Risk assessment of biological nematicides

Sebastian Kiewnick, C. Roumbos and R.A. Sikora

University of Bonn
Institute for Plant Diseases
Phytopathology und Nematology
in Soil Ecosystems

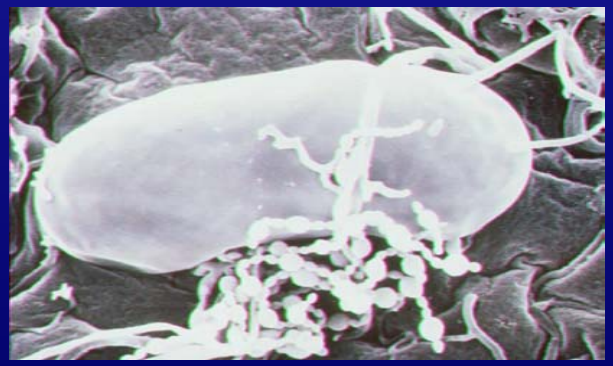
Biological products registered for Nematode Control

Name	Active ingredient	Target	Manufacturer
BIOACT WG *PL*plus	*Paecilomyces lilacinus*	*Radophulus similis* *Meloidogyne* spp. *Rotylenchulus reniformis* *Globodera spp*.	Prophyta BCP
DiTera	*Myrothecium verucaria*	*Meloidogyne* spp. *Pratylenchus* sp. *Radophulus similis* *Trichodorus* spp.	Valent
Deny	*Burkholderia cepacia*	*Helicotylenchus* spp. *Pratylenchus* sp.	CCT Corp.
Invade	*Serratia entomophila*	*Meloidogyne incognita*	AgResearch NZ

Paecilomyces lilacinus

Class: Deuteromycetes (Fungi imperfecti)
Order: Moniliales
Family: Moniliaceae
Genus: *Paecilomyces*
Species: *lilacinus* (Samson 1974)

- ellipsoid, 2,5-3,0 µm long and 2,0-2,2µm wide conidia, lilac tan



- facultative egg pathogen
- attacks also larvae and sedentary females

Paecilomyces lilacinus strain 251

Biological nematicide BIOACT ® WG:

- Produced in solid-state fermentation
- Conidia are separated from the substrate
- Fluidized-bed drying with glucose as carrier

Final product is a water dispersible granule containing at least **1 x 10^{10}** viable conidia/g

Objectives

Paecilomyces lilacinus (strain 251) as a model organism to monitor the fate of fungal biocontrol agents in the environment

Test systems

1. **Growthchamber/greenhouse:**
   - Tomato
   - *Meloidogyne incognita, M. hapla*

1. **Field experiment**
   - Sugar beet
   - *Heterodera schachtii*

Depending on the persistence in soil after application:

What kind of exposure can be expected?

Recovery of *P. lilacinus* from treated soil

Dilution plating on
OHIO-agar

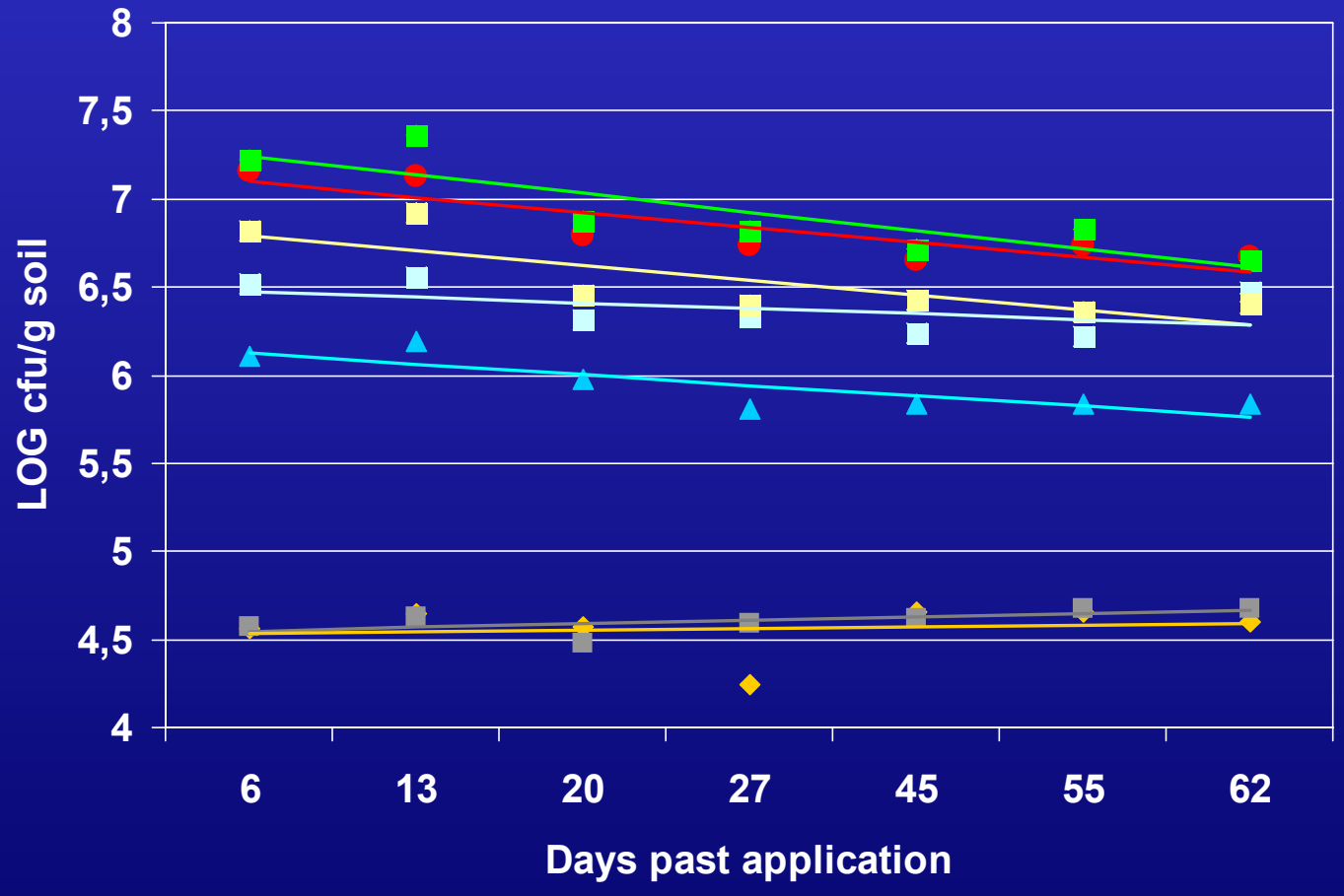
Persistence of *P. lilacinus*

Dose response experiment

Growth chamber, 25°C

Persistence of *P.lilacinus* at 25°C

Growth chamber experiment – Tomato-*M.incognita*



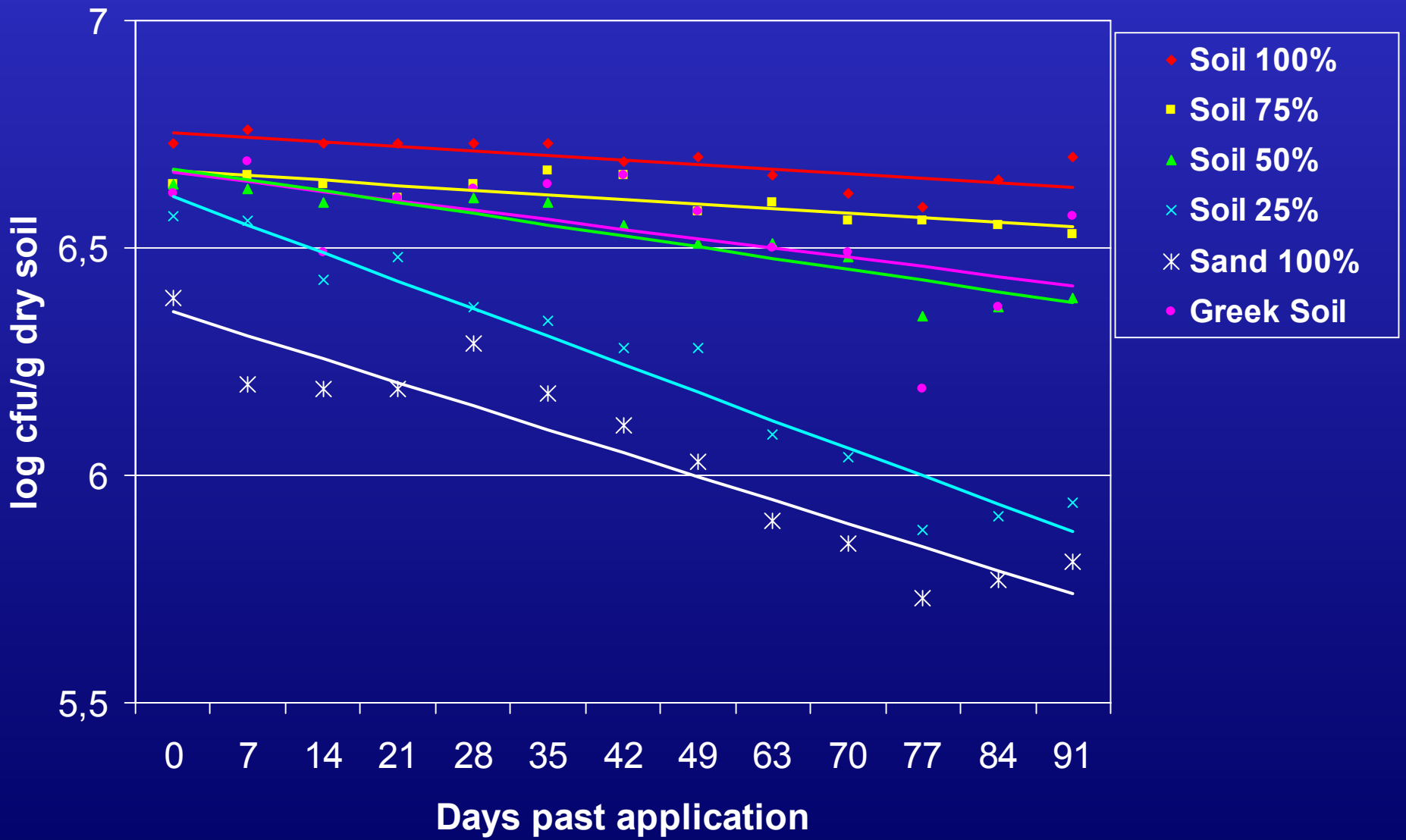
* other filamentous fungi

Persistence of *P. lilacinus*

Effect of substrate

Greenhouse

Persistence of *P. lilacinus* in different substrates
Greenhouse experiment



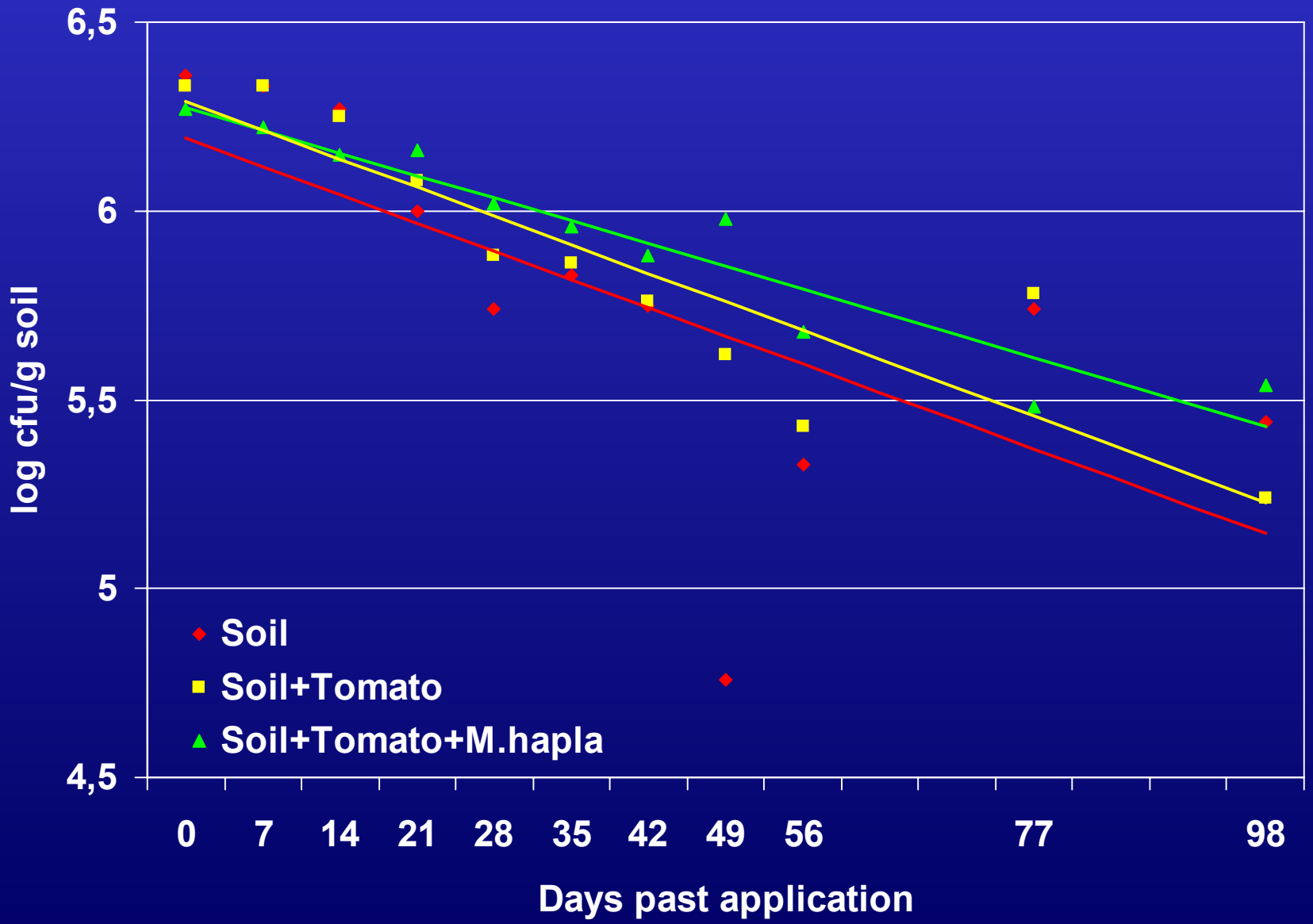

50µm

Persistence of *P. lilacinus*

Effect of host plant and target

Growth chamber 20°C

Persistence of *P.lilacinus*
Growth chamber experiment (20°C), Tomato-*M. hapla*

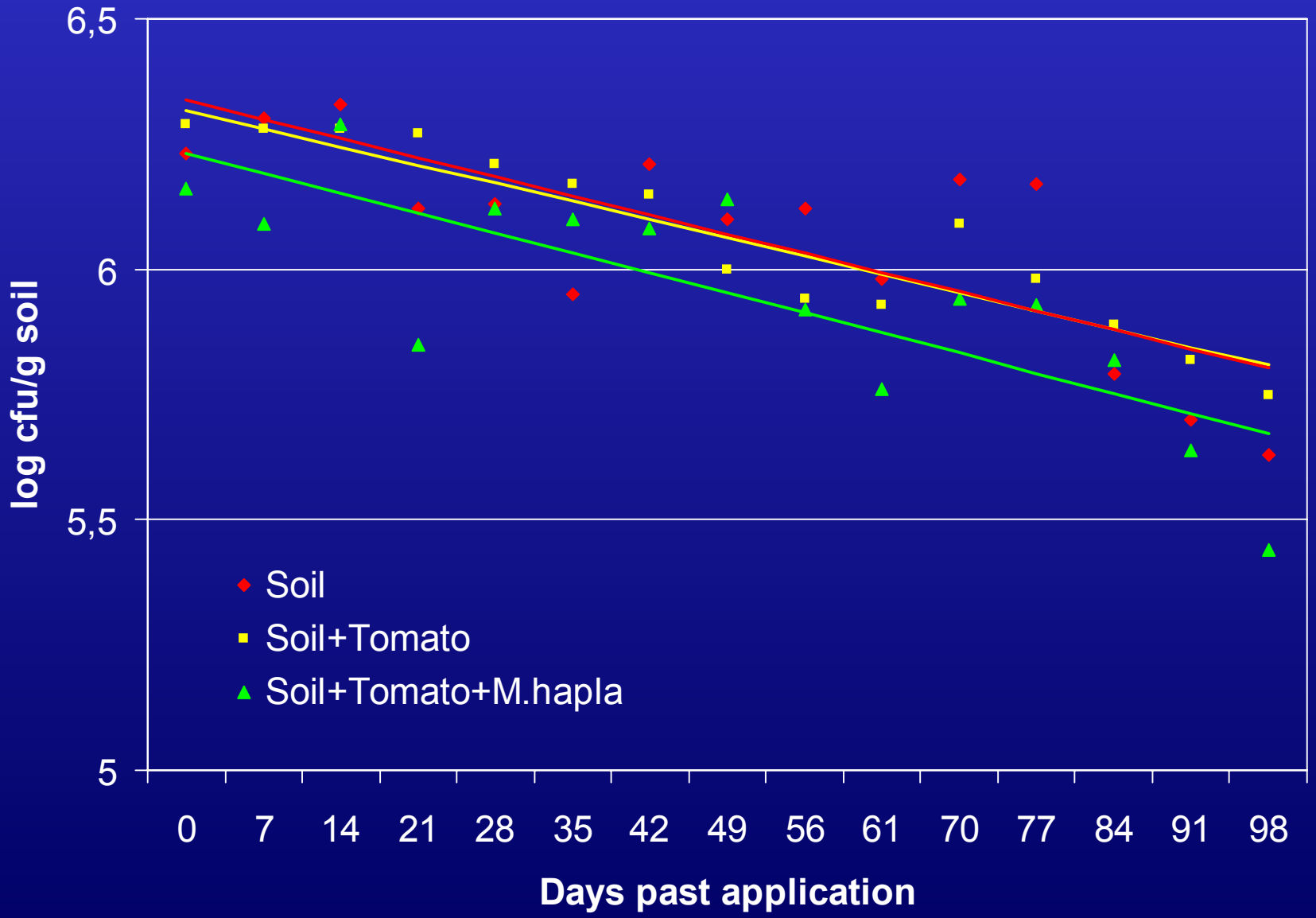


Persistence of *P. lilacinus*

Effect of host plant and target

Greenhouse

Persistence of *P.lilacinus* Greenhouse experiment, Tomato-*M. hapla*

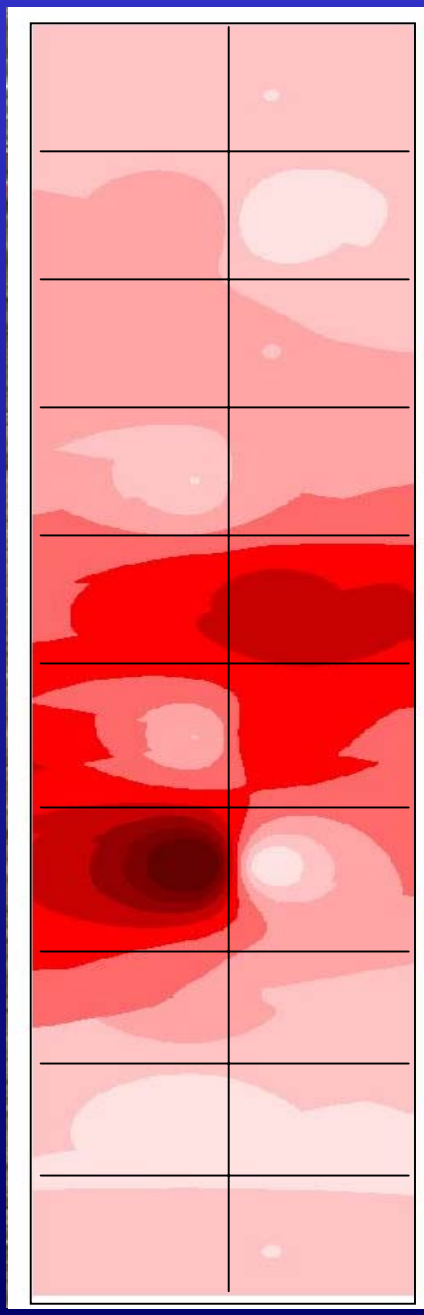


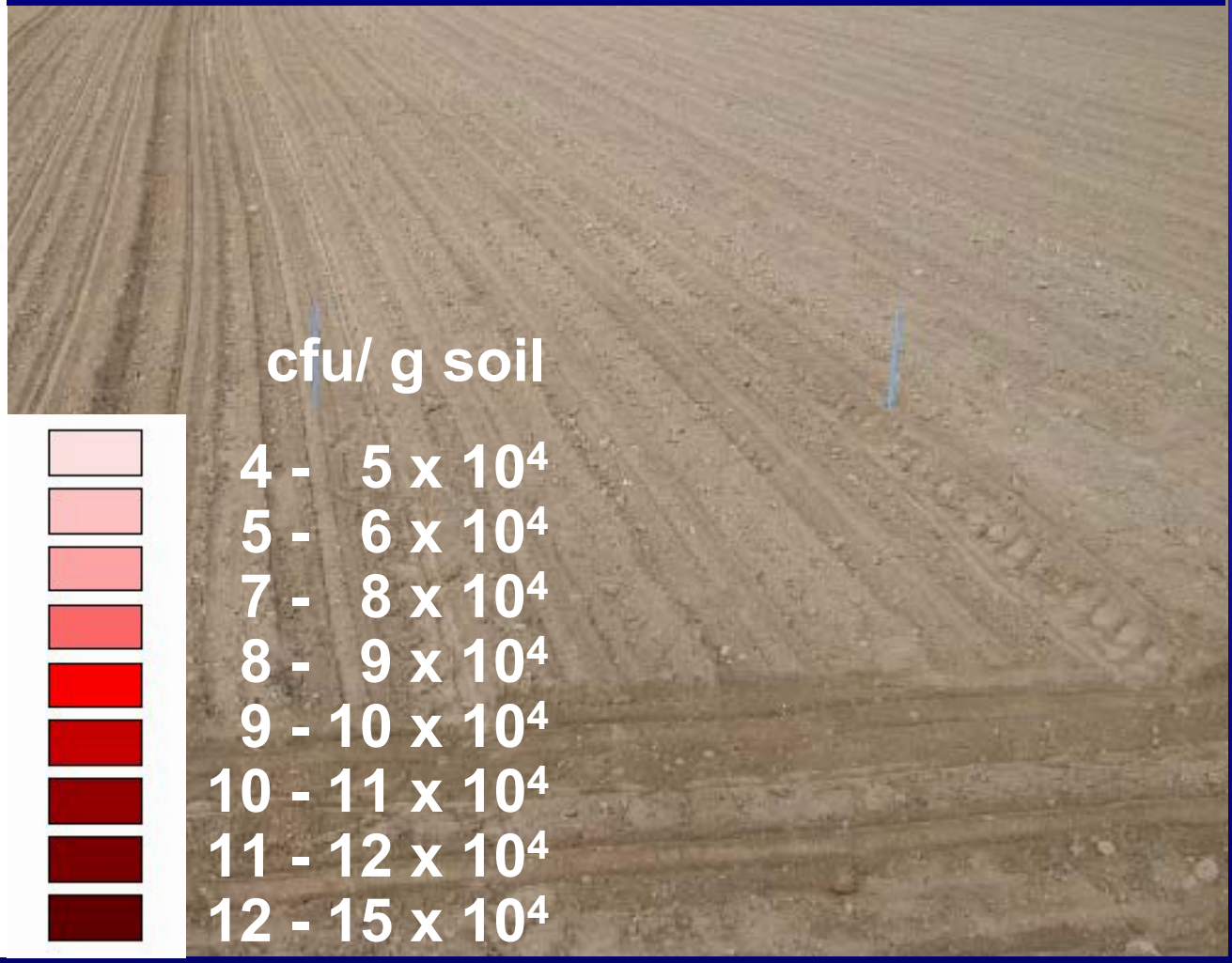
Persistence of *P. lilacinus*

Field experiment, Bonn 2003

Sugar beet - *Heterodera schachtii*

Spatial distribution of *P. lilacinus* (strain 251) in field soil after broadcast application





cfu/ g soil

4 - 5 x 10^4
5 - 6 x 10^4
7 - 8 x 10^4
8 - 9 x 10^4
9 - 10 x 10^4
10 - 11 x 10^4
11 - 12 x 10^4
12 - 15 x 10^4

Recovery of *P. lilacinus* (strain 251) in a sugar beet field after broadcast application

Application rate:	4×10^{13} conidia/ha
Incorporation:	~7-10 cm deep
Expected:	3×10^{5} cfu/g soil
Recovered:	$2{,}5 \times 10^{4}$ to $1{,}4 \times 10^{5}$

12 to 2-fold lower

Semi selective medium for better enumeration of *P.lilacinus* in soil samples Reduction of the detection limit

Semi selective agar

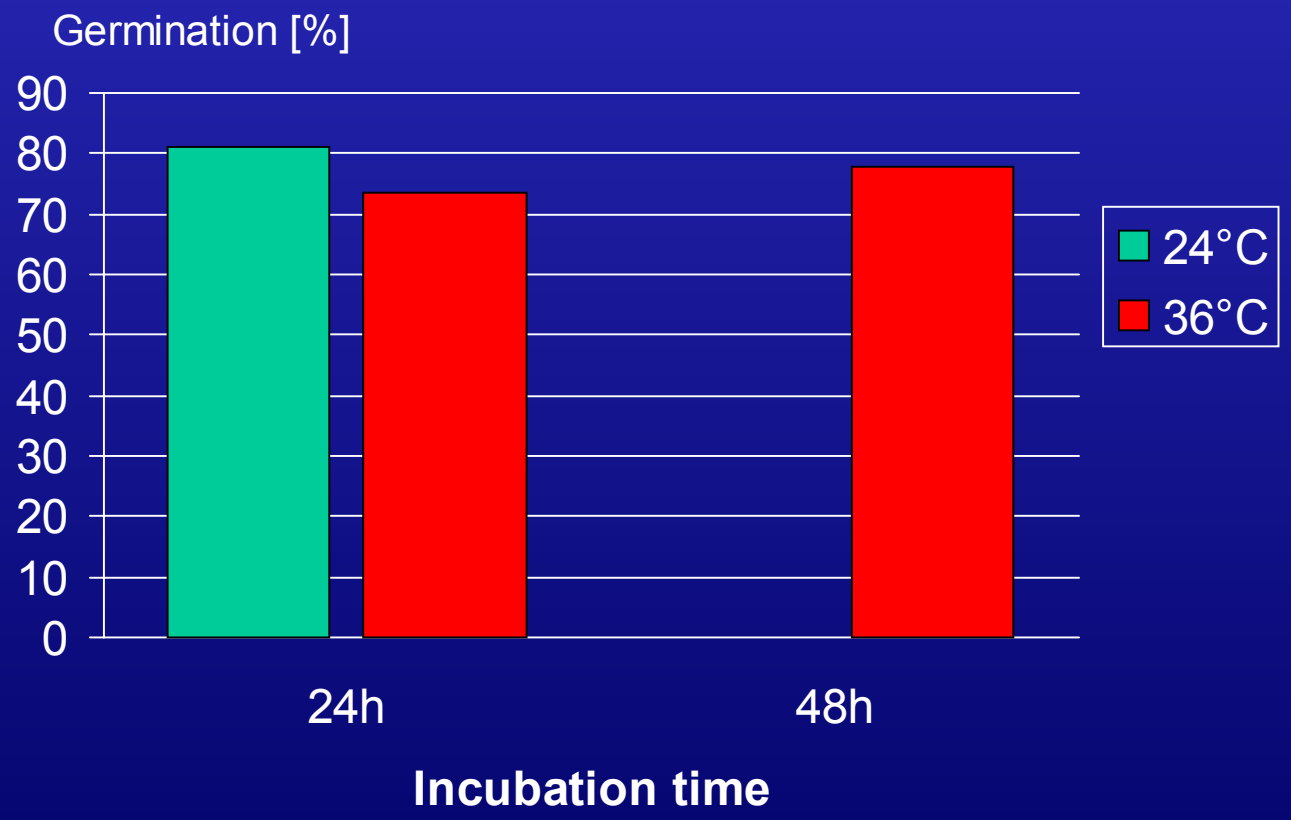
OHIO-agar

Microbial human health risk assessment of fungal biocontrol agents

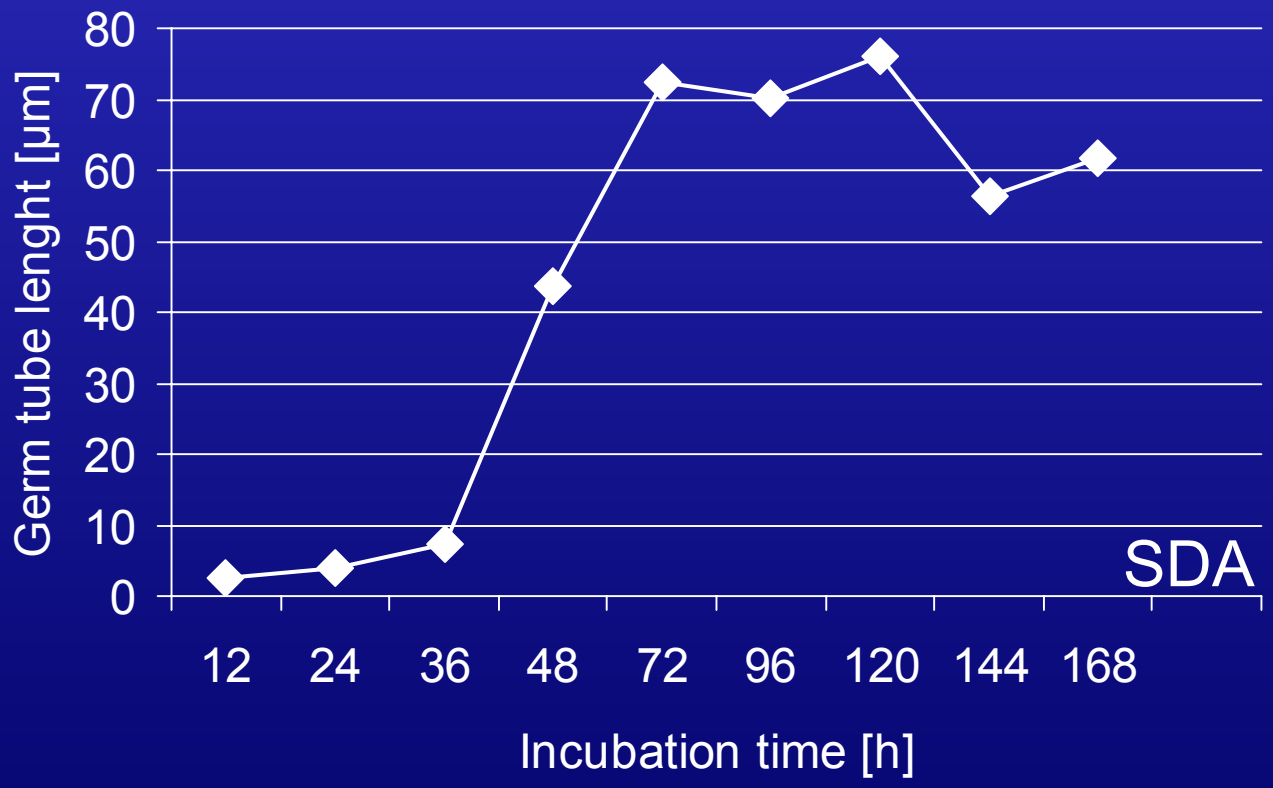
- Determination of the maximum growth temperature
- radial growth at temperatures from 24°C to 36°C
- *P.lilacinus* strain 251 showed no growth at 36°C

- since most products contain formulated conidia or blastospores as active ingredient, these test should be performed with the formulated BCA

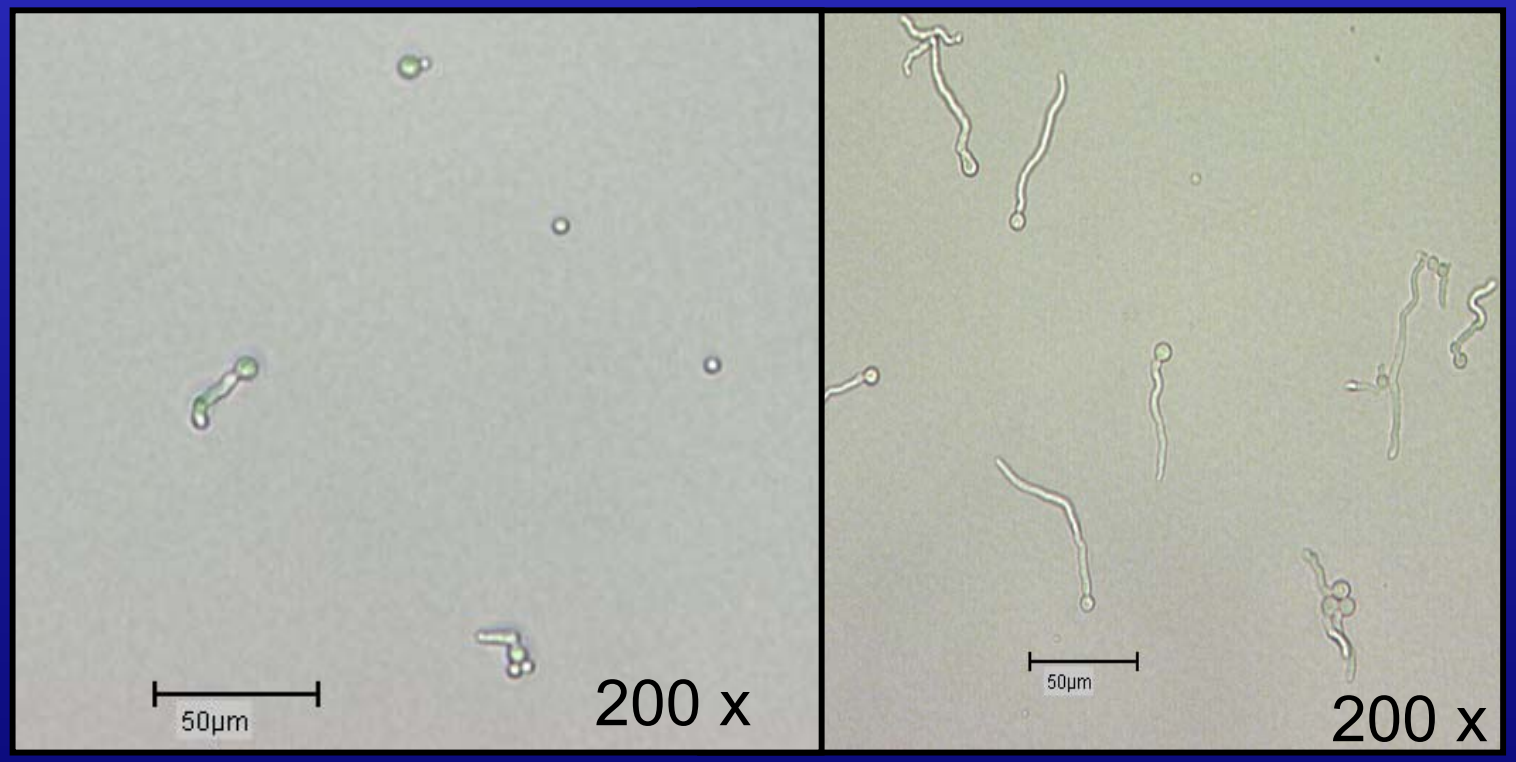
Germination of *P. lilacinus* conidia at 24°C and 36°C - formulated conidia -



Effect of incubation temperature on germ tube development of *P. lilacinus* (251) at 36°C - formulated conidia -



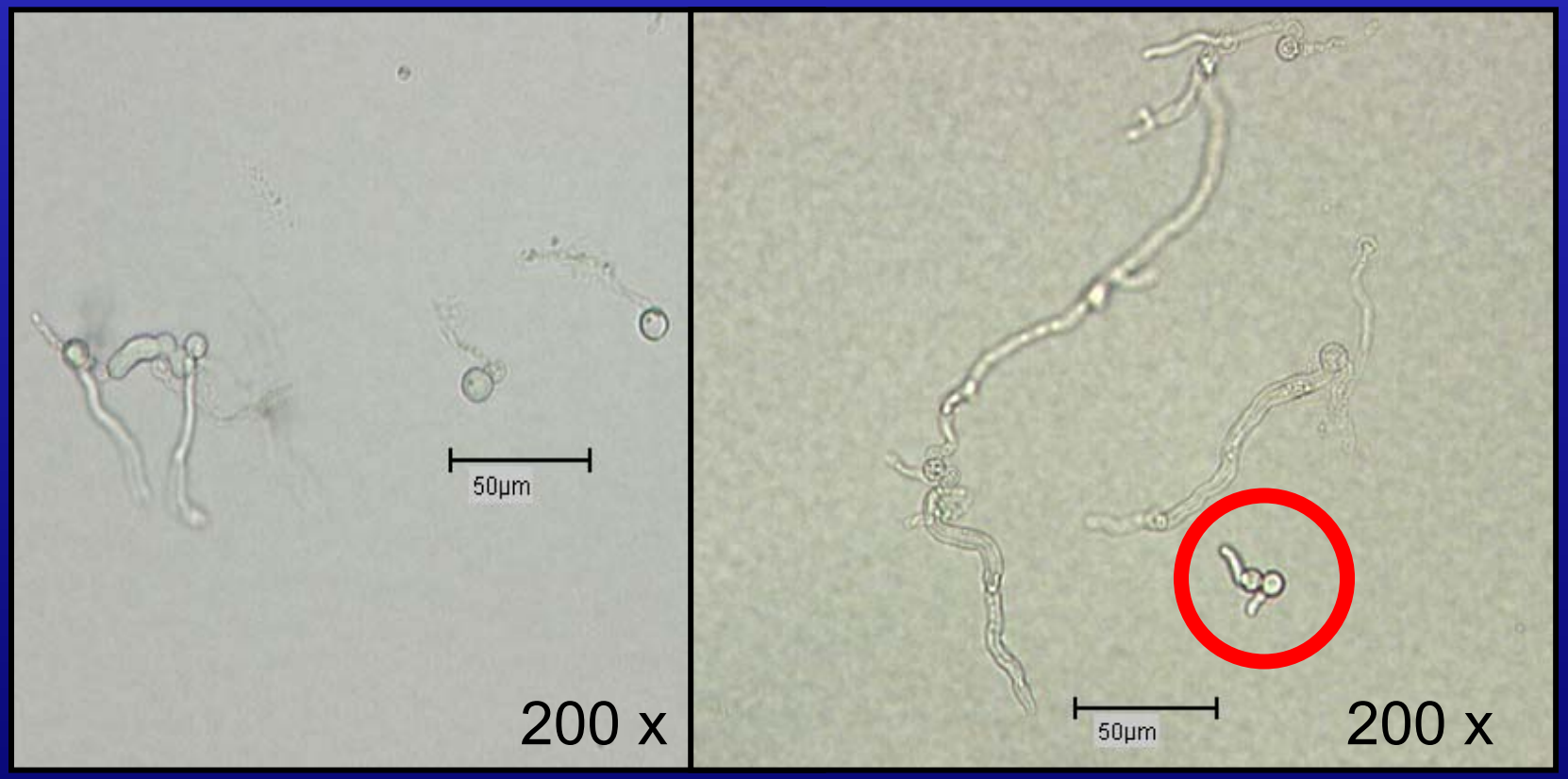
Germination and growth of *P. lilacinus* (251) at 36°C



24 hours (SDA)

48 hours (SDA)

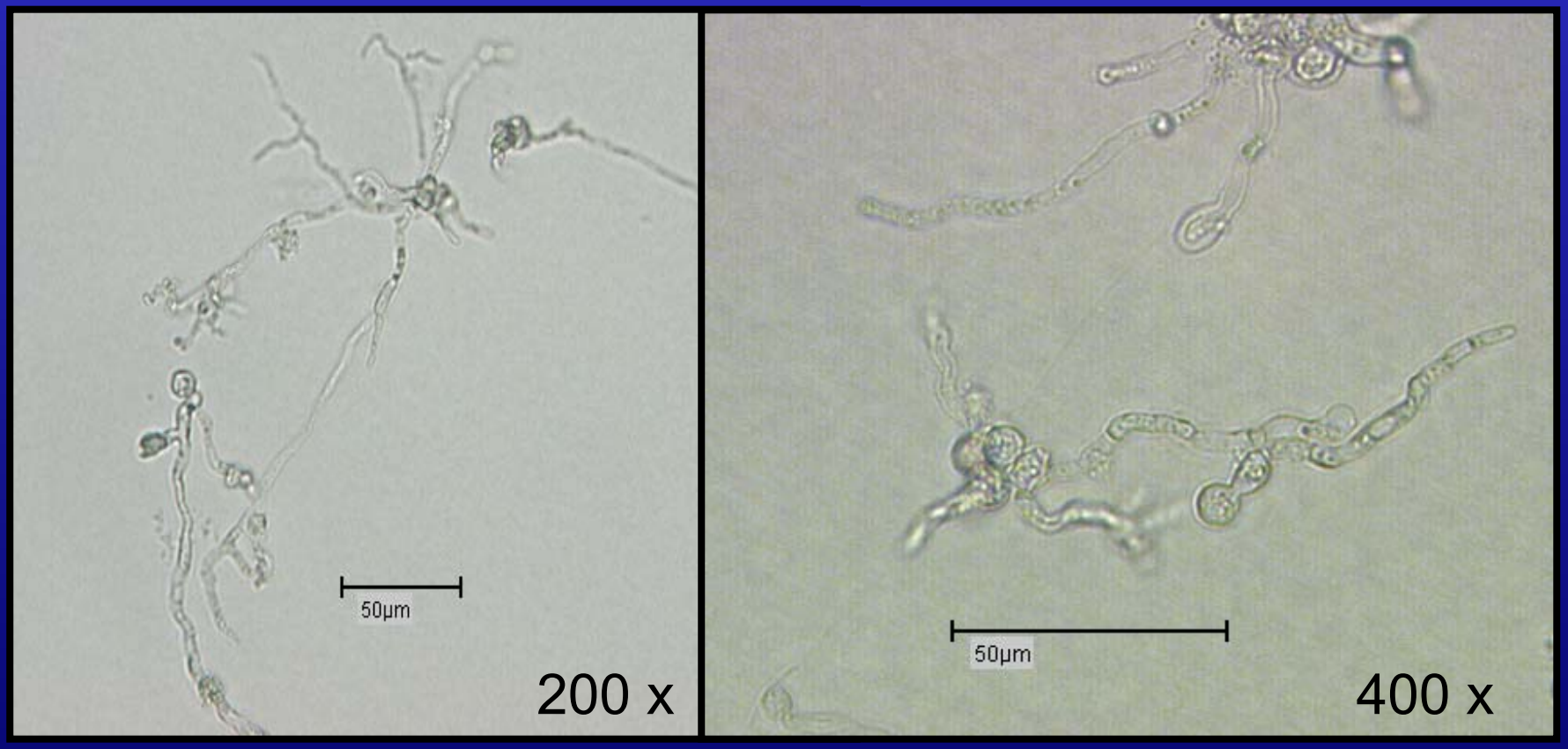
Germination and growth of *P. lilacinus* (251) at 36°C



72 hours (SDA)

96 hours (SDA)

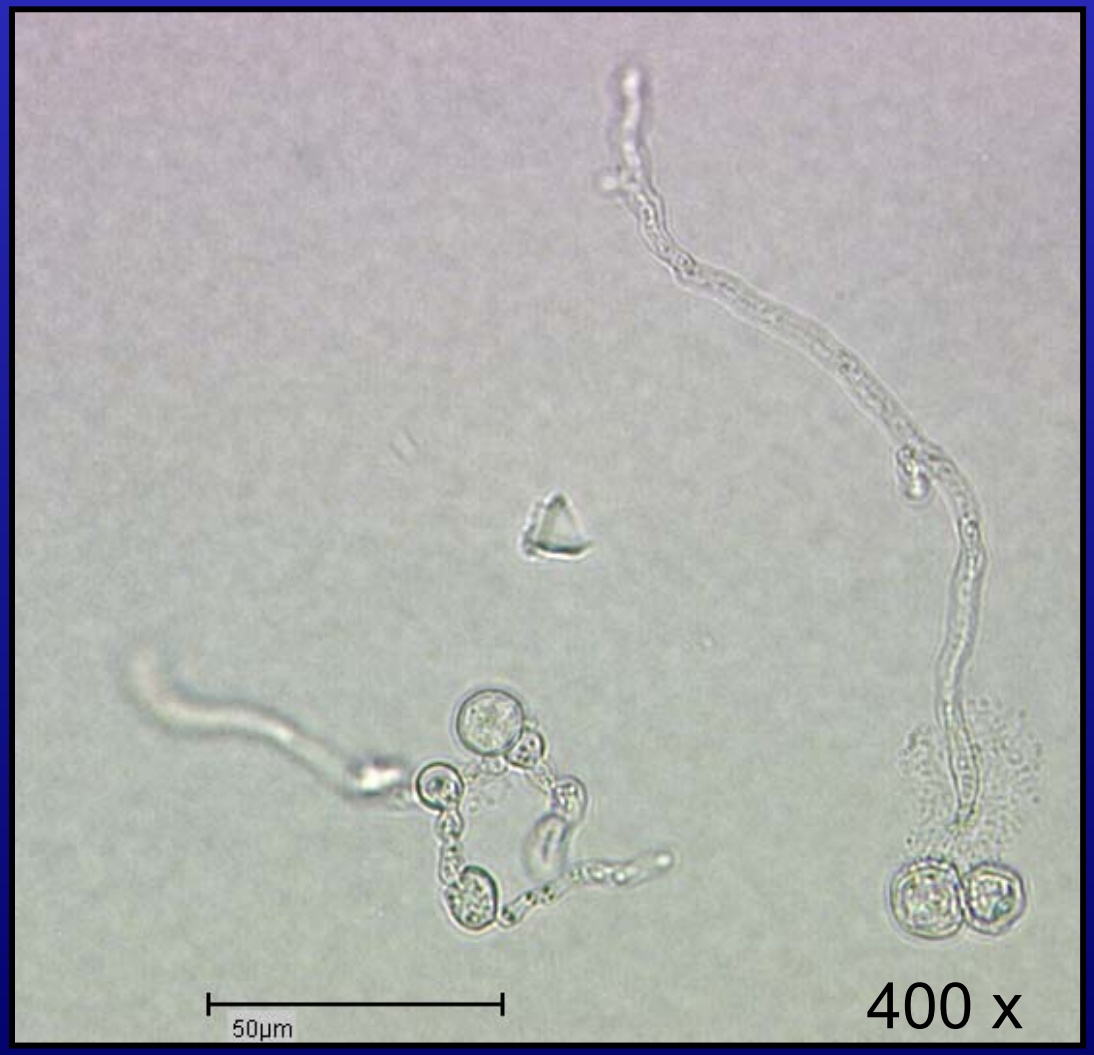
Germination and growth of *P. lilacinus* (251) at 36°C



120 hours (SDA)

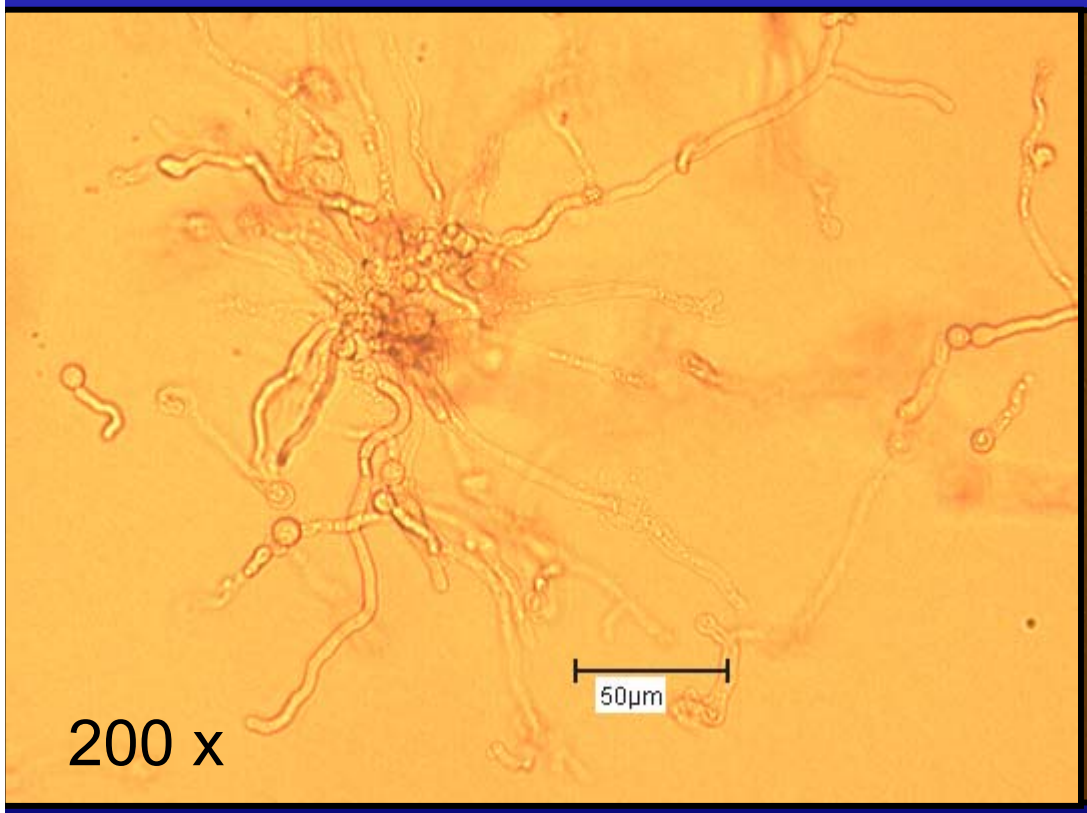
144 hours (SDA)

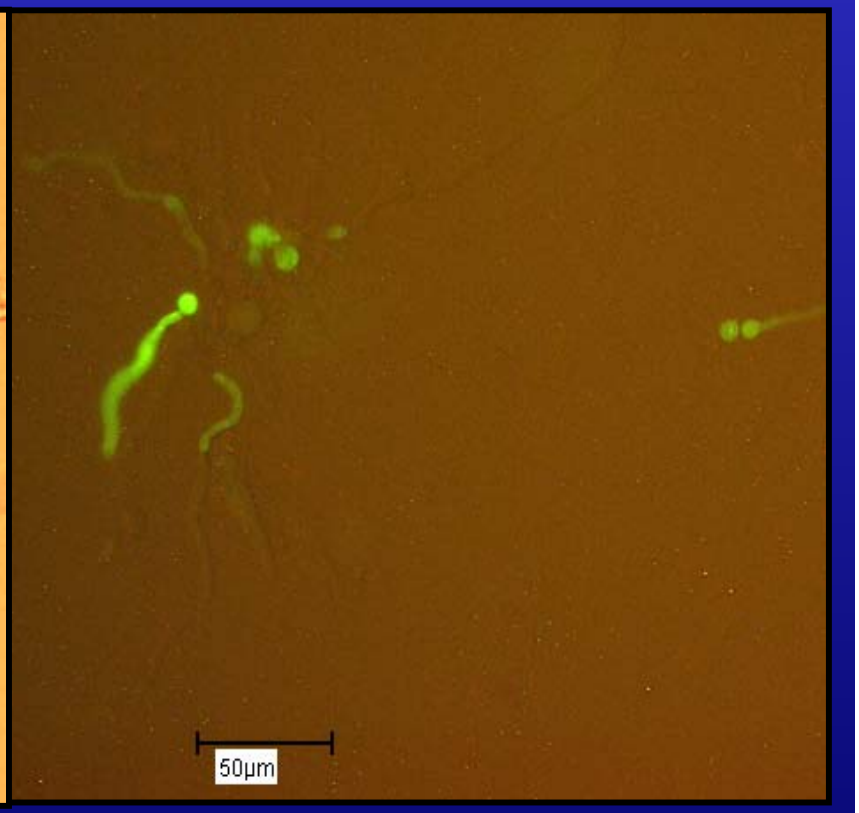
Germination and growth of *P. lilacinus* (251) at 36°C



168 hours SDA

Germination and growth of *P. lilacinus* (251) at 36°C

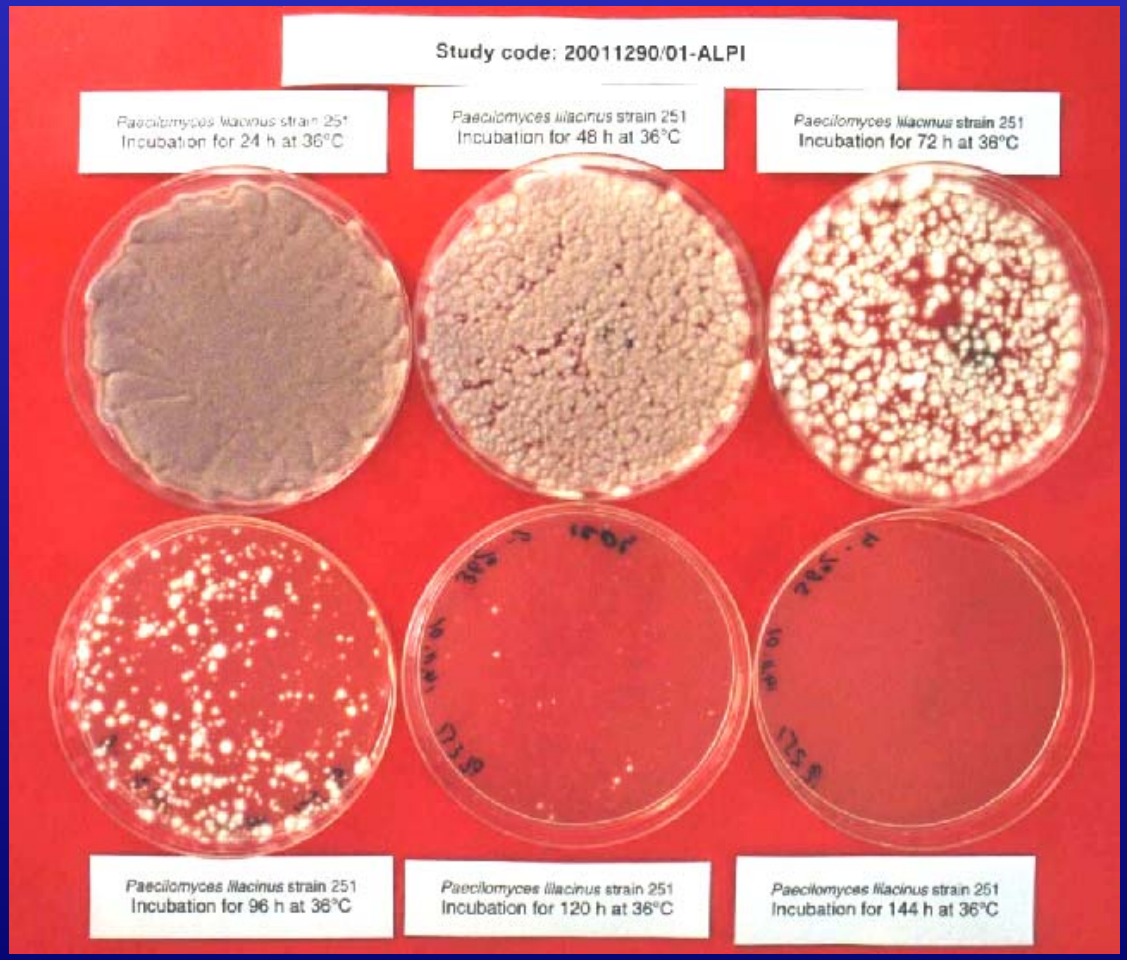




36°C **72** hours SDA + FDA

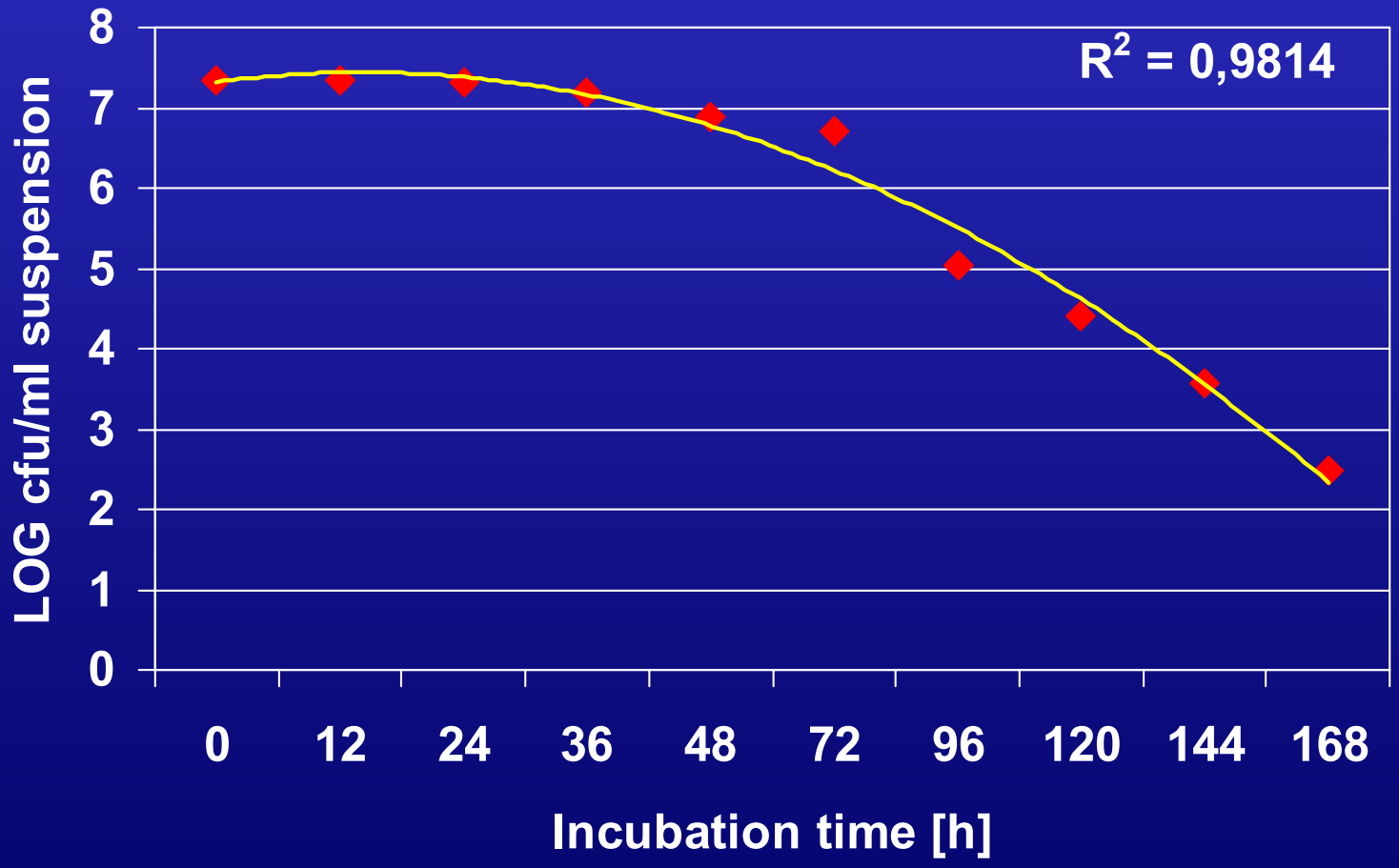
Effect of exposure to 36°C on survival of *P. lilacinus* conidia

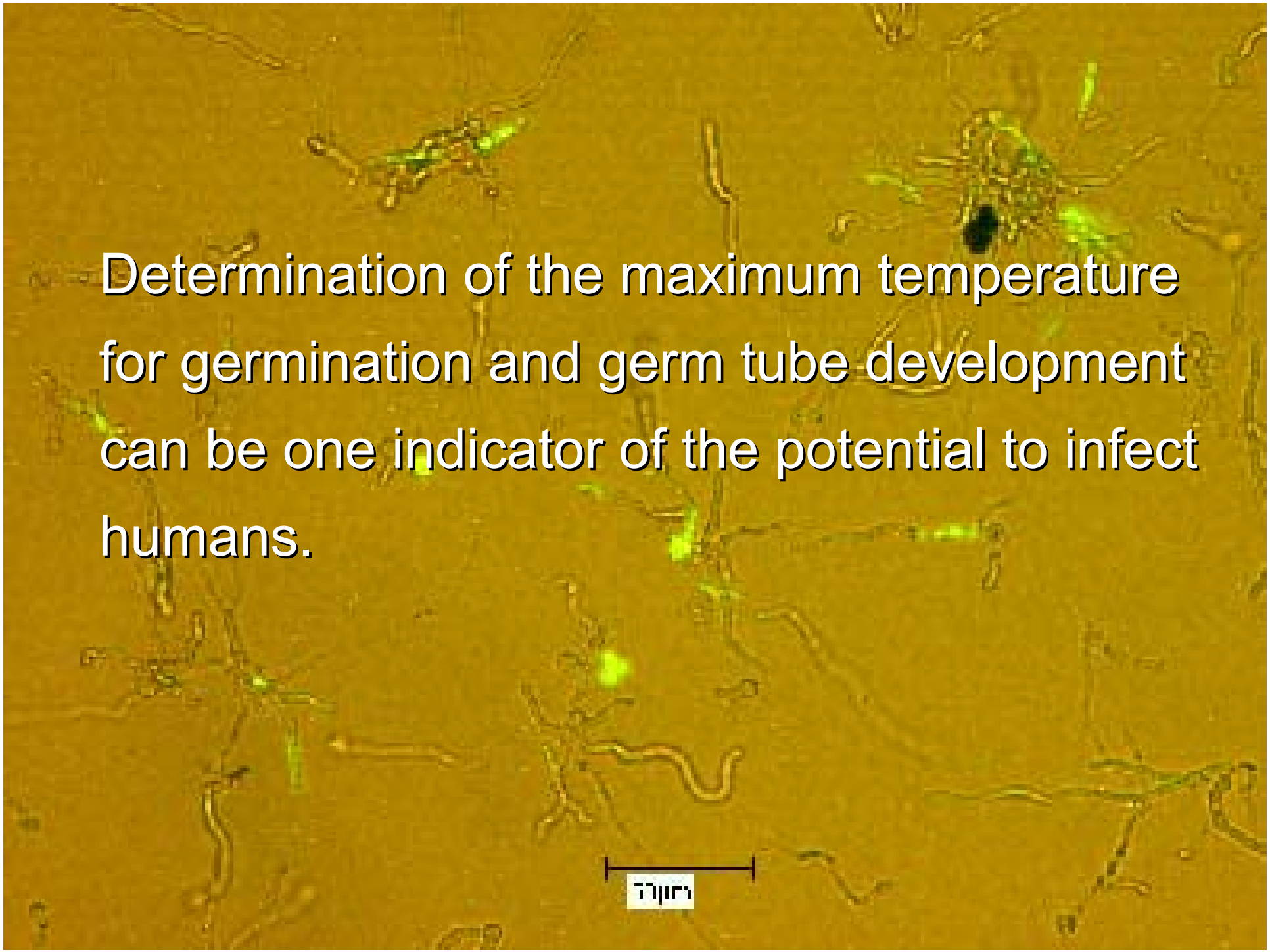
Effect of exposure to 36°C on survival of *P. lilacinus* (251) [2 x 10^4 conidia/plate]



No growth detected after 144 hours exposure

Effect of exposure to 36°C on survival of *P. lilacinus* (251) [up to 2 x 10^6 conidia/plate]




Determination of the maximum temperature for germination and germ tube development can be one indicator of the potential to infect humans.

Summary

Persistence of *P. lilacinus* seems to depend on conditions for saprophytic growth

Presence of the host plant and target (root knot nematodes) had no effect on persistence

Experiments are underway to evaluate the long term persistence (greenhouse and field trials)

Conclusions

Based on initial data, the persistence of *P. lilacinus* seems to be relatively low and predictable

A low exposure is expected

=> low risk

Outlook

Appropriate models to predict the fate of *P.lilacinus* need to be developed

Methods to assess worker and bystander exposure need to be developed

Based on this information, a better risk assessment for biopesticides containing conidia/blastospores would be possible

Worker exposure

Spray drift

Spread by cultivation

Bystander exposure

Thank you!