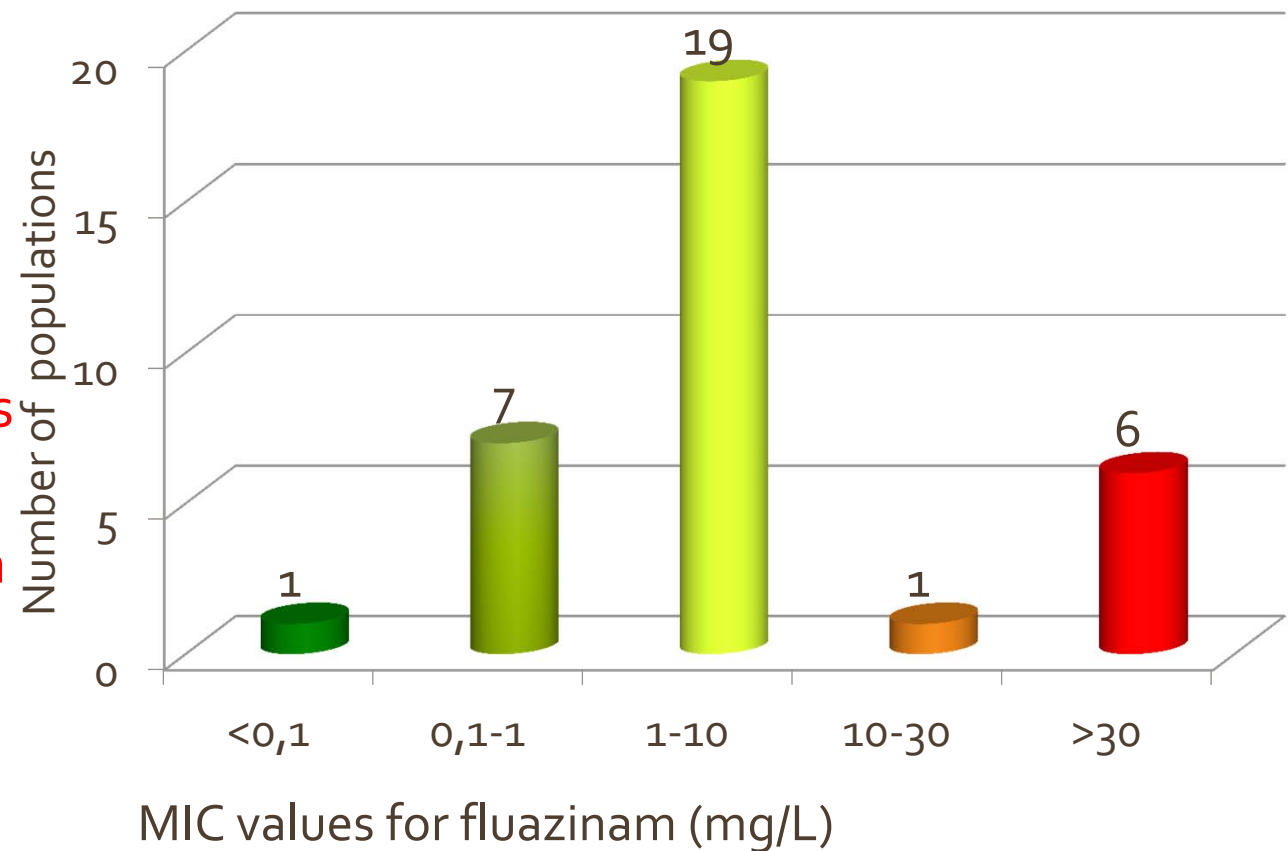
France – 2016 – 34 populations

Discriminatory rate  
of 10 mg/L

**Abnormal situation**

6 populations with  
resistant phenotypes

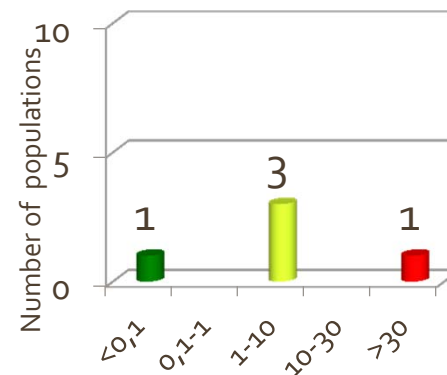
Isolation of many  
resistant strains with  
RF >100 and good  
fitness



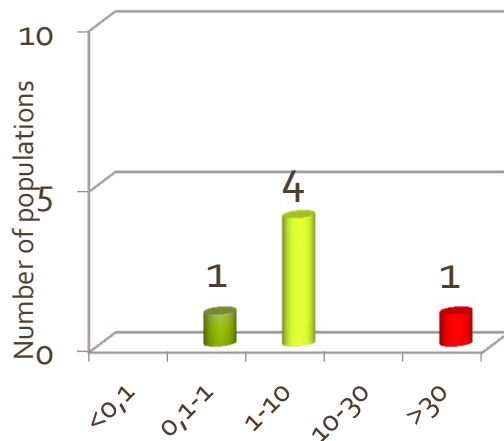
# Results : Sensitivity of the populations to fluazinam

## *Distribution of the populations according to MIC*

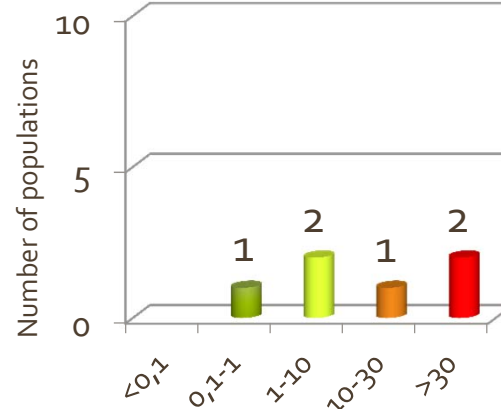
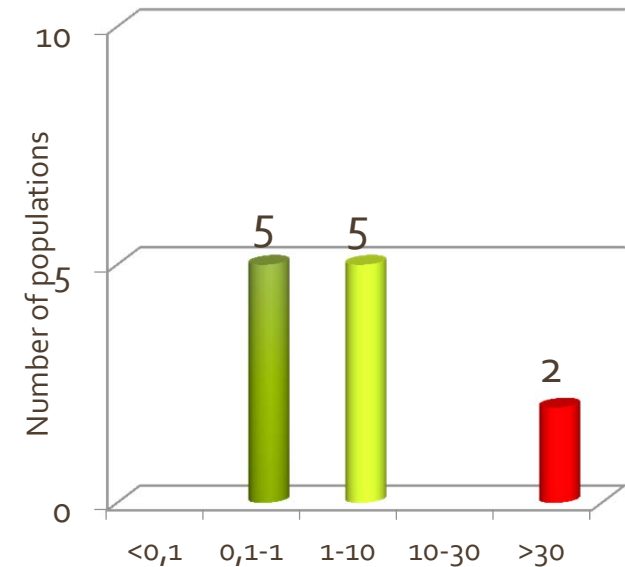
Nord / Pas de Calais  
5 populations



Bretagne  
6 populations



Champagne  
12 populations



Picardie  
6 populations



# CONCLUSIONS :

## Preliminary results on French resistance status

Synthesis on **34 populations** (62 samples) : low number

Caution in the interpretation of the results and conclusions

- **CAA**

- Normal situation except one population non controlled by 3 mg/L
- No fitness for these less sensitive strains

- **Qil**

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

- **Fluazinam**

- Detection of **6 populations non controlled by 30 mg/L**
- Validation of this character of resistance (monosporangial strains)
- Resistance widespread (in all the Potato growing areas)
- **Watch this mode of action!**