

**Visualization of the Zoxamide  
Mode of Action  
towards *Phytophthora infestans***

**Functional Fluorescence Microscopy Study**

**Viviane Calaora<sup>1</sup> and Jean-Marc Seng<sup>1, 2</sup>**

**Euroblight 2010**

**Contact: [viviane.calaora@biotransfer.fr](mailto:viviane.calaora@biotransfer.fr)**

<sup>1</sup>BIOtransfer – 41, rue Emile Zola – F-93100 Montreuil - France

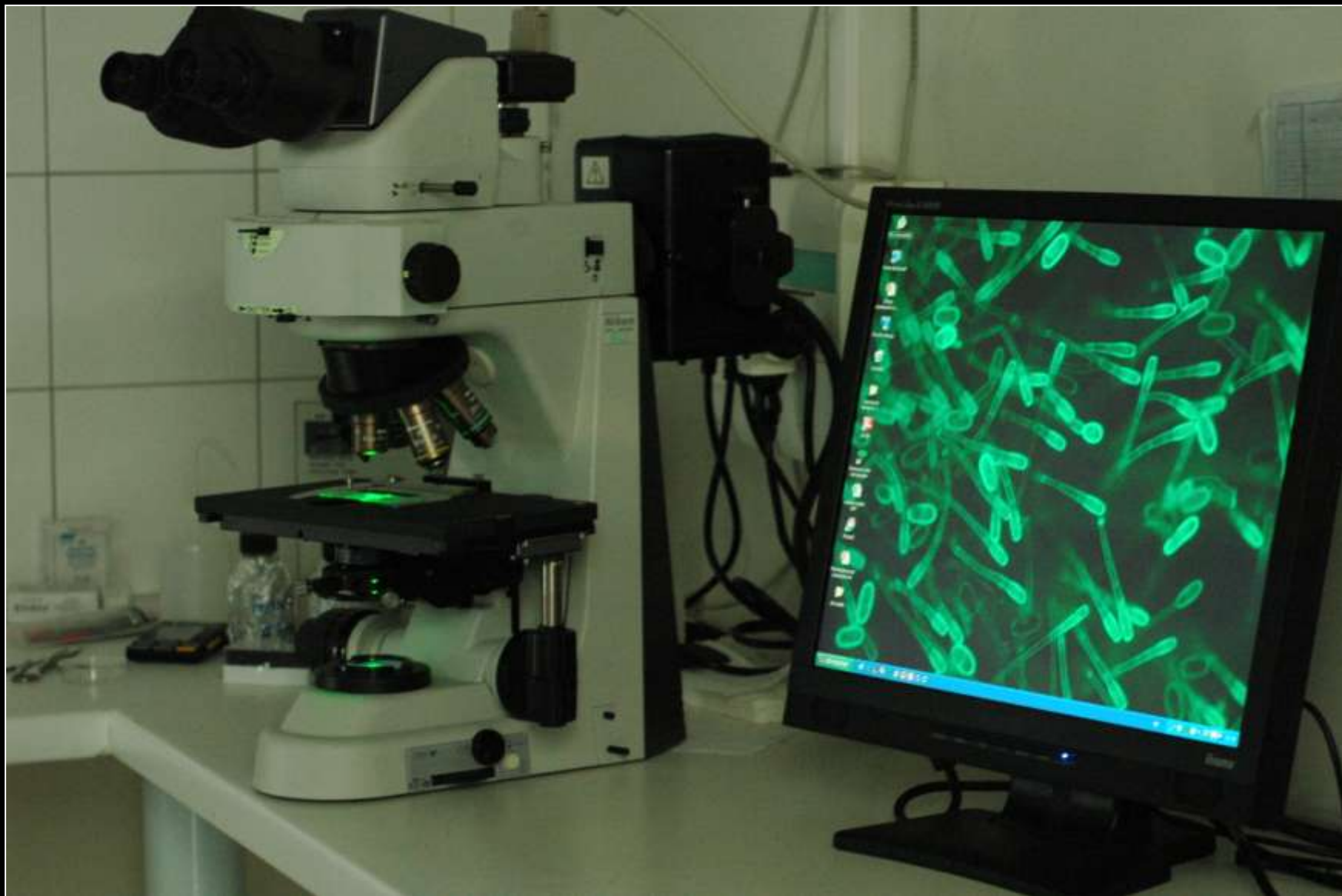
<sup>2</sup>IBP - Institut de Biologie des Plantes - Université Paris XI-Orsay - France

## Aim of the Study

The goal of GOWAN is to visualize the uniqueness of the Zoxamide mode of action comparing it to other fungicide families used in the protection of potatoes towards *Phytophthora infestans*, the causal agent of late blight on potatoes.

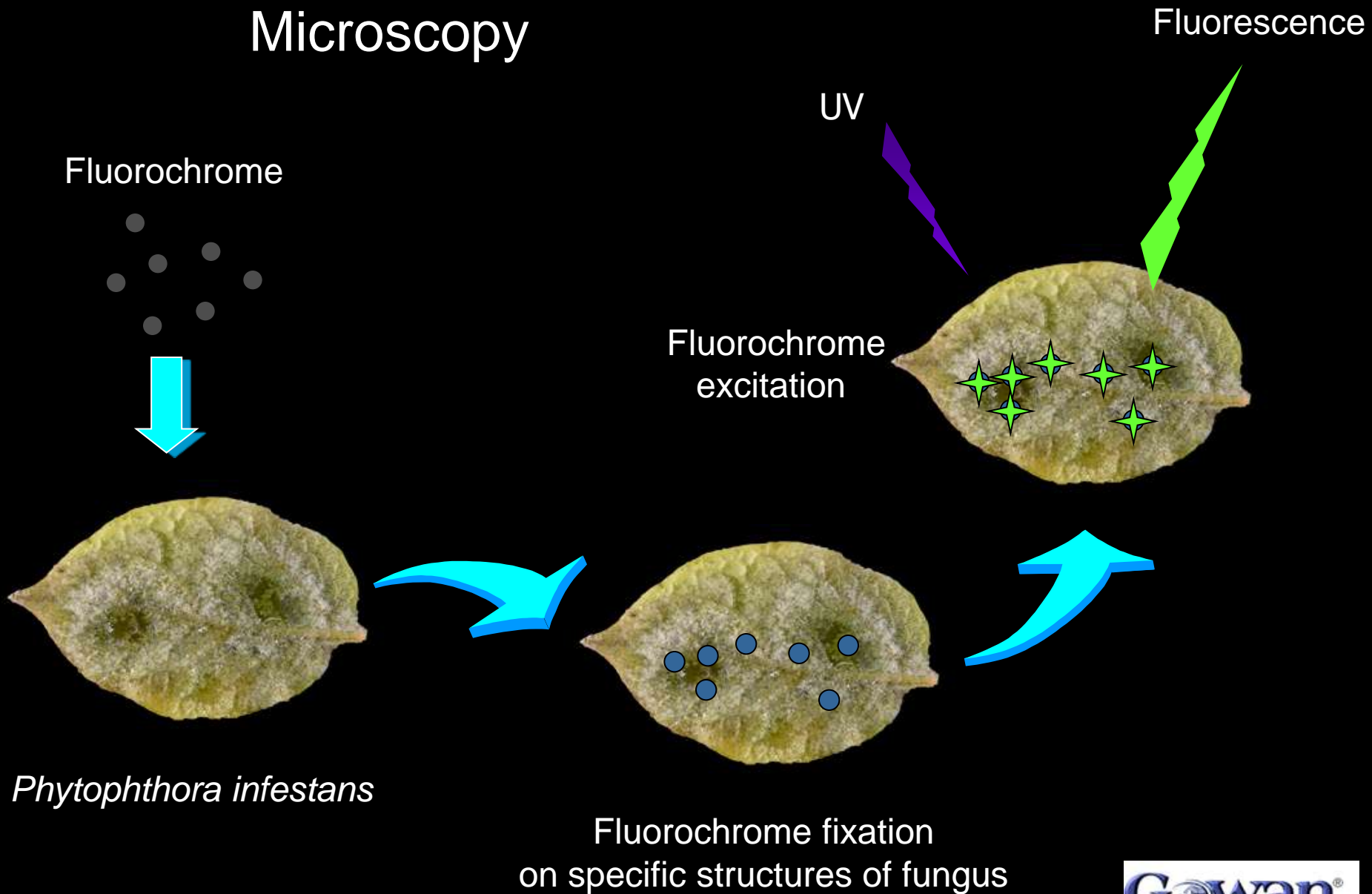
Proposal: illustrate the mode of action of Zoxamide, Cyazofamid and Mancozeb fungicides by the mean of epifluorescence functional microscopy.





# Principle of the Fluorescence

## Microscopy



*Phytophthora infestans*

Fluorochrome fixation  
on specific structures of fungus

# Different Fluorochrome for Different Purposes

Fluorochrome = Fluorophore = Fluorescent dye

## Structure Fluorochromes: Visualization of the fungal structure

### Blue fluorochrome I

Strong binding to  $\beta$ -glycosyl polysaccharides like cellulose, chitin and glycan. Stains in blue the fungus walls and septa.

### Blue fluorochrome II

Strong binding to DNA. Stains in blue cells nucleus.

## Functional Fluorochromes: Differentiate dead or alive and show vitality intensities

### Green/red fluorochrome

Accumulates in active vacuoles. Stains in red highly active vacuoles and in green low active vacuoles of living fungi. Diffuse yellow staining in dead fungi.

### Green fluorochrome

Accumulates in active mitochondria. Stains in green active mitochondria (living fungi). No staining in dead fungi.



Mode of action of Zoxamide, Cyazofamid  
and Mancozeb Fungicides  
on *Phytophthora infestans*  
Germination



# Development of the Experimental Process

1. Development of an experimental model for *in vitro* culture of *Phytophthora* allowing a good visualization with fluorescence microscopy
2. Development and optimization of fluorescent staining for a good visualization of :
  - General morphology
  - Nuclear localization and morphology
  - Mitochondria activities
  - Vacuolar activities
3. Screening by functional fluorescence microscopy of efficacy of different doses for each fungicide to select 2 doses with different effect :

– Zoxamide	0.005 µg/ml	0.05 µg/ml
– Cyazofamid	0.01 µg/ml	0.25 µg/ml
– Mancozeb	0.01 µg/ml	0.1 µg/ml
4. Staining and fluorescence microscopy observation after 48h incubation time

# Experimental Model

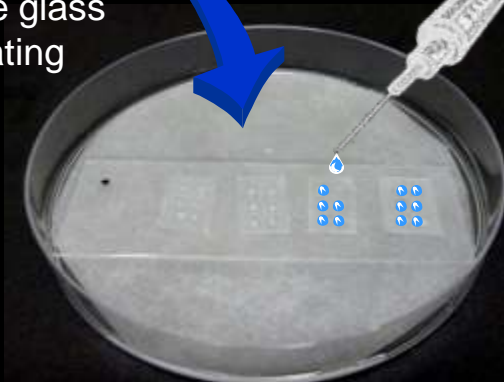
Fungicide doses at various concentration :  
Control → Distilled water  
Zoxamide → Technical formulation  
Cyazofamid → Ranman  
Mancozeb → Dithane neotec



*Phytophthora infestans* culture on a specific agar medium

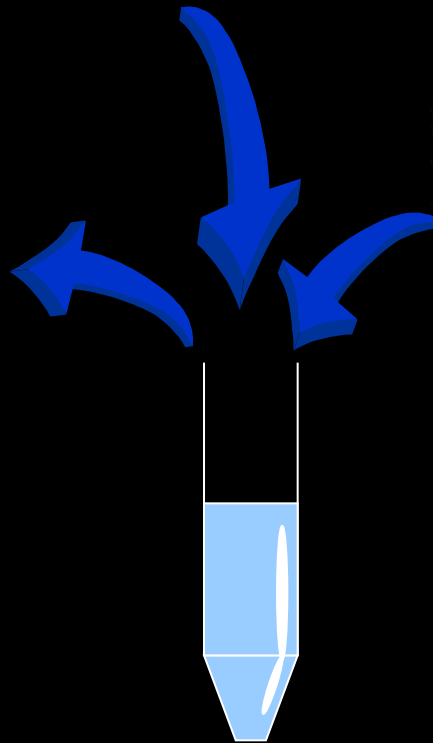


Sterile parafilm fragment deposit on sterile glass slide in incubating chamber



6 droplets of 2µl deposit on parafilm

Various incubation timing before staining and observation



50/50 mixing of :  
sporangia suspension  
( $10^6$  sporangia/ml final)  
Fungicide (dose N final)



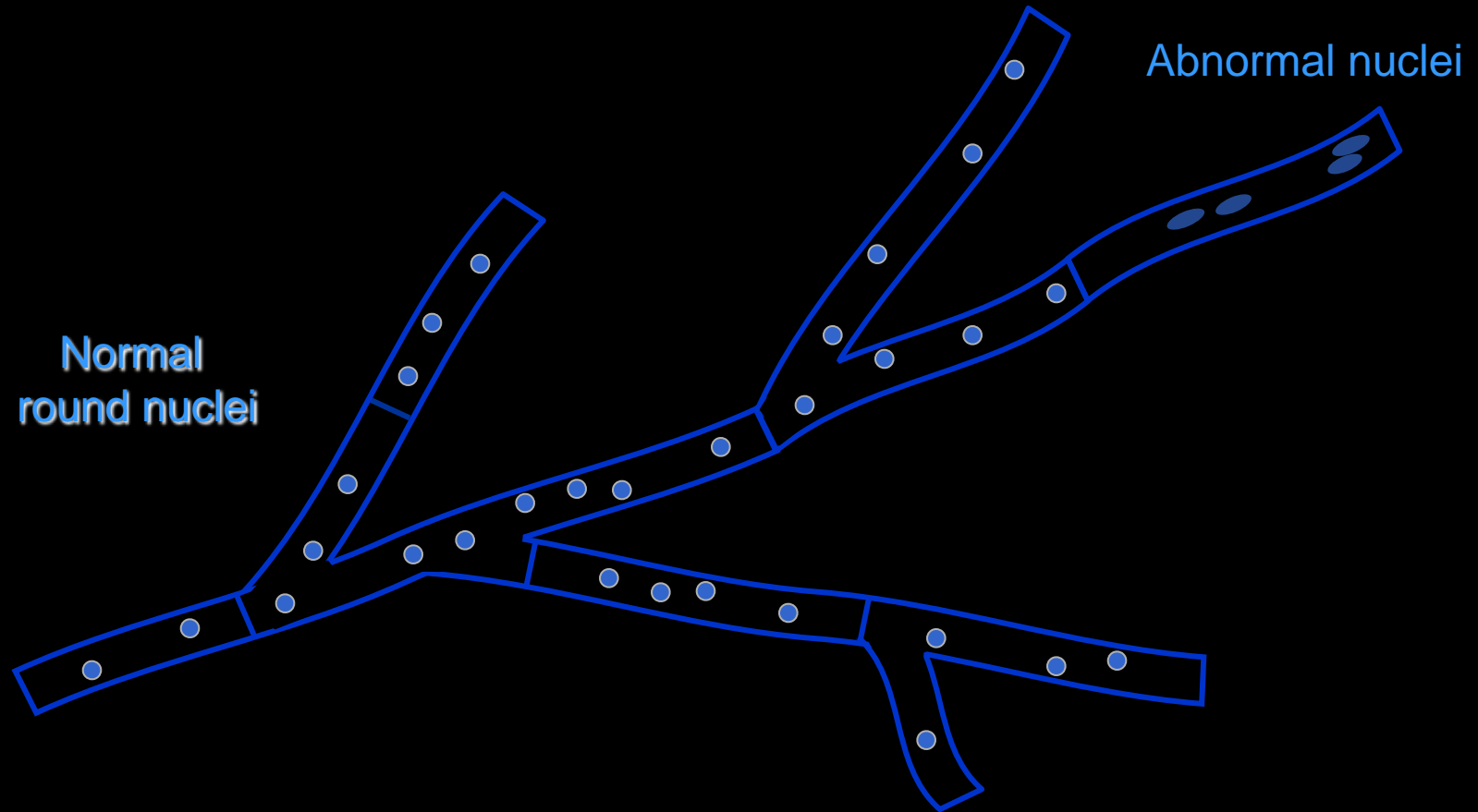
*Phytophthora* sporangia suspension  
 $2 \times 10^6$  sporangia/ml





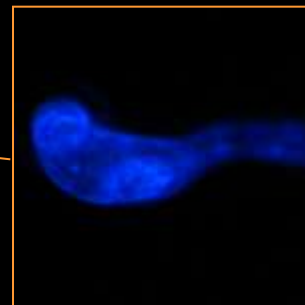
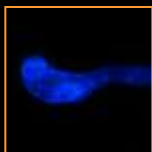
# Illustration of Fungicide Mode d'action :

## Nuclear morphology

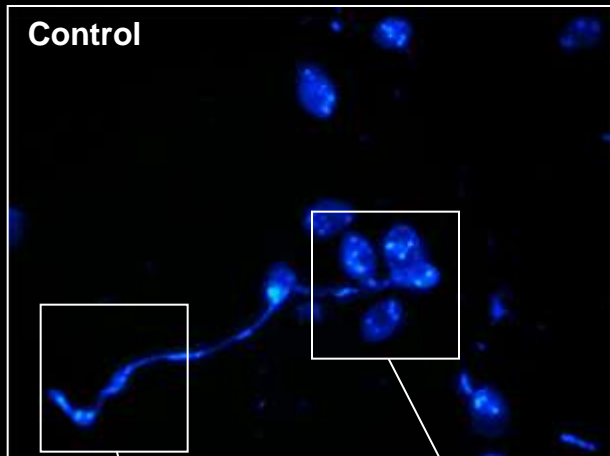


10µm

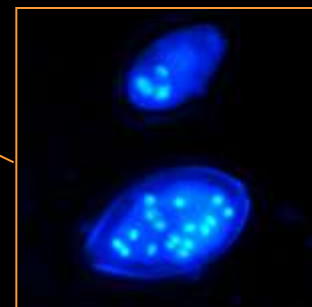
Zoxamide 0.05µg/ml



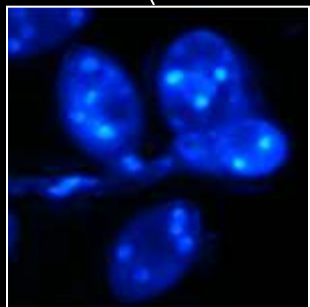
Control



Cyazofamid 0.25µg/ml

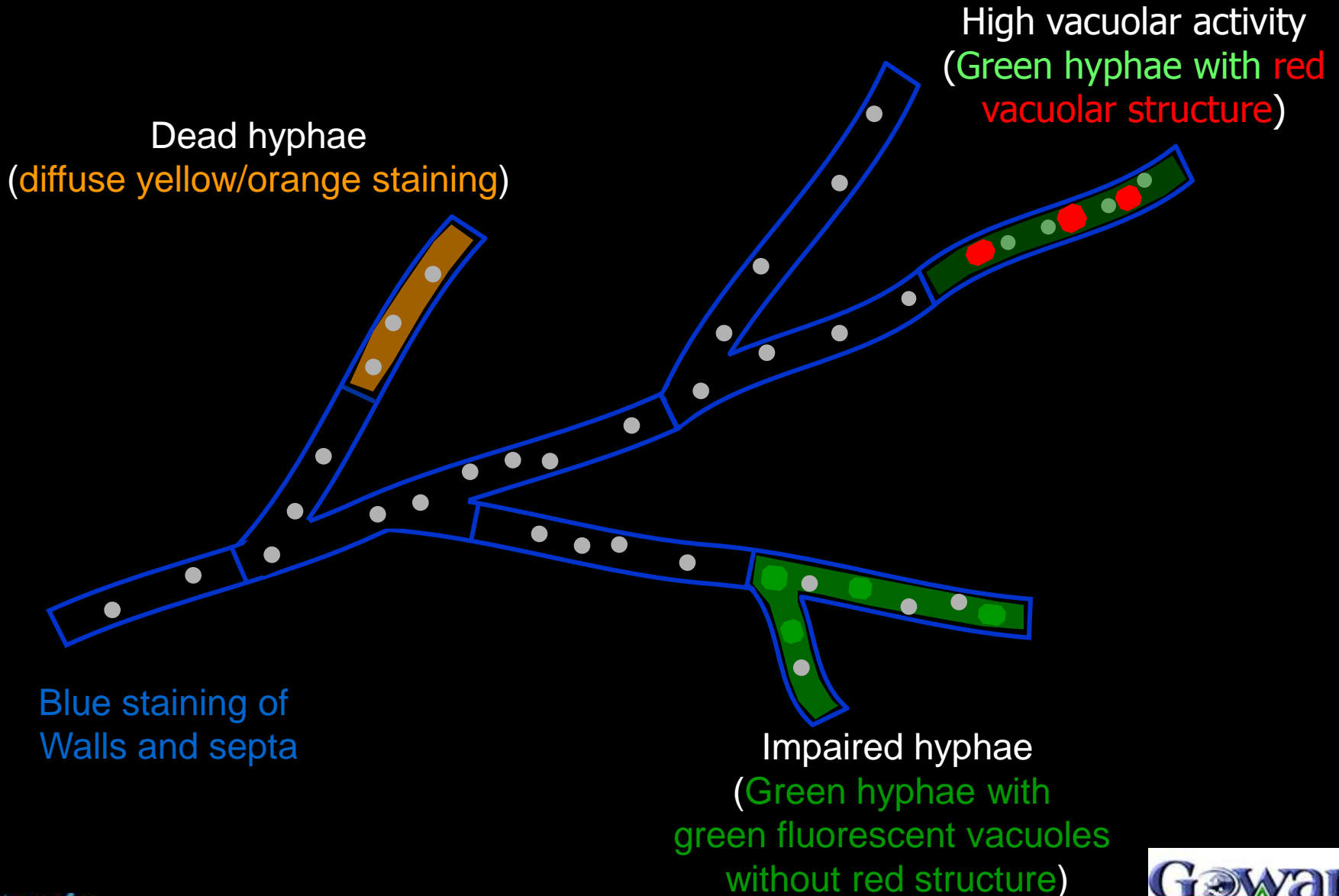


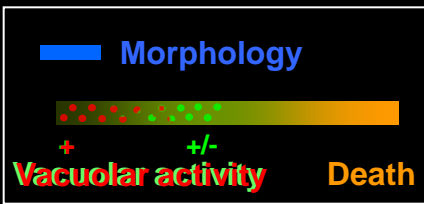
Mancozeb 0.1µg/ml



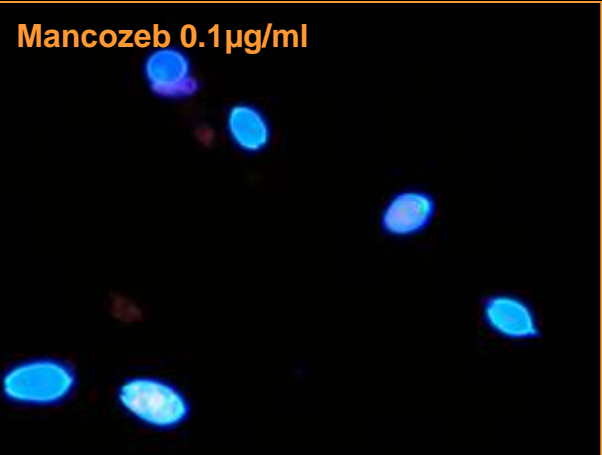
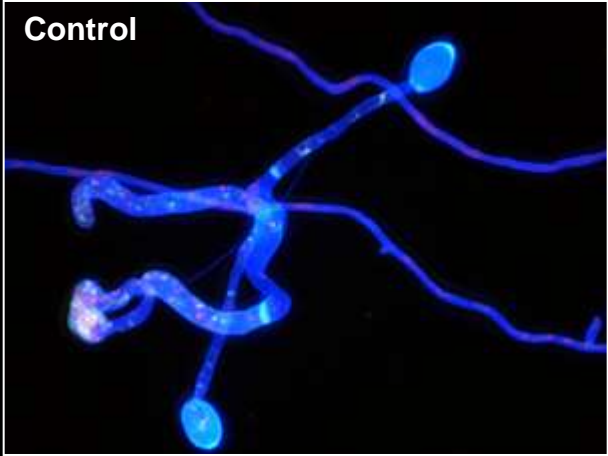
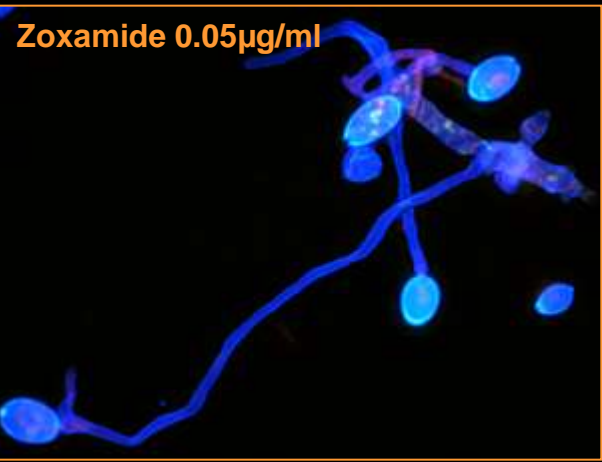
# Illustration of Fungicide Mode d'action :

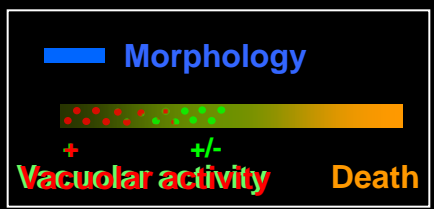
## Vacuolar activities / fungus walls



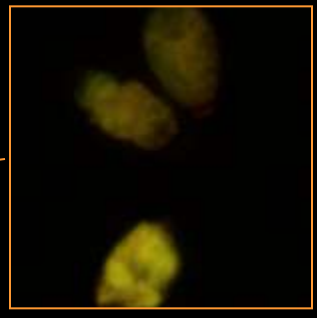
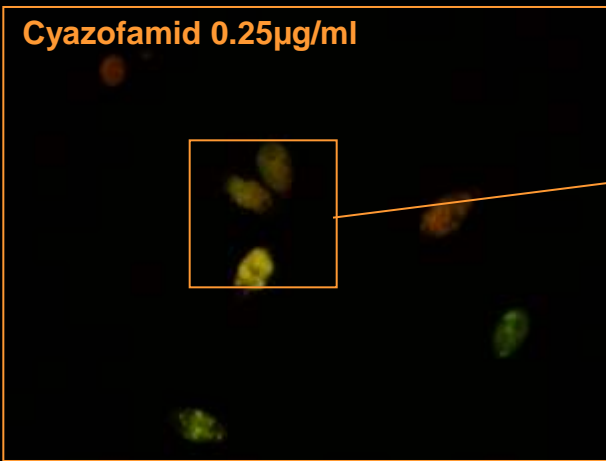
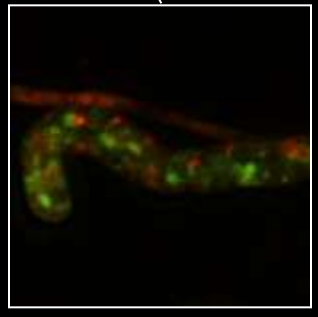
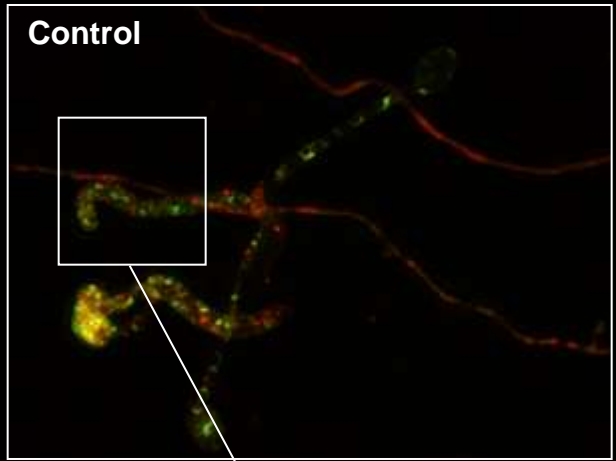
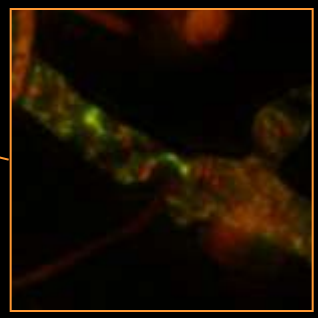
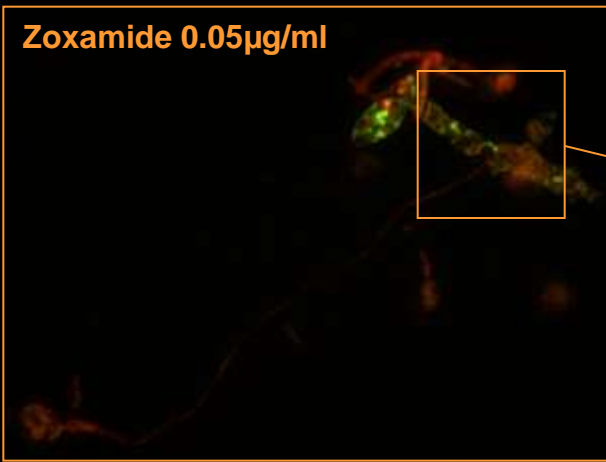


10µm



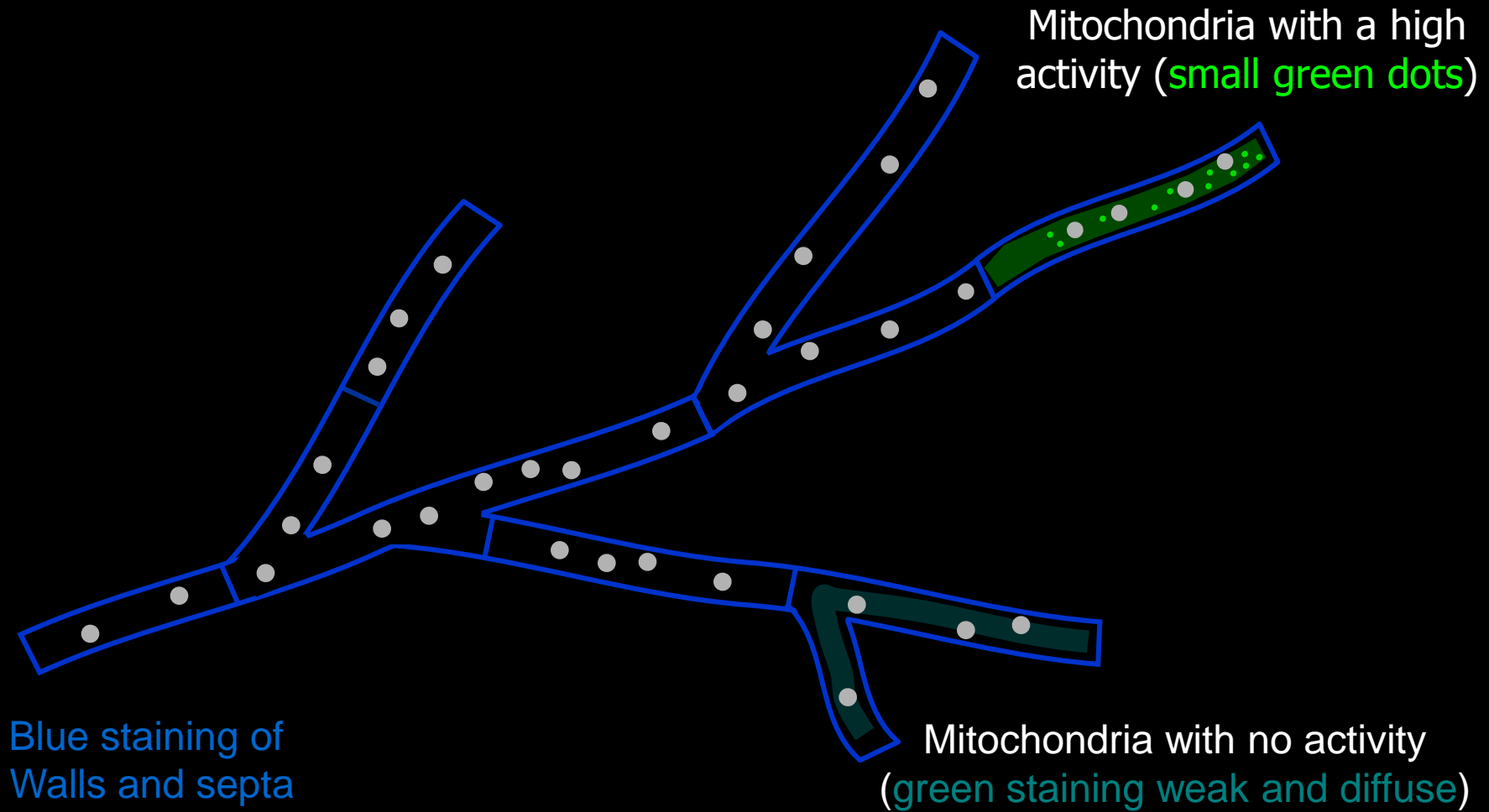


10µm




# Illustration of Fungicide Mode d'action :

## Mitochondria activities / fungus walls

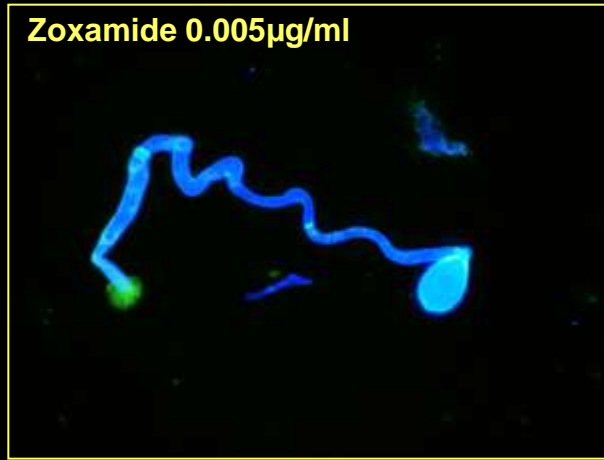


 Morphology

  
+ Mitochondrial activity -

10μm

Zoxamide 0.005μg/ml



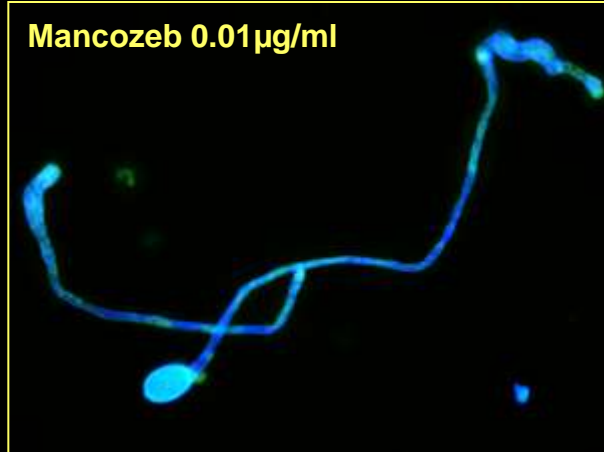
Control



Cyazofamid 0.01μg/ml

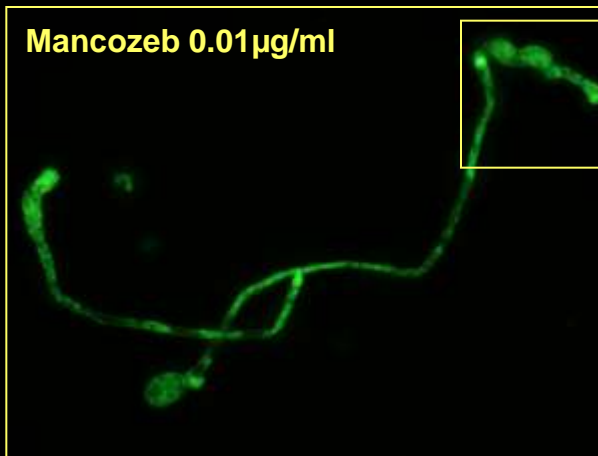
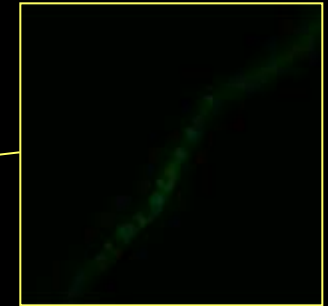
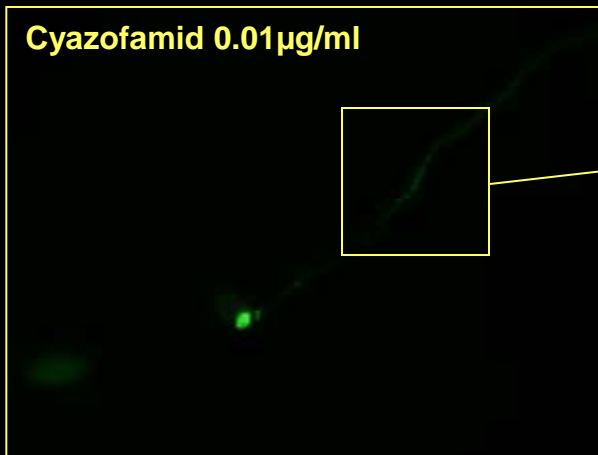
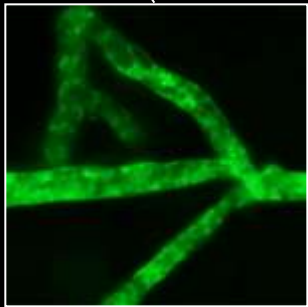
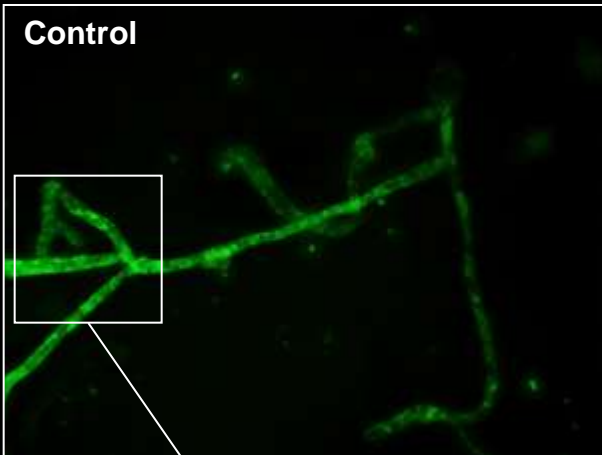
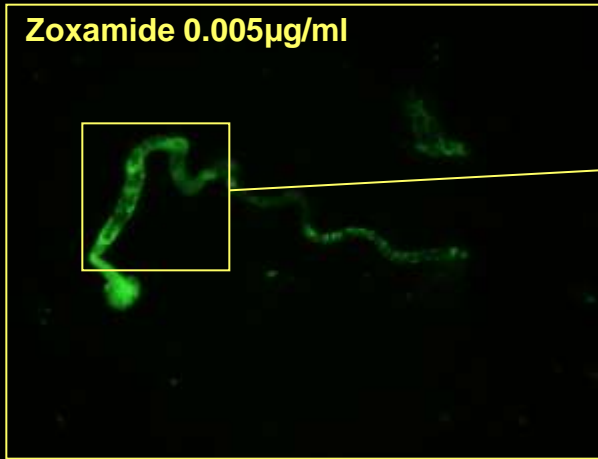


Mancozeb 0.01μg/ml





10µm





Fungicide	µg/ml	Morphology	Nuclear morphology	Vacuolar activity	Mitochondria activities	
Control	0					Healthy
Zoxamide	0.005					Ailing Unwell Distressed
Technical formulation	0.05					Ill Sick unhealthy
Cyazofamid	0.01					Critically ill or Dying
Ranman®	0.25					
Mancozeb	0.01					
Dithane neotec®	0.1					

## Conclusions and perspectives

- Each evaluated fungicide causes impairment of growth with reduced metabolic activity and cell death but presents a distinct pattern when stained with fluorochromes :
  - Zoxamide is characterized by an enlarged germ tube with abnormal nuclei.
  - Cyazofamid presents a strong reduction in vacuolar and mitochondrial activities.
  - Mancozeb shows strong effect in germ tube development with abnormal nuclei and strong reduction in vacuolar activity.