

# Nematicidal properties of fruit cysteine proteinases

Rosane Curtis

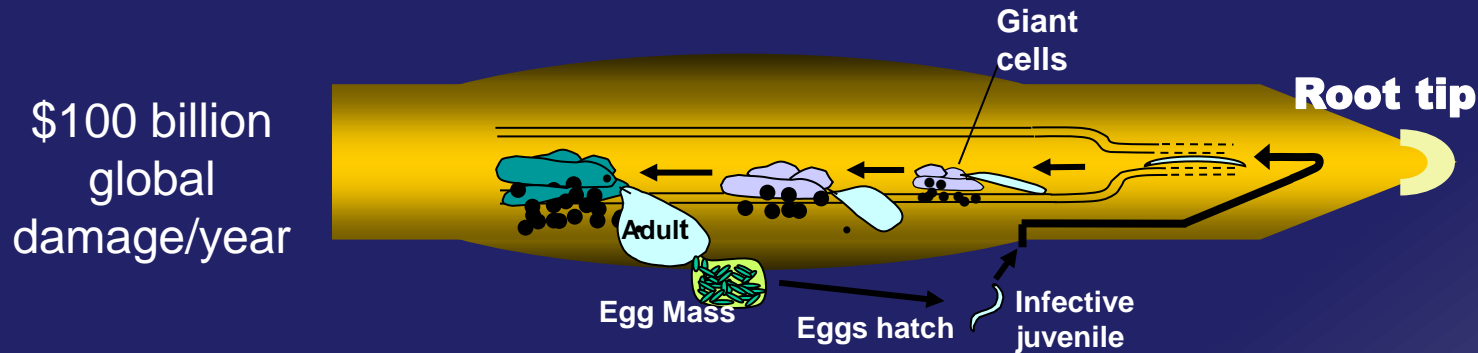


**Rothamsted-Research**





# Life cycle of root-knot nematode (~30 days)



Most resistant strategies have been directed at disrupting feeding.

Preventing the nematode entering the root (disrupting host recognition) would protect the plant from the damage associated with invasion as well as feeding.

**Rhizosphere can be manipulated to disorientate nematodes**



# **Nematicidal properties of natural products - Animal Nematodes -**

- **Plants and fruits have been used for centuries against gastro intestinal nematode infections of humans and livestock in the tropics.**
- **In the Amazon basin, crude extracts of plant latex delivered for 3 consecutive days (1ml/kg) matched the efficacy of synthetic antihelmintics.**



# **Nematicidal properties of natural products Plant Nematodes**

- **Soil amendments with natural products (leaves, stems, fruits, seeds, oil extracts, etc..) have been incorporated into infested soil and shown to reduce the rate of nematode infection of plants.**
- **The mode of action of most of these nematicidal extracts/products is unknown and the rates of application of most plant materials are too high to be used in practice.**
- **Organic amendments increase microbial activity in the soil, therefore accumulation of by products of decomposition of added organic matter and microbial metabolites might also play a role in the reduction of nematode infection of plants.**



# Sources of fruit cysteine proteinases



*Ananas comosus* - Bromelain



*Carica papaya* - papain



*Actinidia chinensis*

Actinidin



*Ficus glabrata* - Ficin



# Fruit cysteine proteinases

- Occur in the latex, in juices from the fruit and in the stem of the source plants. Their structures and catalytic mechanisms are identical. They can differ in their substrate specificity.
- Crude preparations of papain, bromelain and actinidin are used primarily in the food industry as meat tenderizer.
- These enzymes are licensed as drugs and the main medical application is a digestive aid as it has a soothing effect on the stomach protein digestion.
- Other uses: to treat edemas, inflammatory processes and in the acceleration of wound healing. In addition, bromelain has fibrinolytic and antithrombotic activities.
- In immunology: Preparations of Fab antibody fragments.
- Ingredient of toothpastes, cosmetics etc..





# Nematicidal properties of fruit cysteine proteinases

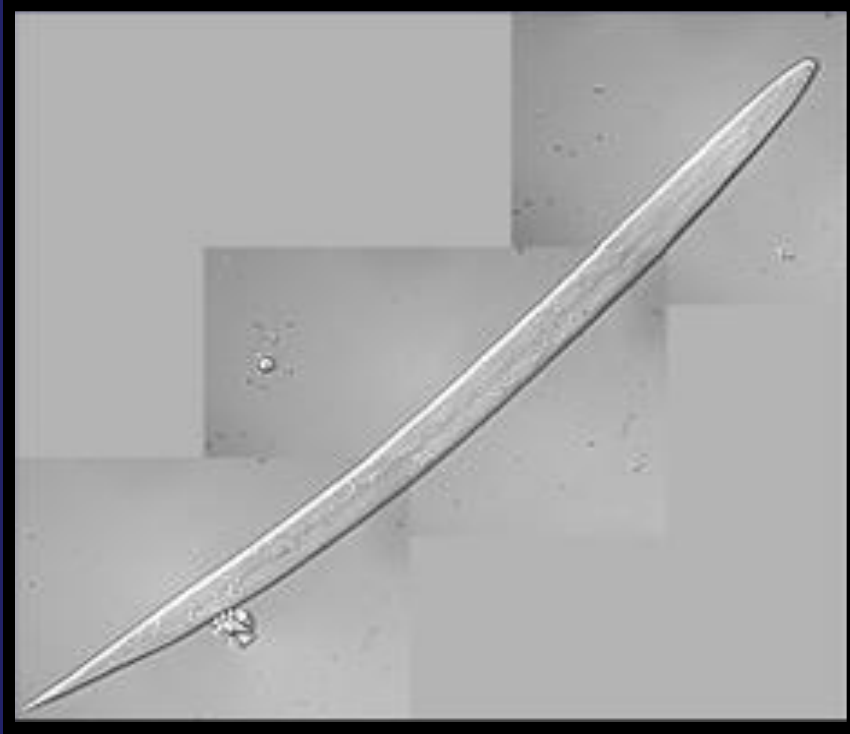


## Objectives

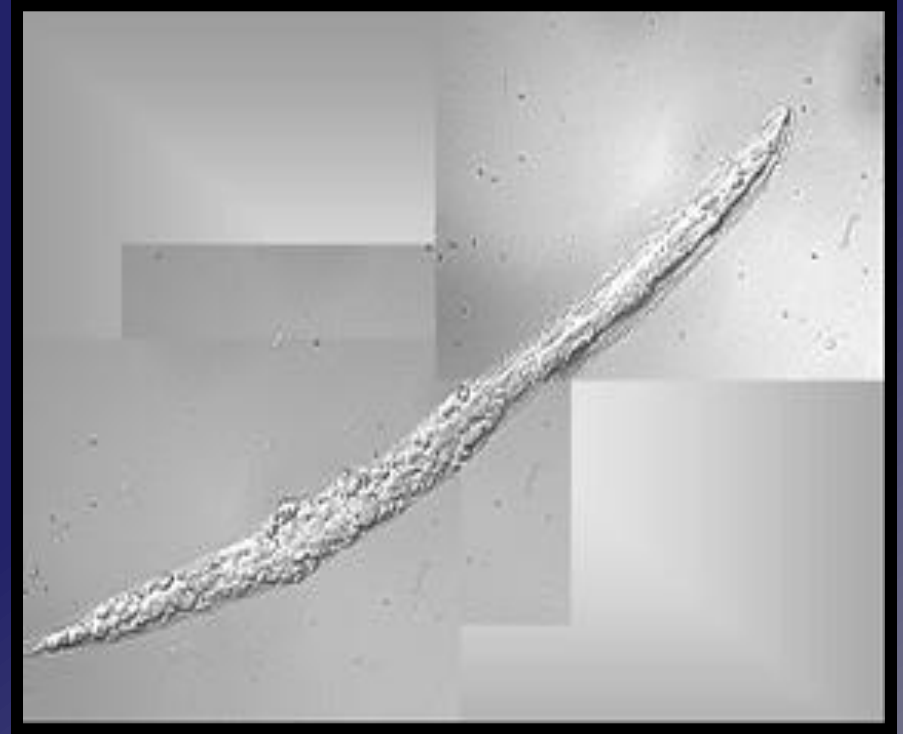
- To test the effect of fruit cysteine proteinases on sedentary and migratory plant parasitic nematodes and also beneficial nematodes.
- To determine whether soil application of these enzymes to infested soils could affect nematode infection of plants.
- Can soil application of these enzymes render nematodes more vulnerable to harmless concentrations of nematicides?



# Exposure to plant cysteine proteinases damages the cuticle of plant parasitic nematodes J2

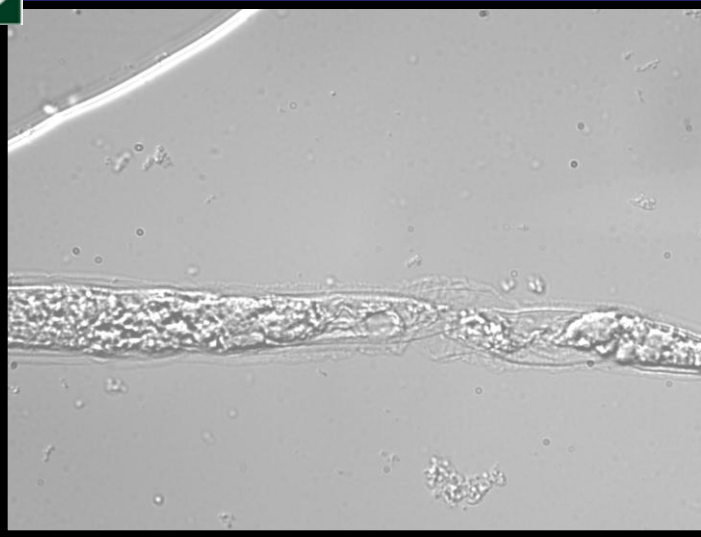


*M. incognita* J2 (control)

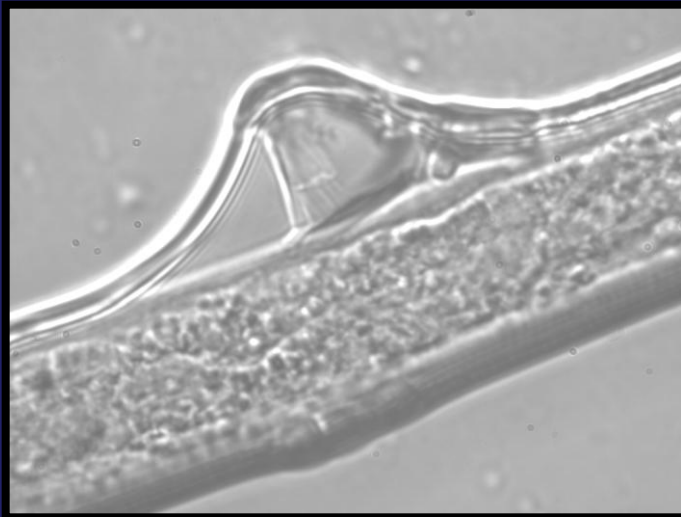


*M. incognita* J2 ( papain latex)

Enzymes tested: crude papaya latex, papain, stem bromelain and kiwi fruit extracts  
Specific inhibitor E-64 blocks the effect of CP



*M. incognita* – papain latex (60 min)



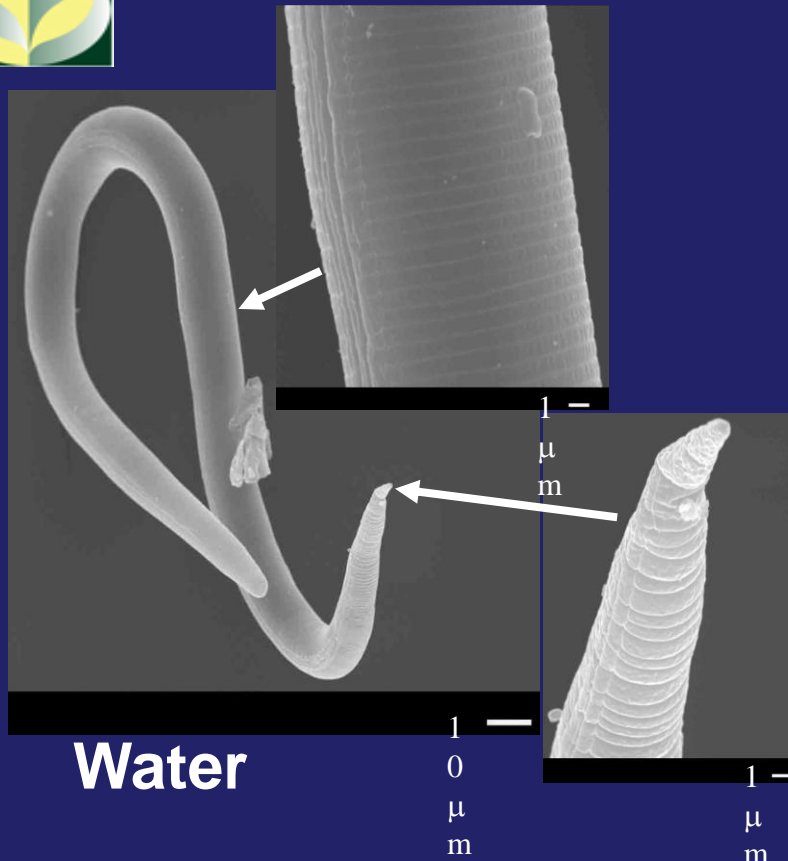
*M. incognita* + stem bromelain

**Fruit cysteine  
proteinases cause  
nematode cuticle  
to blister and  
rupture**

Parasitology, 2007



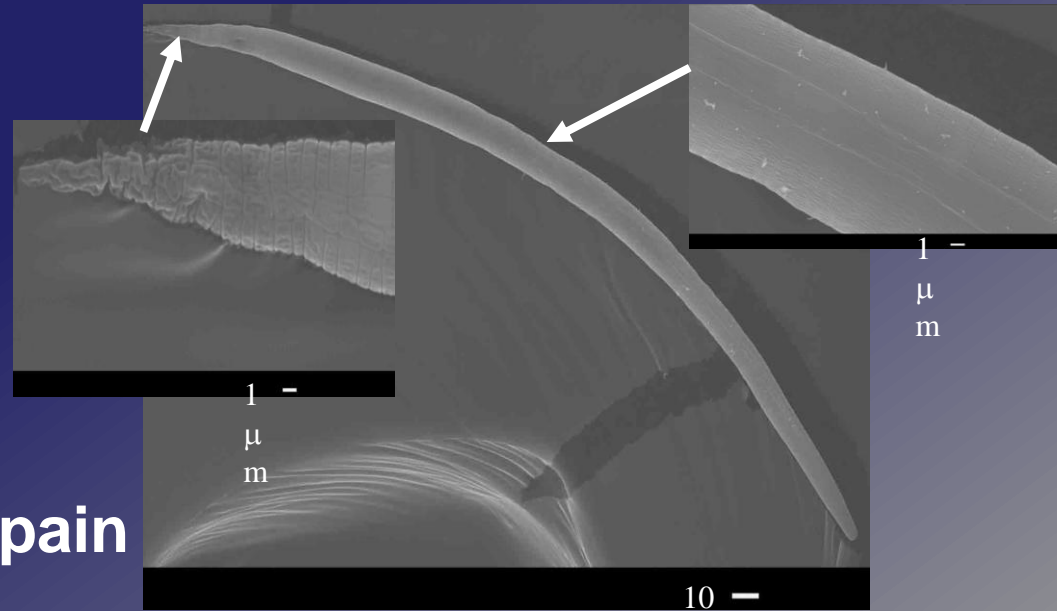
# Scanning electron micrographs of *Meloidogyne* spp. J2s exposed to papain 4 h



Water

100  
μm

1  
μm



Papain

1  
μm

1  
μm

10  
μm



# Differences in the cuticular profile of nematodes

- GI nematodes (adult stages) are affected by Fruit CP, with exception of actinidin.
- Actinidin damage the cuticle of plant parasitic nematodes but causes no damage to the cuticle of animal nematodes.
- *C. elegans* and other free-living nematodes present in soil are not affected by fruit cysteine proteinases.
- Beneficial nematodes were affected differently by fruit cysteine proteinases: Papain latex has no effect on the mobility of *Heterorhabditis megidis* but affected slightly movement of *Steinernema feltiae*. All concentrations of actinidin were nematocidal towards *S. feltiae* but only higher concentrations affected *H. megidis*.



# *In planta* bioassays

## Infested soil:

- soil mixture: 16% OM
- 1000 J2 of *M. incognita*

## Soil treatments:

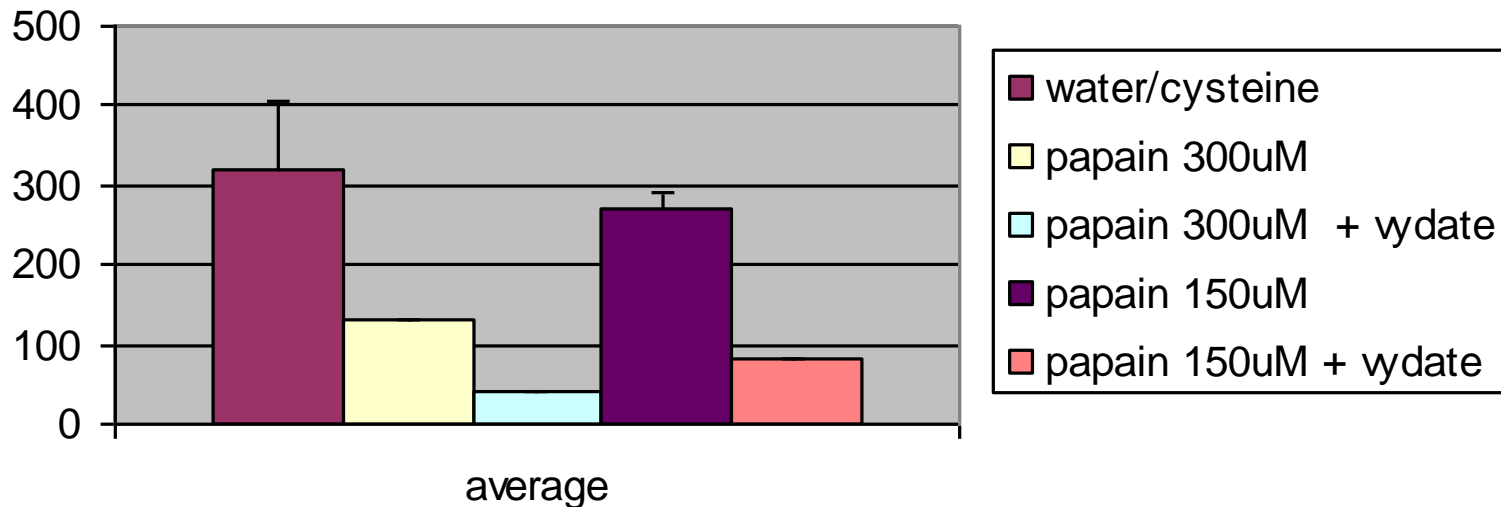
- one or two applications of fruit CP for 48 hours prior to planting tomato seedlings
- one application of a mixture of CP and a nematicide at a concentration which give partial effect.
- Nematode infection of tomato plants was analysed 11dpi.





# *In planta* bioassays

## One soil treatment with papain and papain/ vydate



Reduction of  
nematode  
infection

=60%

=87%

=14%

=74%

**Oxamyl (10% liquid Vydate)**

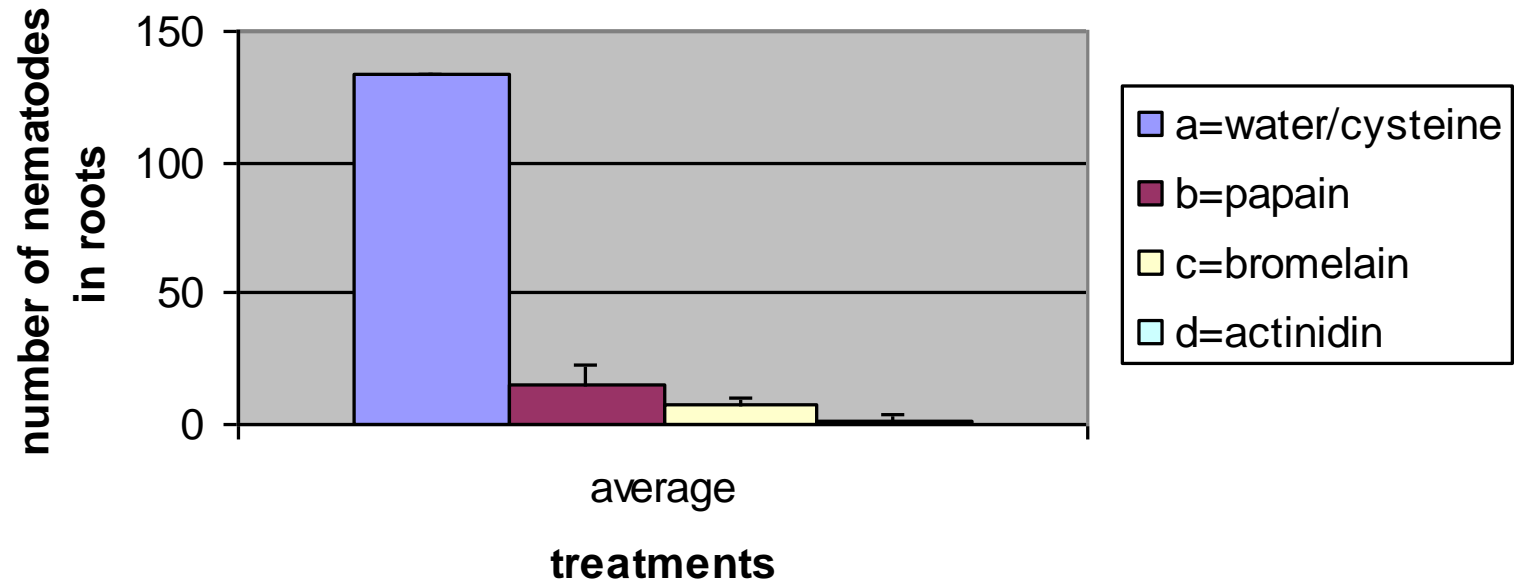
**125µl of 1000x dilution of 10% Vydate**

**gives 35% of nematode mortality 'in vitro'.**



# *In planta* bioassays

## Two soil treatments with cysteine proteinases



**Reduction of nematode infection:**  
= 89% for papain  
= 95% for bromelain  
= 99% for actinidin



# *In vitro* Bioassays

➤ *In vitro* assays demonstrated that the paralysis or permanent damaged caused to plant nematodes is concentration dependent.

= active concentration of 100uM papain latex causes permanent damage to the nematodes

= active concentration of the enzyme below 100uM causes reversible nematode paralysis (24hs)

➤ *In vitro* soil assays demonstrated that two applications of the fruit CP with active concentrations of 250uM to infested soil samples (16% OM) caused 100% of nematode mortality.



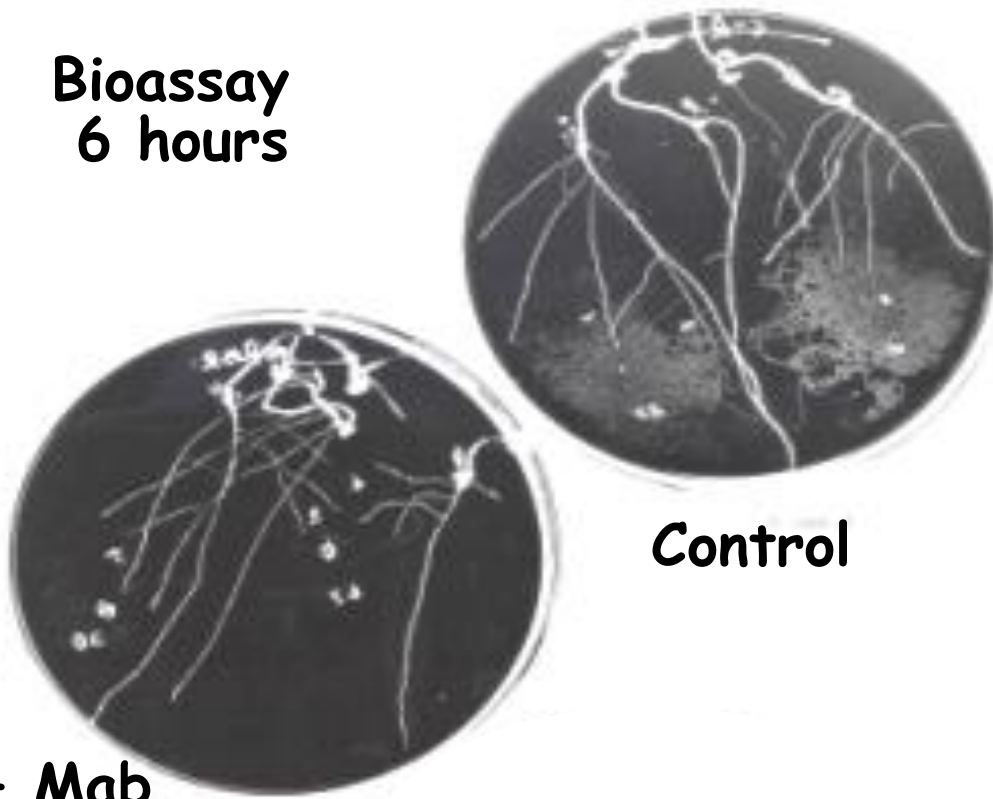
# Summary

- **The use of plant cysteine proteinases to destroy these cuticle-related targets represents a novel approach and *they are potential candidates for a novel class of bio-nematicides for soil application and for the production of transformed plants resistant to nematode infection.***
- **Plant cysteine proteinases could probably control nematodes when applied to infested soil by rendering nematodes immobile and unable to reach host plants**
- **Transgenic plants over-expressing cysteine proteinases have the potential to stop nematode invasion of roots when secreted into the rhizosphere.**



# Root penetration blocked by treatment of J2 with Mabs

Bioassay  
6 hours



J2 + Mab

Control



CP secreted in the  
apoplast or rhizosphere:  
Promoters: epidermal cell,  
lateral root cap, root  
border cells.

J. Int.Parasitology 2002  
Parasitology, 2002  
GB 46082

➤ Patent in the US - International application number  
PCT/IB2008000522