# IDENTIFICATION OF FREE-LIVING NEMATODES

# European Union quarantine nematodes

- **List 1; annex 1, part A, section 1**
  - *Hirschmaniella spp* (except *H. gracilis*)
  - *Longidorus diadecturus*
  - *Nacobbus aberrans*
  - *Xiphinema americanum sl* (not European species)
  - *Xiphinema californicum*

# European Union quarantine nematodes

- **List 2; annex 1, part A, section 2**
  - *Globodera pallida*
  - *Globodera rostochiensis*
  - *Meloidogyne chitwoodi*
  - *Meloidogyne fallax*

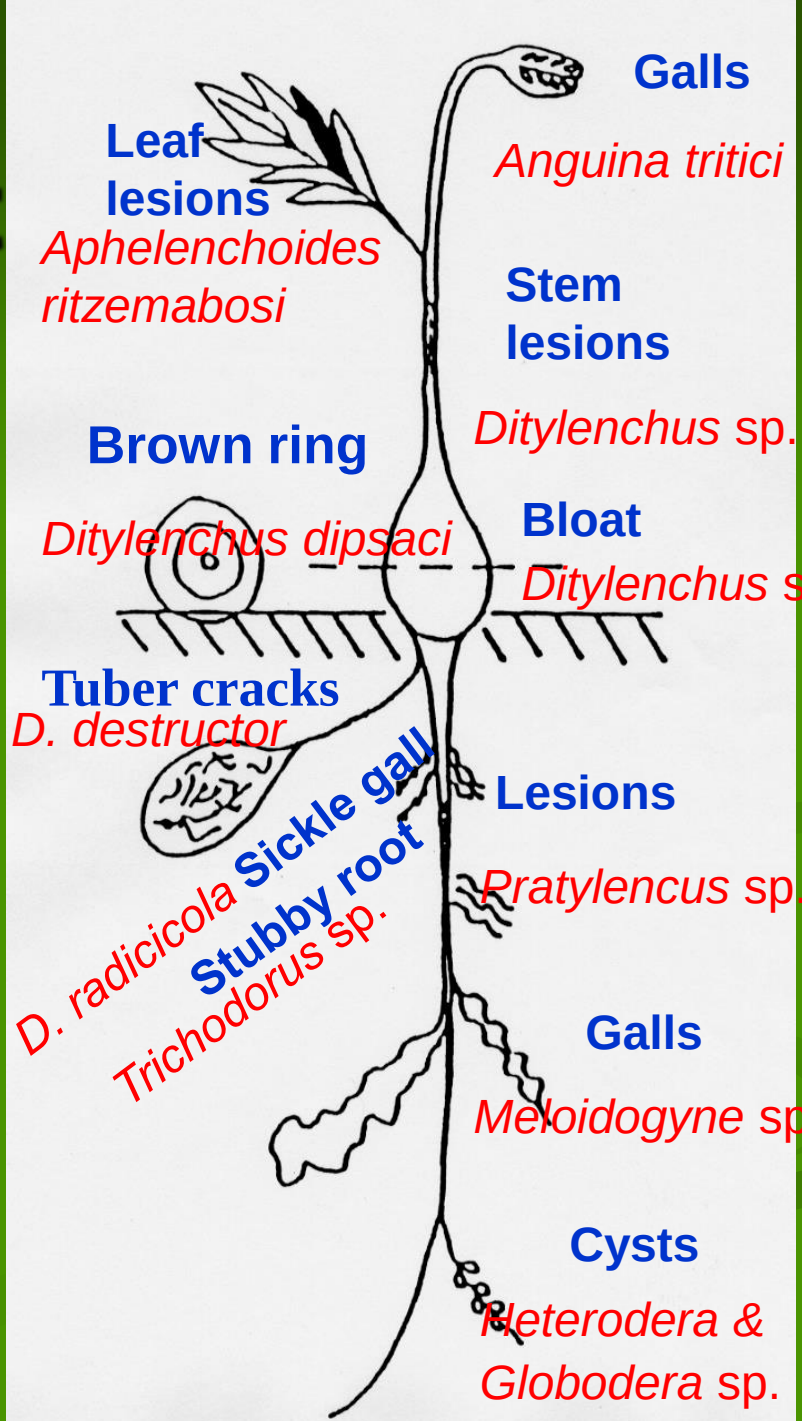
- **List 3; annex 1, part B**
  - *Globodera pallida*

# European Union quarantine nematodes

- **List 4; annex 1, part A, section 1**
  - *Aphelenchoides besseyi*
  - *Bursaphelenchus xylophilus*
  - *Radopholus citrophilus* (*=R. similis*)
- **List 5; annex 1, part A, section 2**
  - *Aphelenchoides besseyi*
  - *Ditylenchus destructor*
  - *Ditylenchus dipsaci*
  - *Radopholus similis*

# Composite plant

Nematodes causing symptoms



# Free-living (primary parasite)

*Ditylenchus destructor (*Root rot nematode)

*Ditylenchus dipsaci* (Stem & bulb nematode)

*Aphelenchoides besseyi* (White tip nematode)

# ***STEM & BULB NEMATODE (Ditylenchus* sp.)**

Fungiverous - ***D. myceliophagus***

Facultative - ***D. destructor***

Obligate - ***D. angustus*** (stem ectoparasite)
***D. dipsaci*** (stem endoparasite)
***D. radicicola*** (root endoparasite)

# Name: ***Ditylenchus destructor***

Common Name: **Potato rot nematode**
**Potato tuber nematode**

Hosts: Potatoes, also occasionally found on carrots, garlic, bulbous *Iris, Tulip*

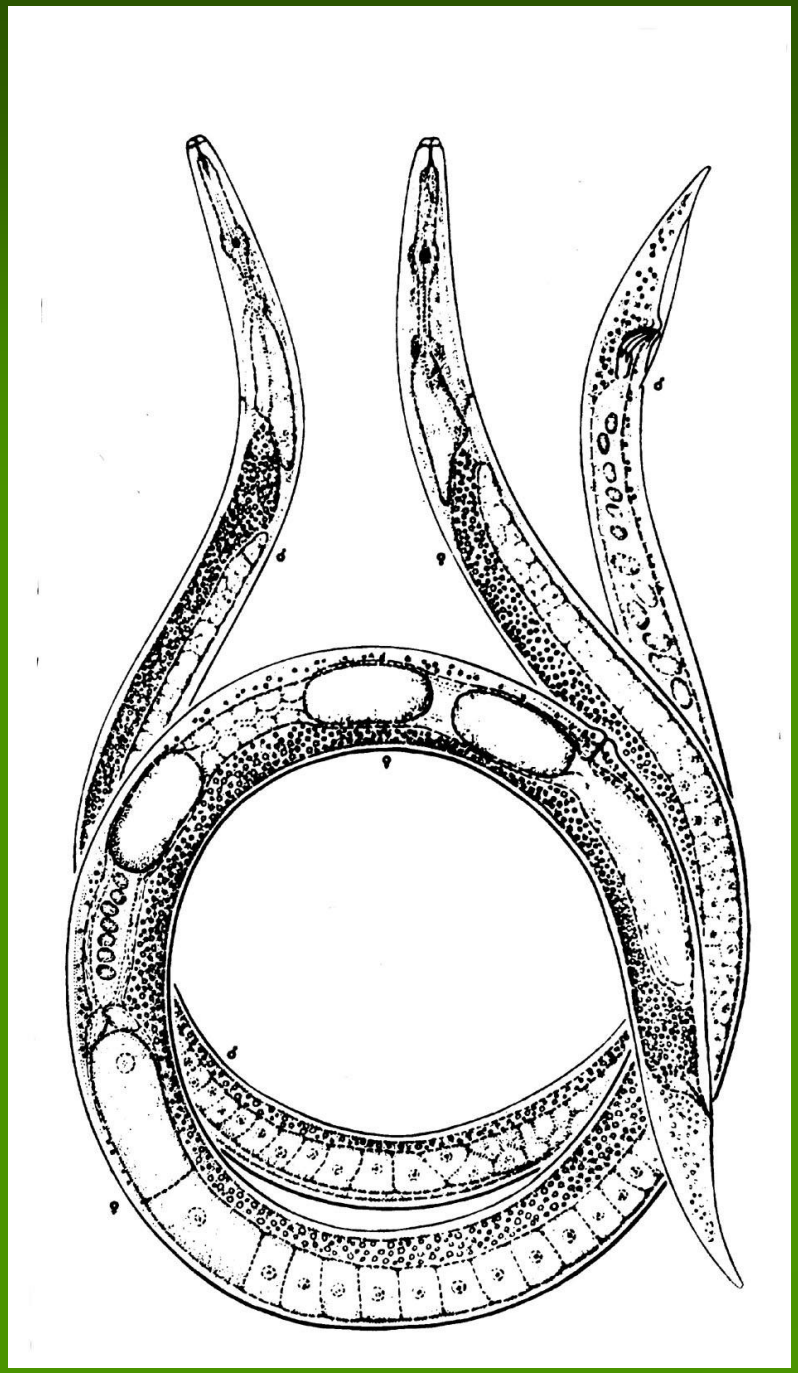
Geographical distribution: Widespread throughout the world

Symptoms:

No obvious symptoms in aerial part of infested plants.

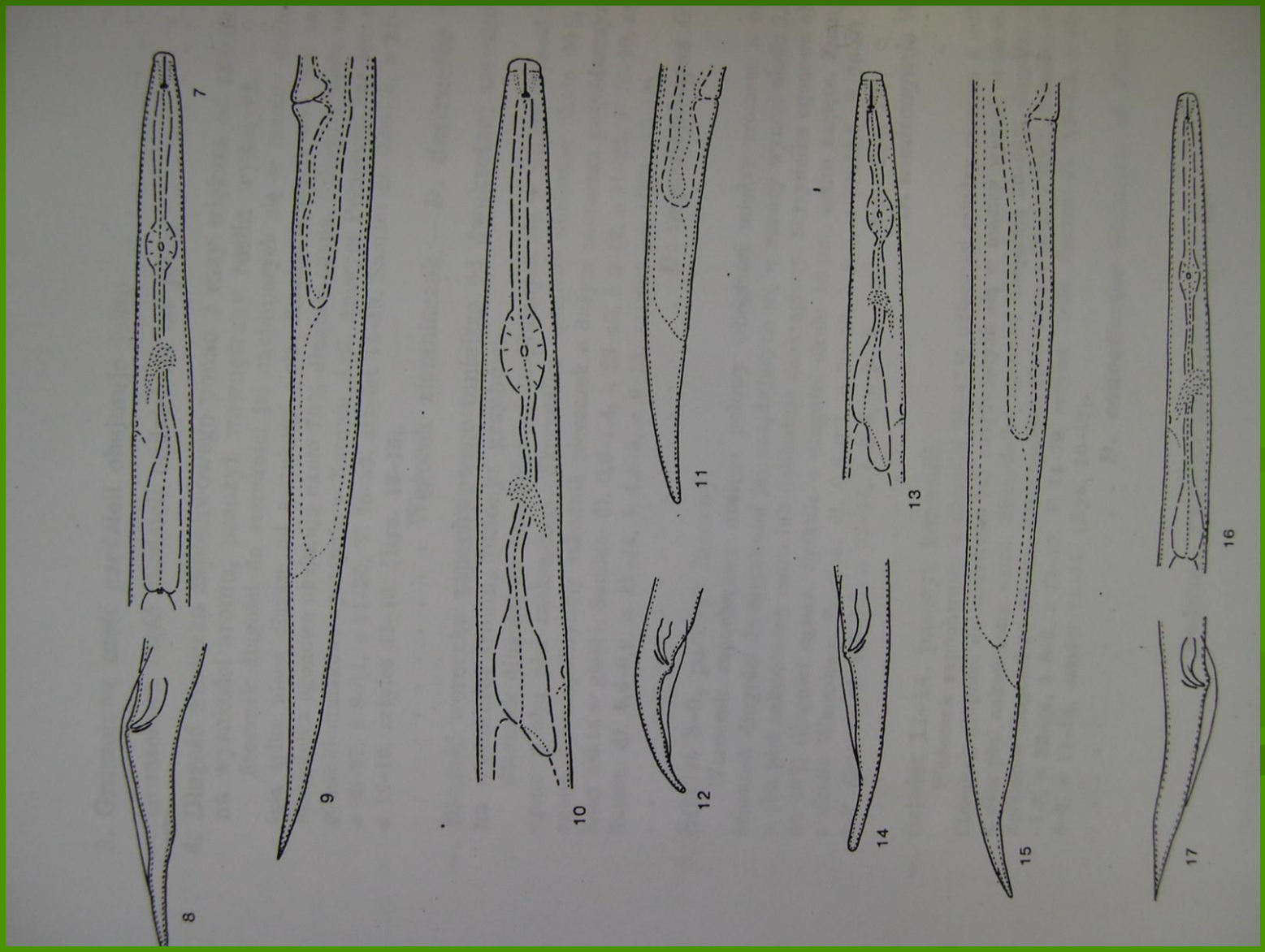
Badly affected tubers have sunken areas with cracked & wrinkled skin, discoloration due to secondary invasion of fungi and bacteria

# Symptoms on tubers



# *Ditylenchus destructor* male & female

*D. dipsaci* *D. myceliophagus* *D. destructor*



Inspection methods:
Sample tubers cut or peeled for characteristic whitish pockets containing nematodes.
Microscopic examination necessary for correct identification

Phytosanitary risk: minor importance with widespread distribution, major local importance

Control: Crop rotation with non-hosts *e.g.* Sugarbeet, use of nematode-free seed potatoes

Name: ***Ditylenchus dipsaci***

Common Name: **Stem nematode, Onion bloat, Stem & bulb eelworm**

Hosts: Widespread, over 450 plant species

Biological races: 10 with restricted host range

Geographical distribution: Most temperate areas of the world (Europe, Americas, Africa, Asia, Oceania)

# Main Races of *D. dipsaci* in Europe

**Oat race - polyphagus** (hosts oat, rye, beans, sugar beet, carrot, onion)

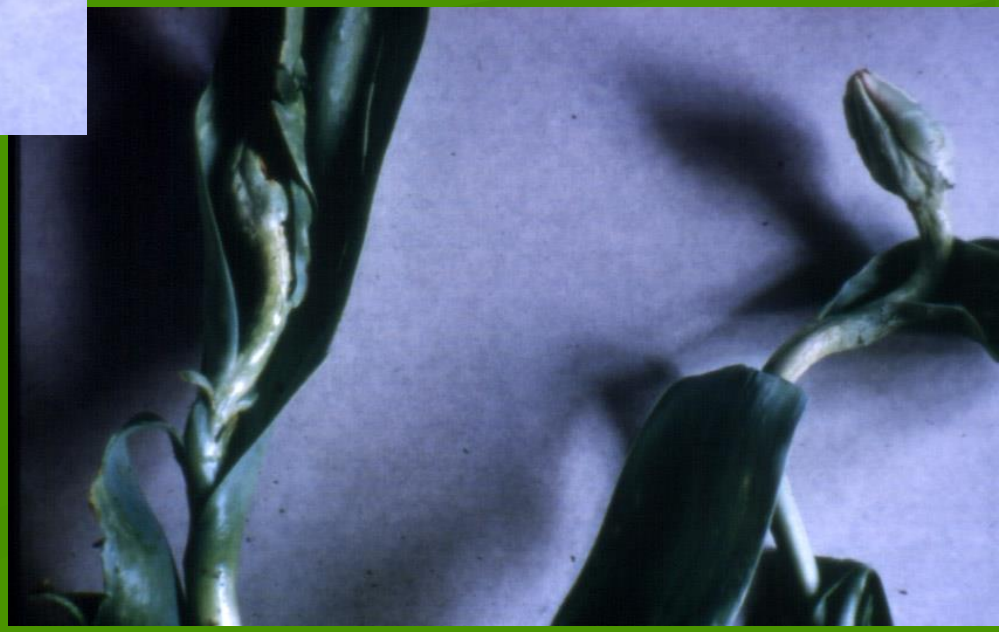
**Forage crops (3 races)**
Red clover
Lucerne
White clover

**Bulbs (3 races)**
Tulip
Daffodil
Hyacinth

# *Ditylenchus dipsaci* - infested oat field

# *Ditylenchus dipsaci* infested clover field

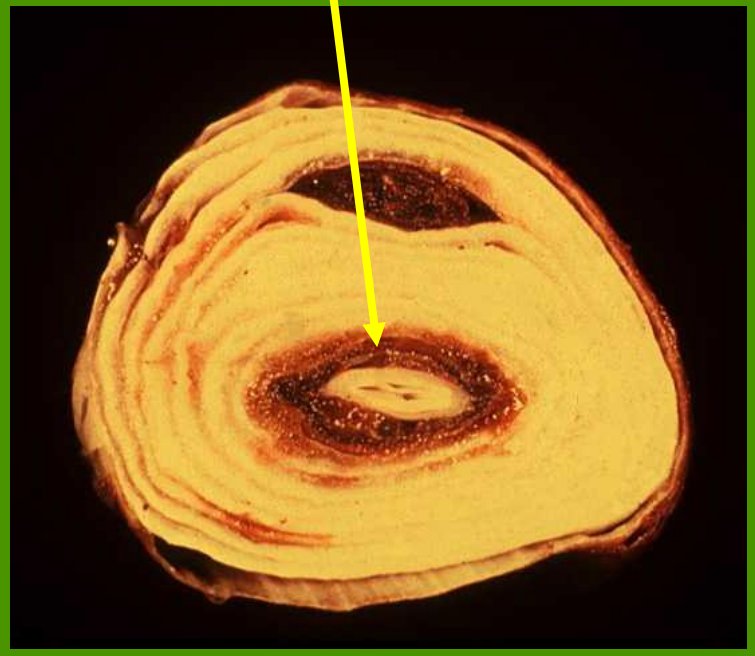
# *Ditylenchus dipsaci* - infested tulip stems



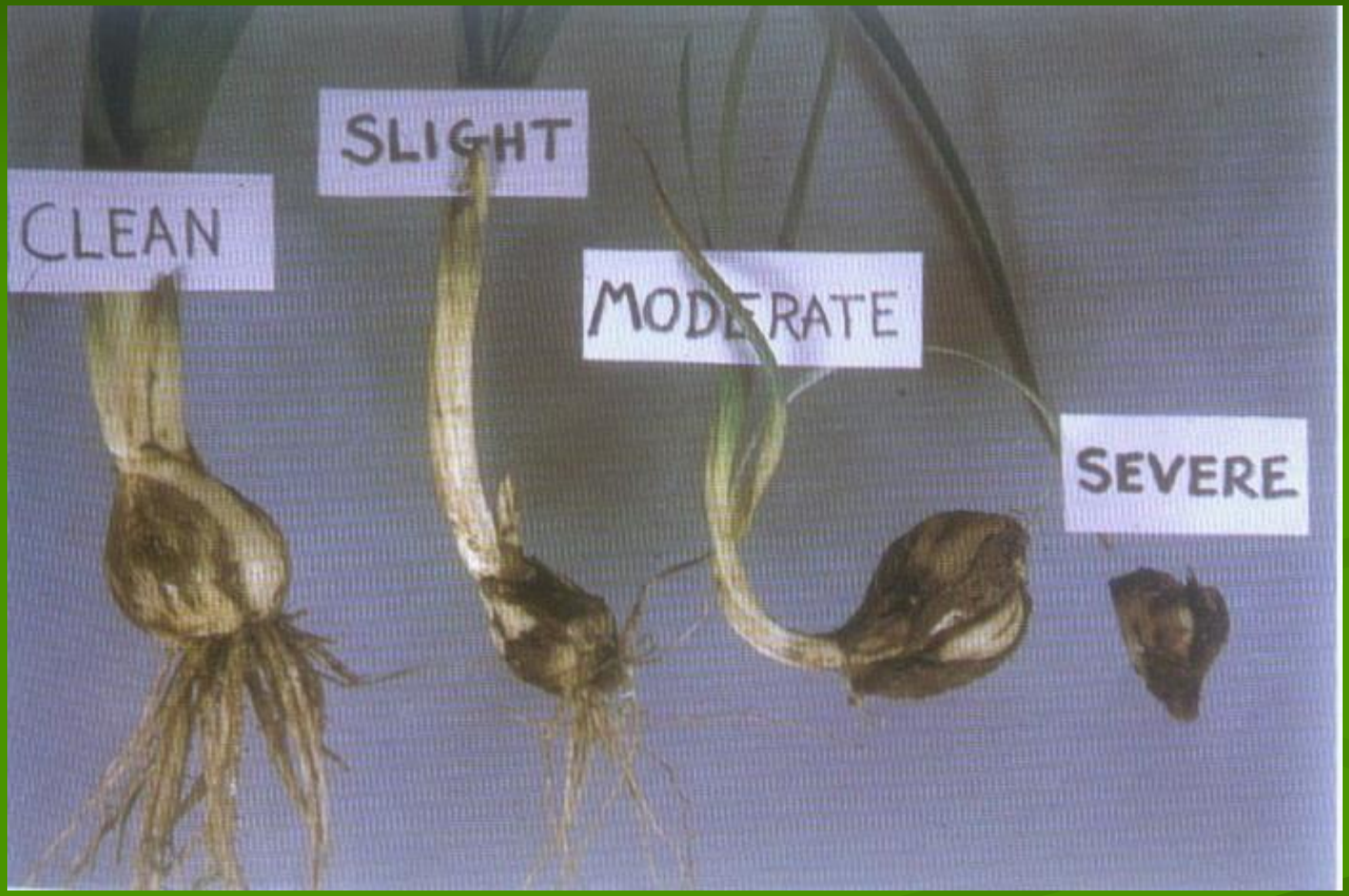
# *Ditylenchus dipsaci* - infested narcissus leaf

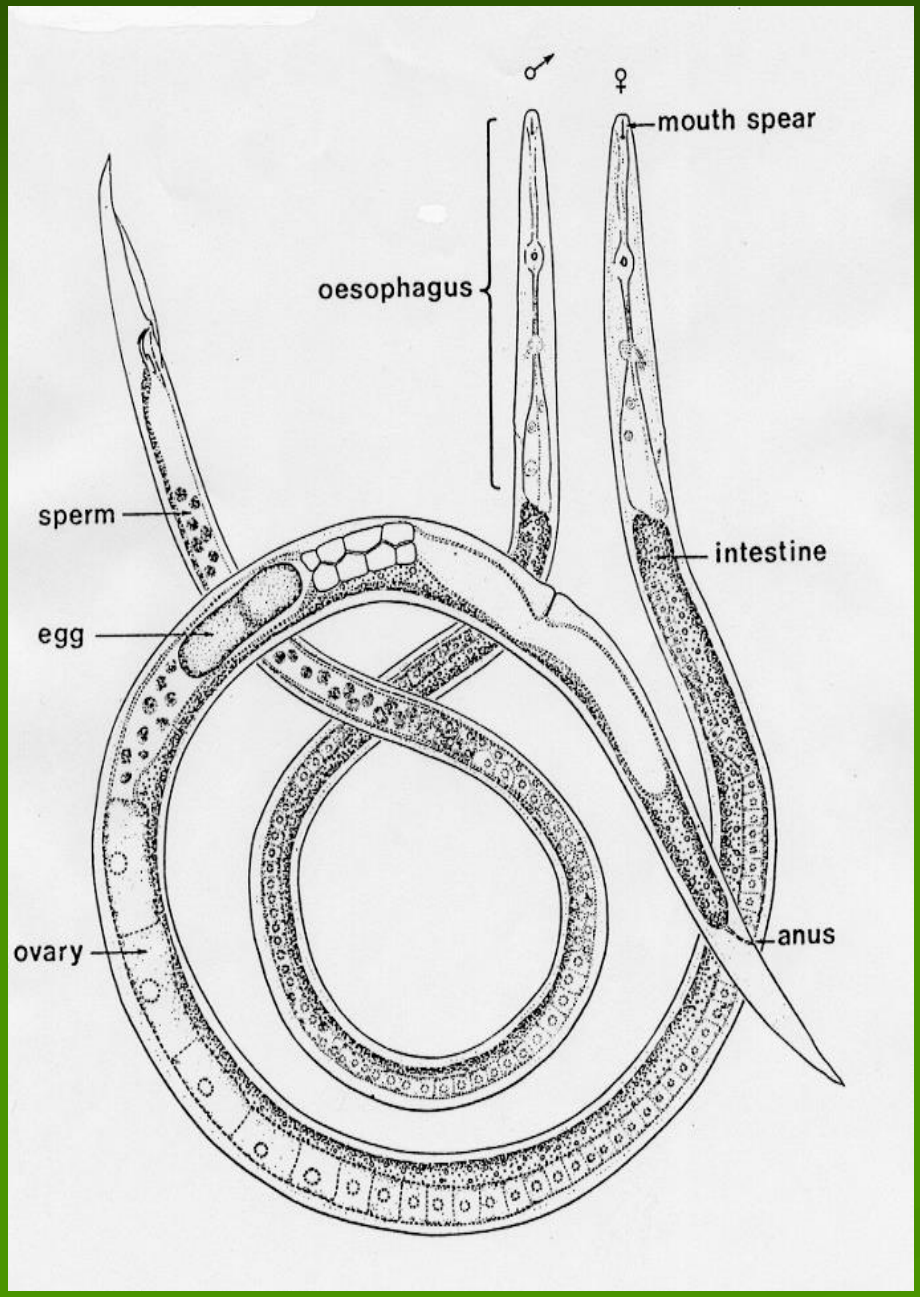
‘spikkles’

‘brown ring’



# *Ditylenchus dipsaci* - infested garlic


CLEAN
SLIGHT
MODERATE
SEVERE




# *Ditylenchus dipsaci male & female*

# Extraction of *D. dipsaci* and *D. destructor*

- Present

  *D. dipsaci*: in all plant parts

  *D. destructor*: in underground plant parts
- The Baermann funnel or the mistifier can be used
- If present in soil the Oostenbrink elutriator can be used

# Name: ***Aphelenchoides besseyi***

Common Name: **Rice white tip nematode**
**Strawberry crimp disease nematode**

Hosts: Rice and Strawberries, also some ornamentals and grasses

Geographical distribution: not beyond latitude 43 N (rice), 40 N (strawberries)

EPPO region: Bulgaria, Hungary, Italy, Russia
Slovakia

Worldwide: Asia, Africa, Americas, Oceania

Plant symptoms:

Rice – whitening of top of leaf tips, crinkling, necrosis

Strawberries – leaf crinkling, distortion & dwarfing

# Symptoms on rice

# Symptoms on strawberries

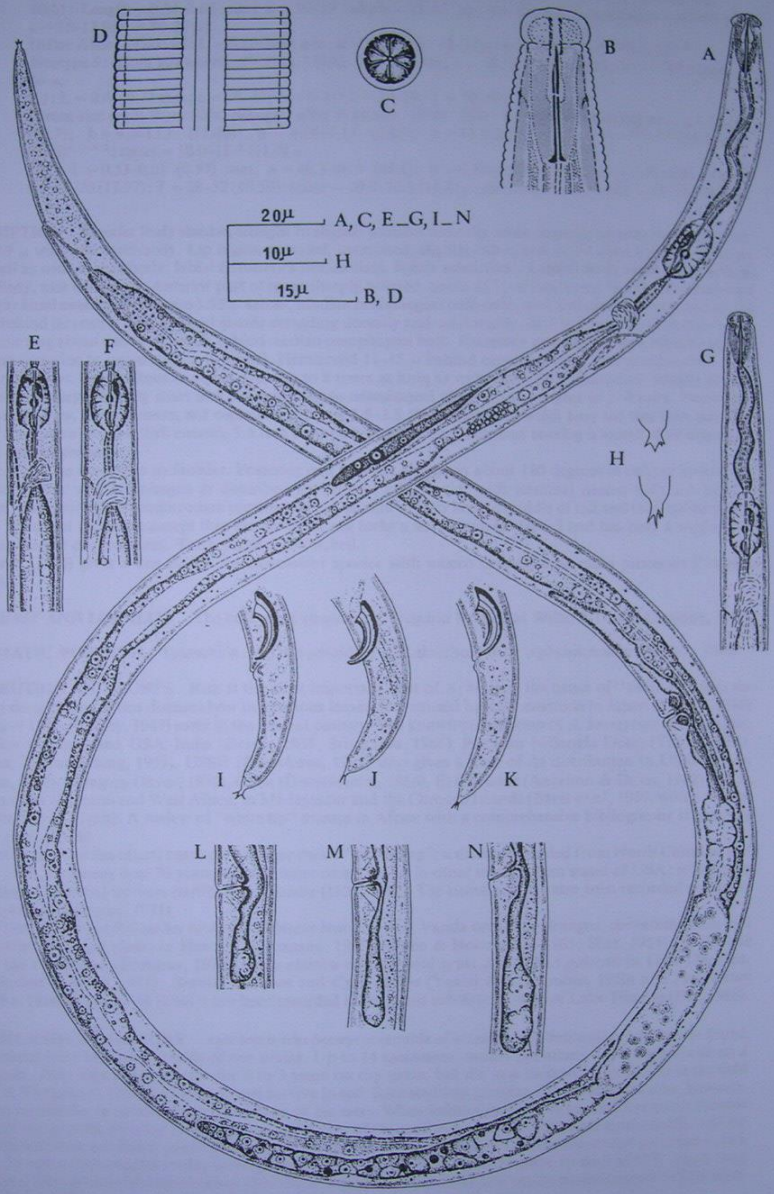

2,


13

# Extraction of *A. besseyi*

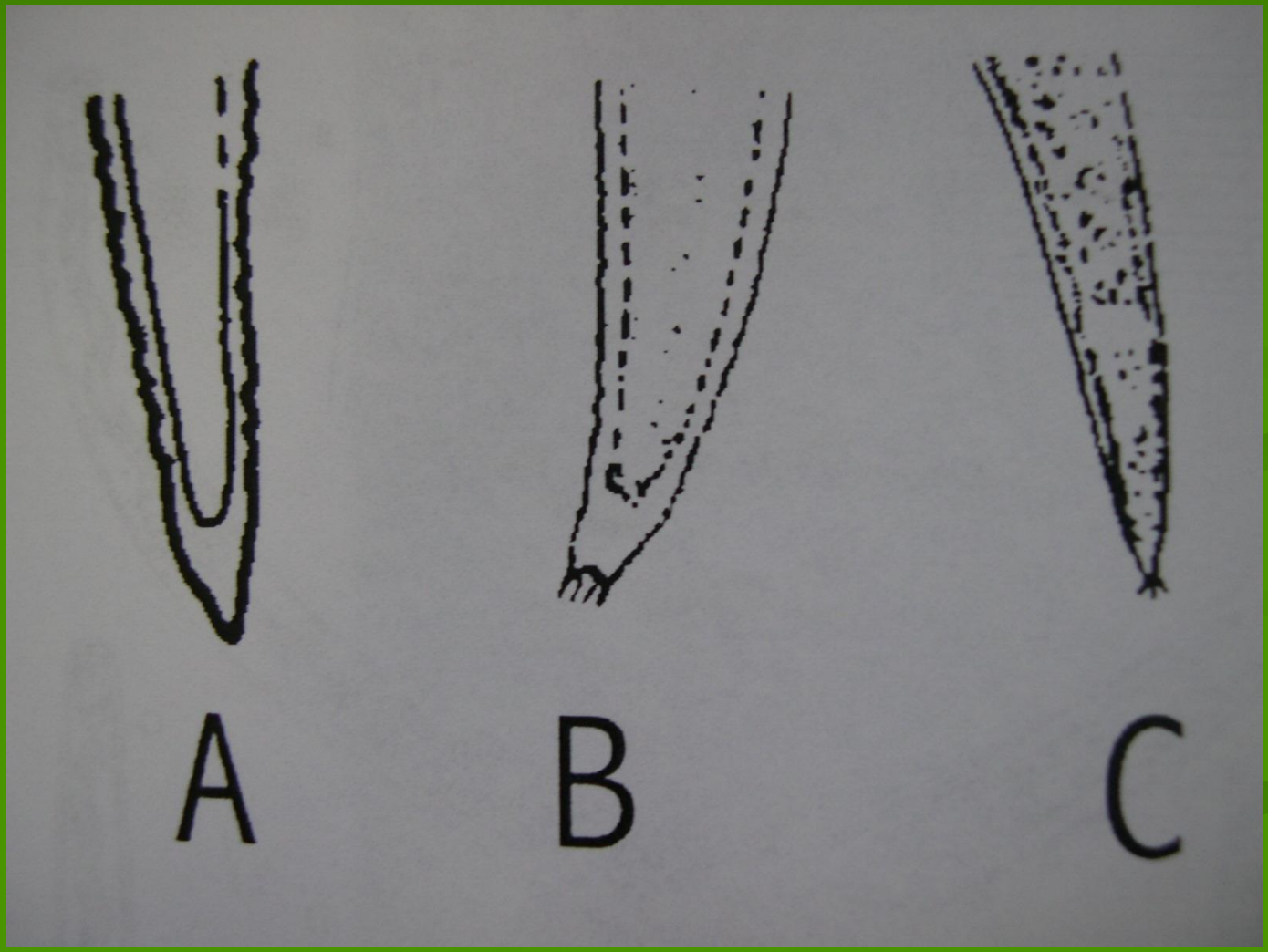
- Plant tissue and seeds
- The Baermann funnel or the centrifuge floating method can be used

# *APHELENCHOIDES BESSEYI*




*Aphelenchoides besseyi* Christie. A. Female. B. Female head end. C. Female *en face* view. D. Lateral field. E & F. Variation in female median oesophageal bulb and position of excretory pore with respect to nerve ring. G. Male anterior end. H. Female tail termini showing variation in shape of mucro. I–K. Male tail ends. L–N. Variation in post-vulval uterine sac. (B and D original: the rest after Fortuner, 1970.)

***A. fragariae*** ***A. ritzemabosi*** ***A. besseyi***



Phytosanitary Measures:
Rice seed from infested countries should be tested as nematodes leave tissues of rice seed soaked in water for several days.

Plants for planting strawberries from infested countries should be tested as treated is required (hot-water treatment – 10 minutes at 46 C)

# *Aphelenchoides* spp.

*A. ritzemabosi*

*A. fragariae*

# Free-living nematodes – virus vectors

***Xiphinema* spp. (Dagger nematode)**

***Longidorus* spp. (Needle nematode)**

***Trichodorus* spp. (Stubby root nematode)**

# Free-living Plant Nematodes

Class **NEMATODA**

Order **TYLENCHIDA** | **DORYLAIMIDA**

Genus

**TYLENCHIDA**

- ***Pratylenchus*** **(Lesion)**
- ***Rotylenchus*** **(Spiral)**
- ***Radopholus*** **(Burrowing)**

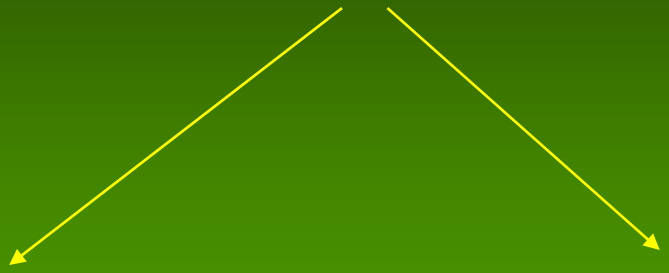
**DORYLAIMIDA**

- ***Longidorus***
- ***Xiphinema***
- ***Trichodorus***

**no known as virus vectors**

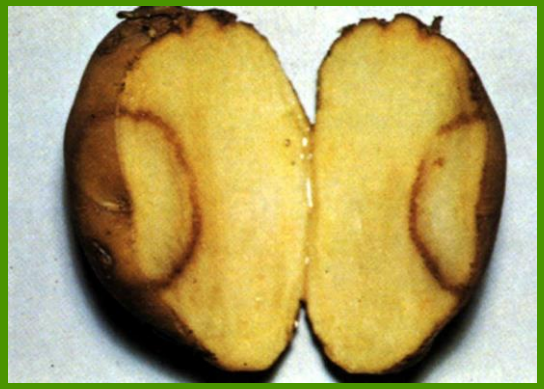
**virus vectors**

Order **DORYLAIMIDA**



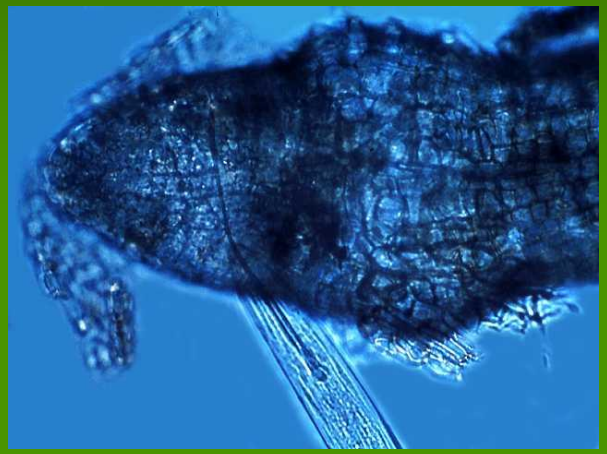
| | | |
|---|---|---|
| Family | **LONGIDORIDAE** | **TRICHODORIDAE** |
| | \| | \| |
| Genus | ***Longidorus*** | ***Trichodorus*** |
| | (Needle) | (Stubby root) |
| | ***Paralongidorus*** | ***Paratrichodorus*** |
| | ***Xiphinema*** | |
| | (Dagger) | |

## *Trichodorus*



Tobacco Rattle Virus
'spraing'

## *Xiphinema*



Tomato Ringspot

## *Longidorus*

Raspberry Ringspot

***Xiphinema diversicaudatum*** (vector)
Virus : **Arabis mosaic** (cherry, cucumber, grape-vine, hop, lettuce, raspberry, strawberry, rhubarb)
**Carnation ringspot** (carnation)
**Cherry leaf roll** (cherry)
**Strawberry latent ringspot** (cherry, blackcurrant, celery, peach, plum, raspberry, rose, rhubarb, strawberry )

***Longidorus elongatus*** (vector)
Virus : **Raspberry ringspot** (blackberry, raspberry, redcurrant, strawberry)
**Tomato blackring (**celery, cherry, lettuce, onion, peach, potato, raspberry, sugar beet, tomato)

***Longidorus attenuatus*** (vector)
Virus : **Tomato blackring**
(as above)

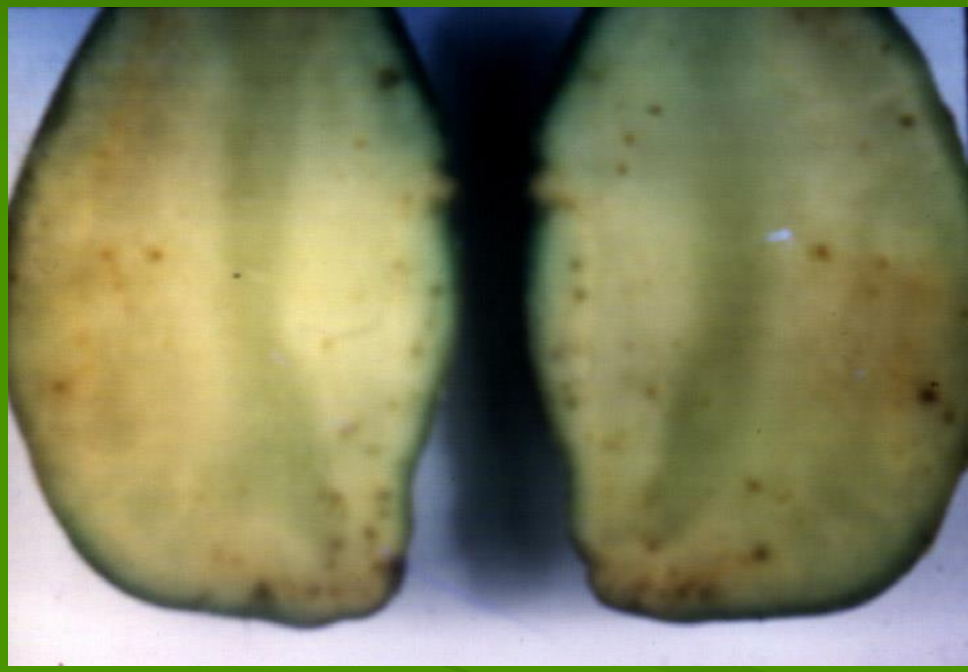
***Longidorus macrosoma*** (vector)
Virus: **Raspberry ringspot**
(as above)

***Trichodorus primitivus***
***T. similis***
***T. Viruliferus***

virus: **Tobacco rattle virus**

Causes ‘SPRAING’ in potatoes

# Internal tuber necrosis caused by Tobacco Rattle Virus ('spraing')



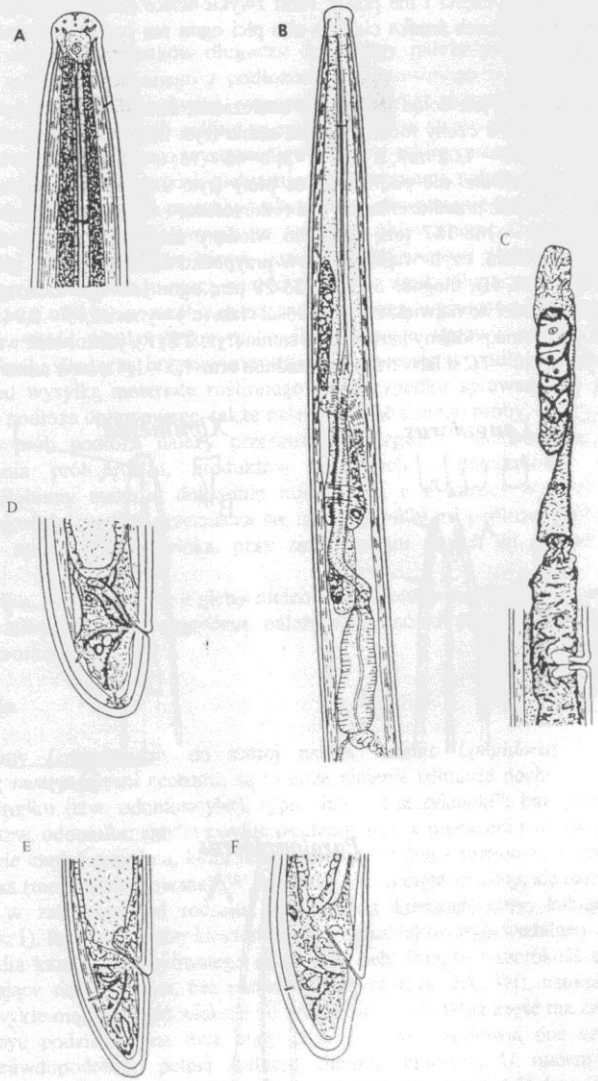
# Name: ***Longidorus diadecturus***

North America

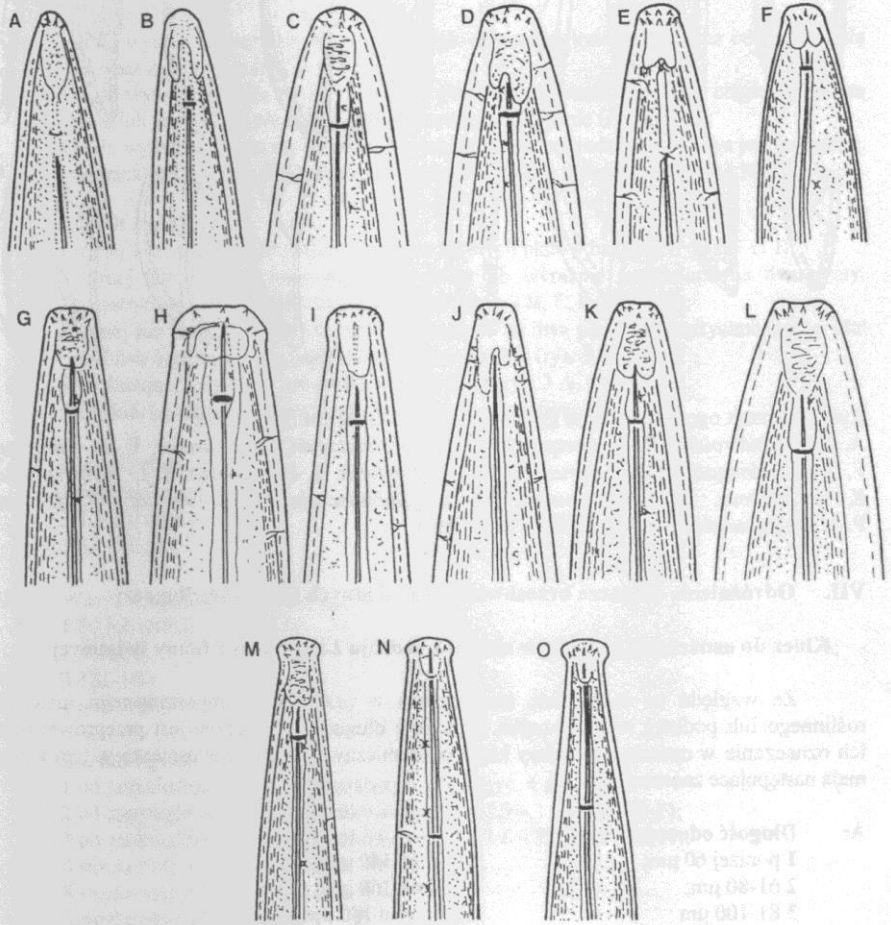
Hosts: grape, peach, cucumber

Vector: Peach rosette mosaic virus
Tomato black ring virus

No particular symptoms (terminal swelling, hooked deformation, brown necrosis)

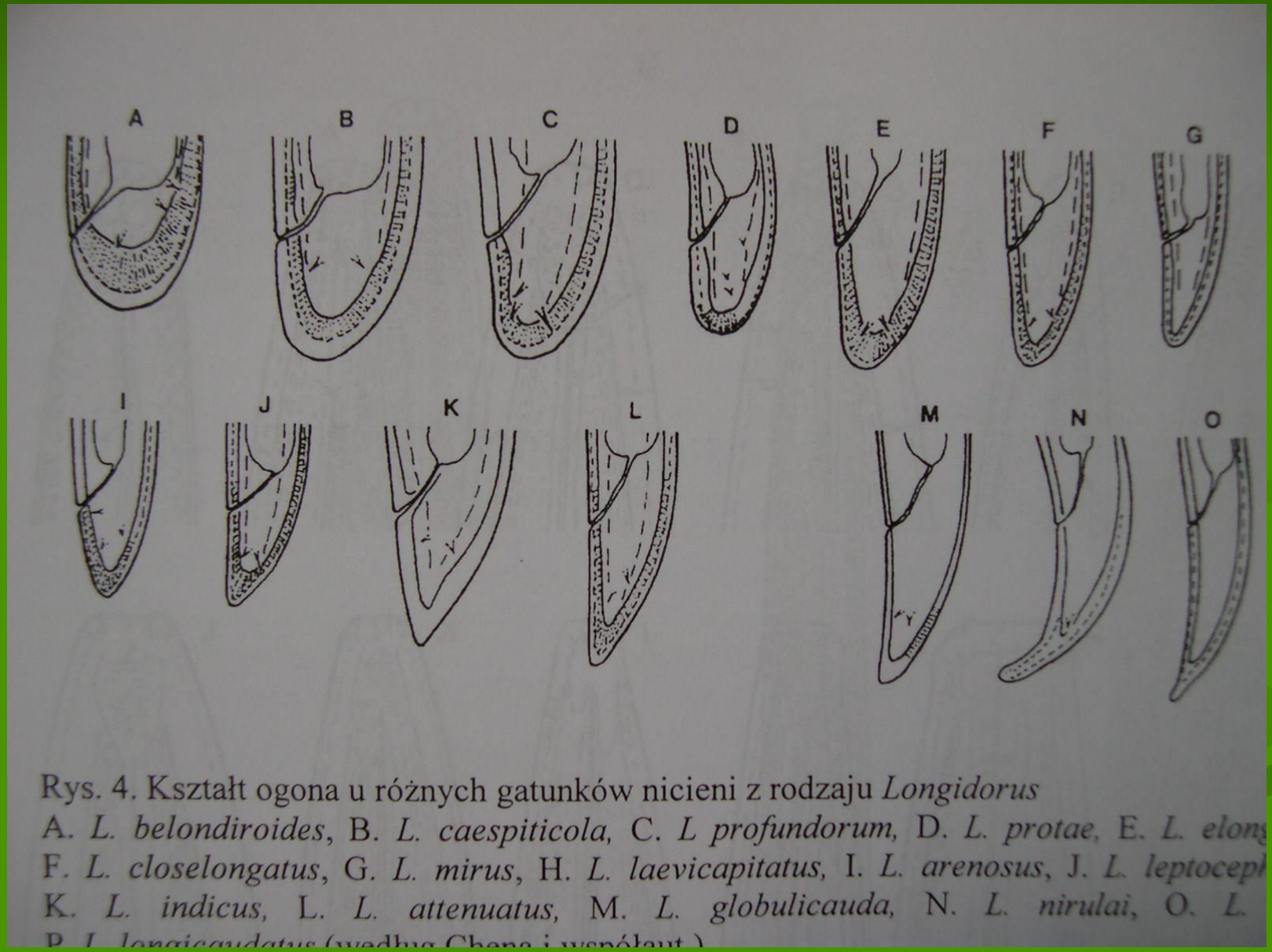



Rys. 2. Długacz brzoskwiniowiec (*Longidorus diadecturus*)
A. Przednia część ciała samicy; B. Przednia część ciała samicy z widocznym przełykiem; C. Fragment układu rozrodczego samicy; D. ogon samicy; E. ogon larwy trzeciego stadium; F. ogon larwy czwartego stadium (według Eveleigha i Allena).



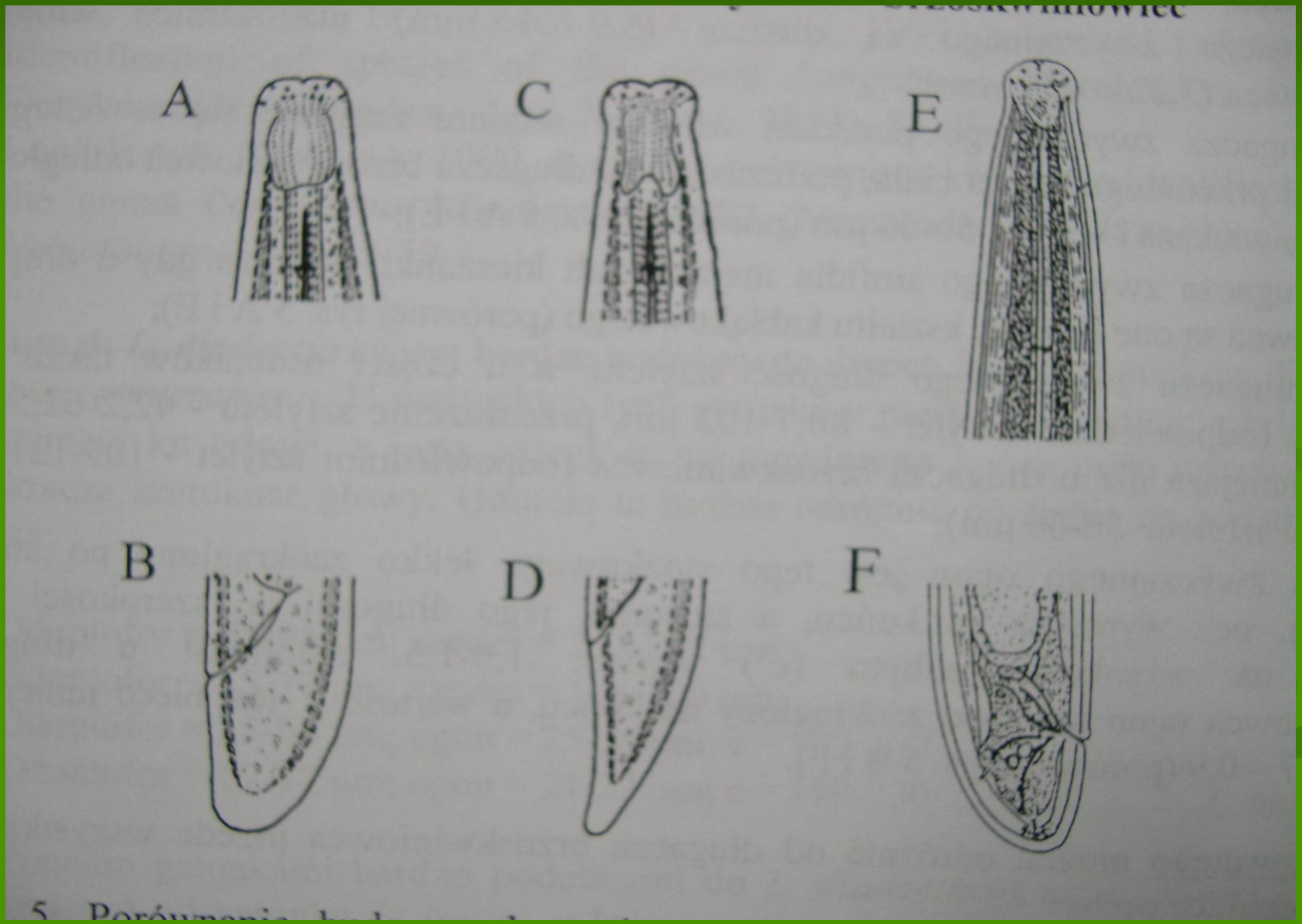

Rys. 3. Budowa przedniej części ciała samic różnych gatunków nicieni z rodzaju *Longidorus*: A. *L. belondiroides*; B. *L. orientalis*, C. *L. caespiticola*, D. *L. goodeyi*, E. *L. litchii*, F. *L. juveniloides*, G. *L. elongatus*, H. *L. kuiperi*, I. *L. proximus*, J. *L. belloi*, K. *L. profundorum*, L. *L. macrosoma*, M. *L. attenuatus*, N. *L. fursti*, O. *L. mobae* (według Chena i współaut.)

# Tail shape

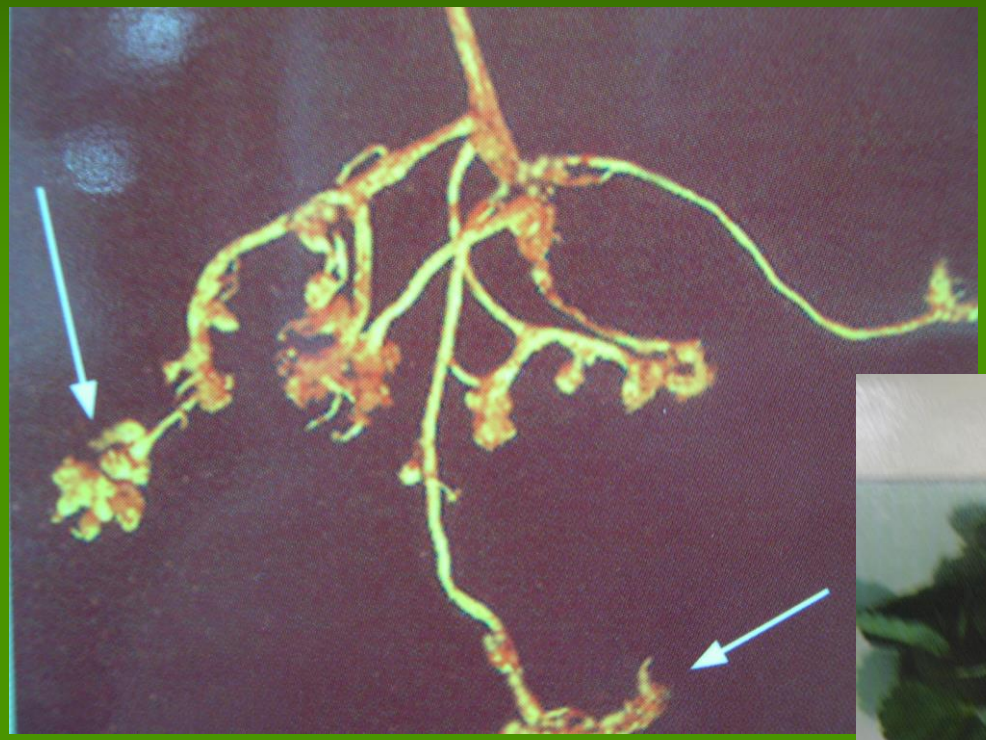



Rys. 4. Kształt ogona u różnych gatunków nicieni z rodzaju *Longidorus*
A. *L. belondiroides*, B. *L. caespiticola*, C. *L profundorum*, D. *L. protae*, E. *L. elong*
F. *L. closelongatus*, G. *L. mirus*, H. *L. laevicapitatus*, I. *L. arenosus*, J. *L. leptocepl*
K. *L. indicus*, L. *L. attenuatus*, M. *L. globulicauda*, N. *L. nirulai*, O. *L.*
P. *L. longicaudatus* (według Chena i współaut.)

***Longidorus elongatus*** ***L. attenuatus*** ***L. diadecturus***



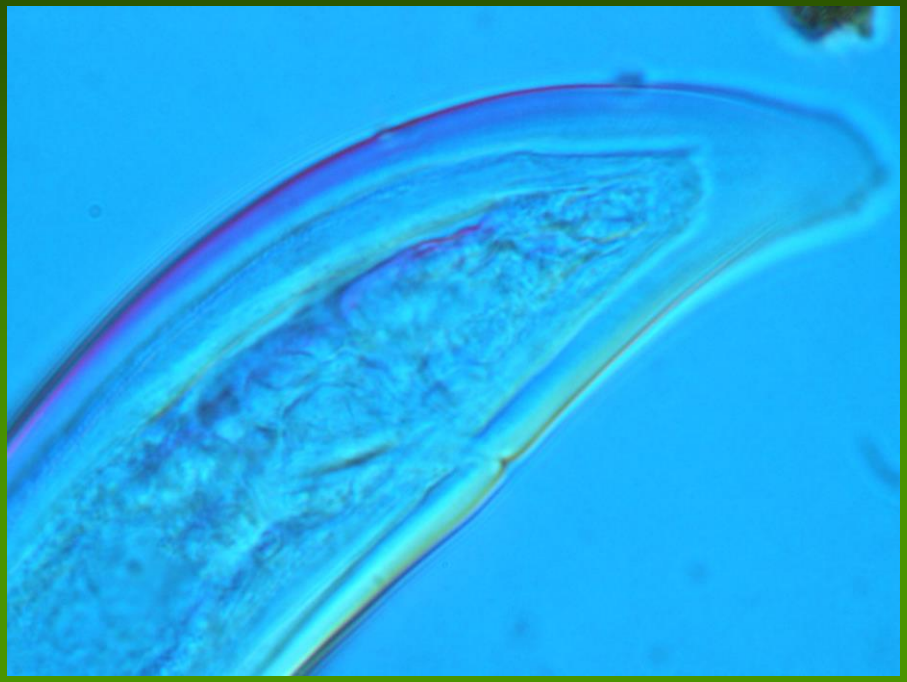
# Symptoms on roots / strawberries

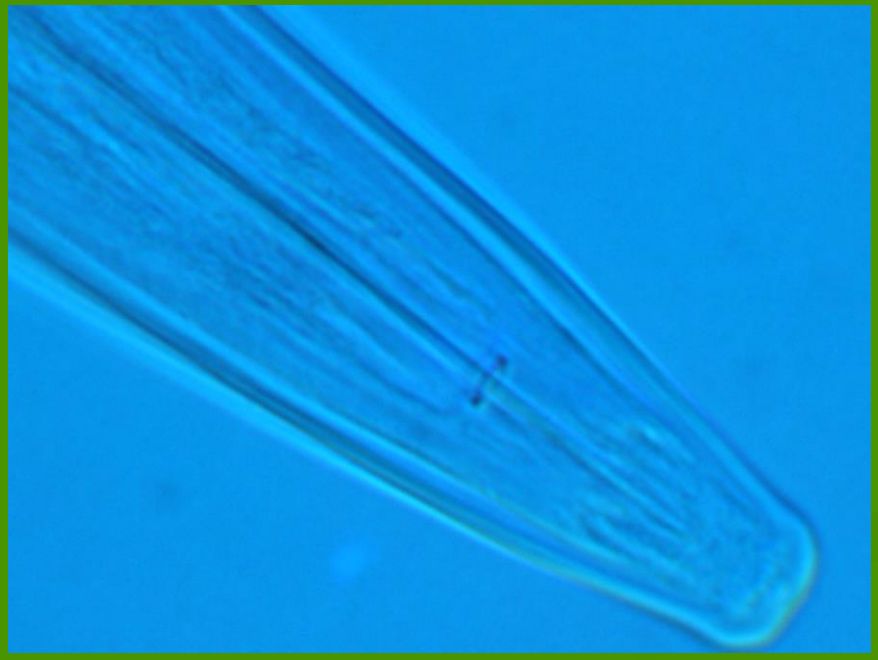
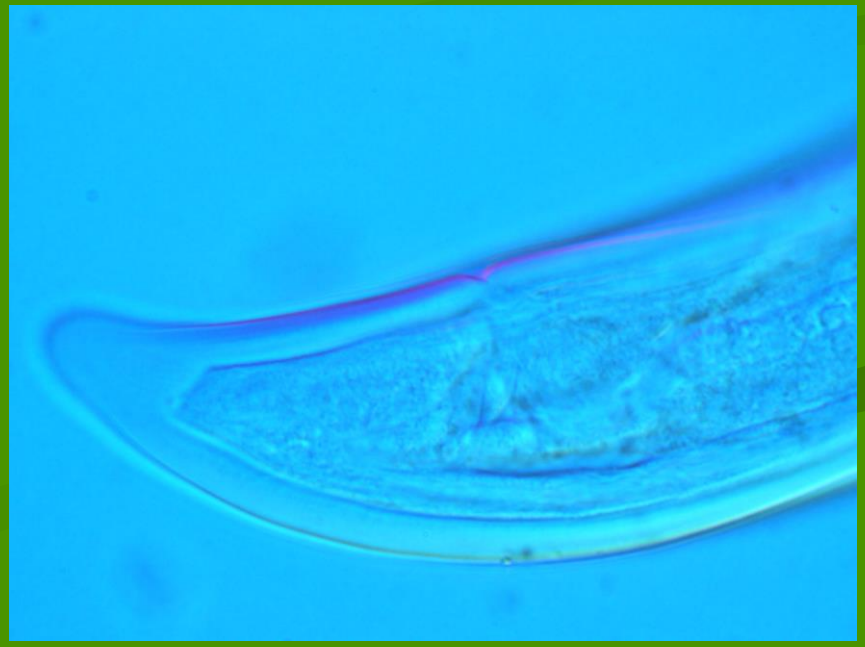


# Extraction of *L. diadecturus*

Only present in soil

The Oostenbrink elutriator can be used using two 160 um sieves



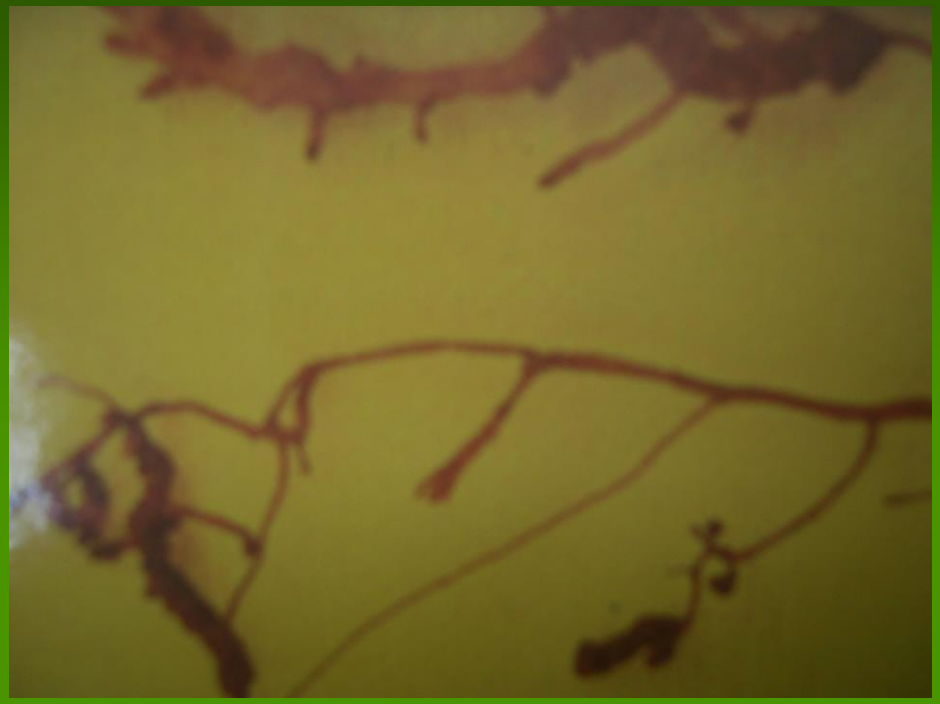
# *Xiphinema americanum sl* (non European species)

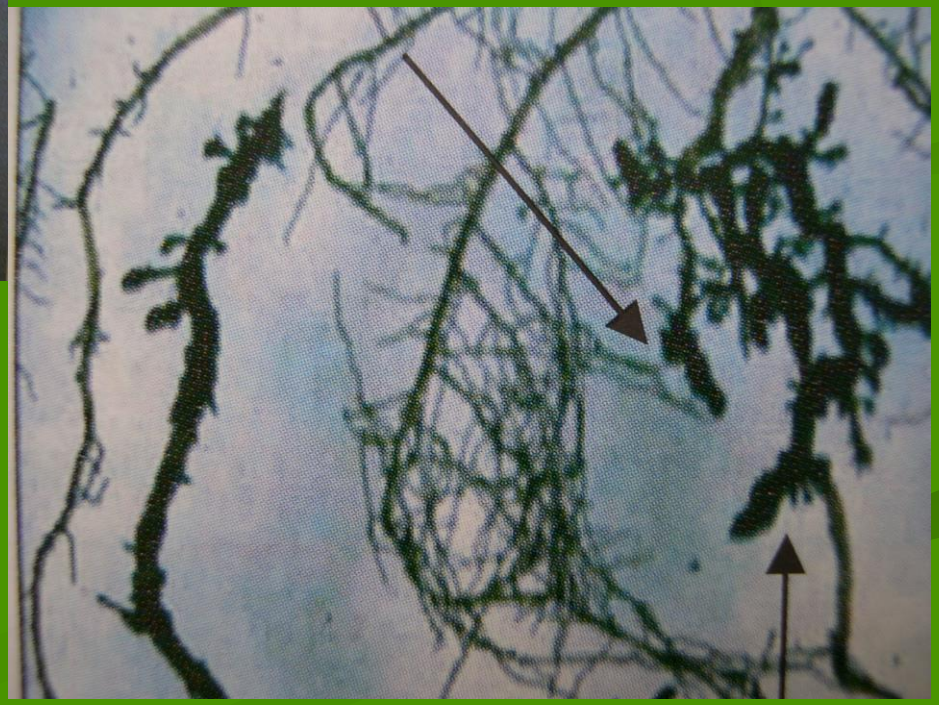
Non European populations

Very wide host range (appricot, strawberry, raspberry, pear, bonsai)

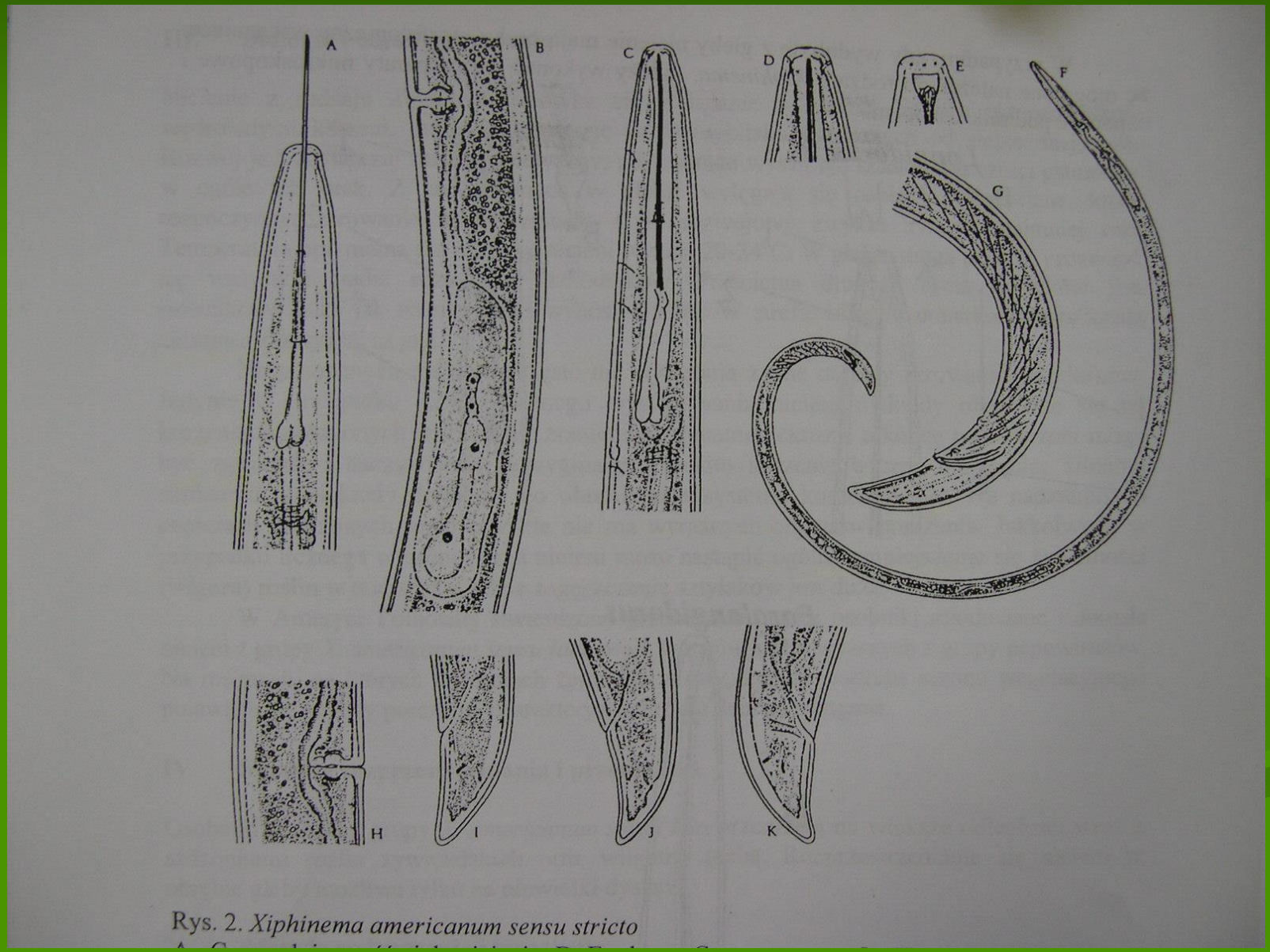
Vector: North American Nepo viruses

No particular symptoms on the plants (terminal swelling, hooked deformation, brown necrosis)





# *Xiphinema americanum ss*




Rys. 2. *Xiphinema americanum sensu stricto*

# *X. index*

# Extraction of *X. americanum sl* (no European species)

Present in soil

The centrifuge floating method or the Oostenbrink elutriator can be used

Identification by morphology and PCR-techniques

# *Xiphinema californicum*

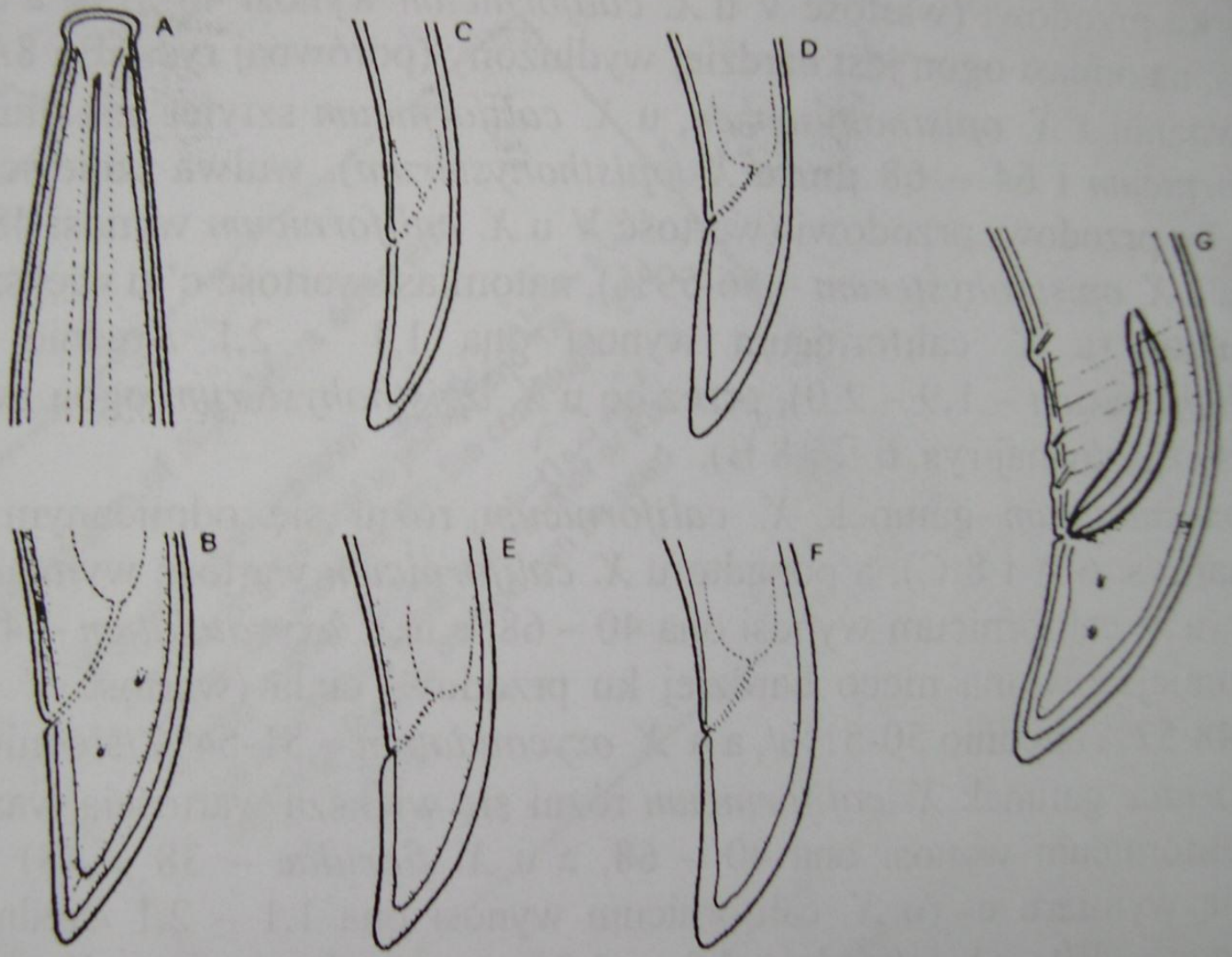
Belongs to the group of *X. americanum sl*

Mexico, USA (California), Brazil, Chile and Peru

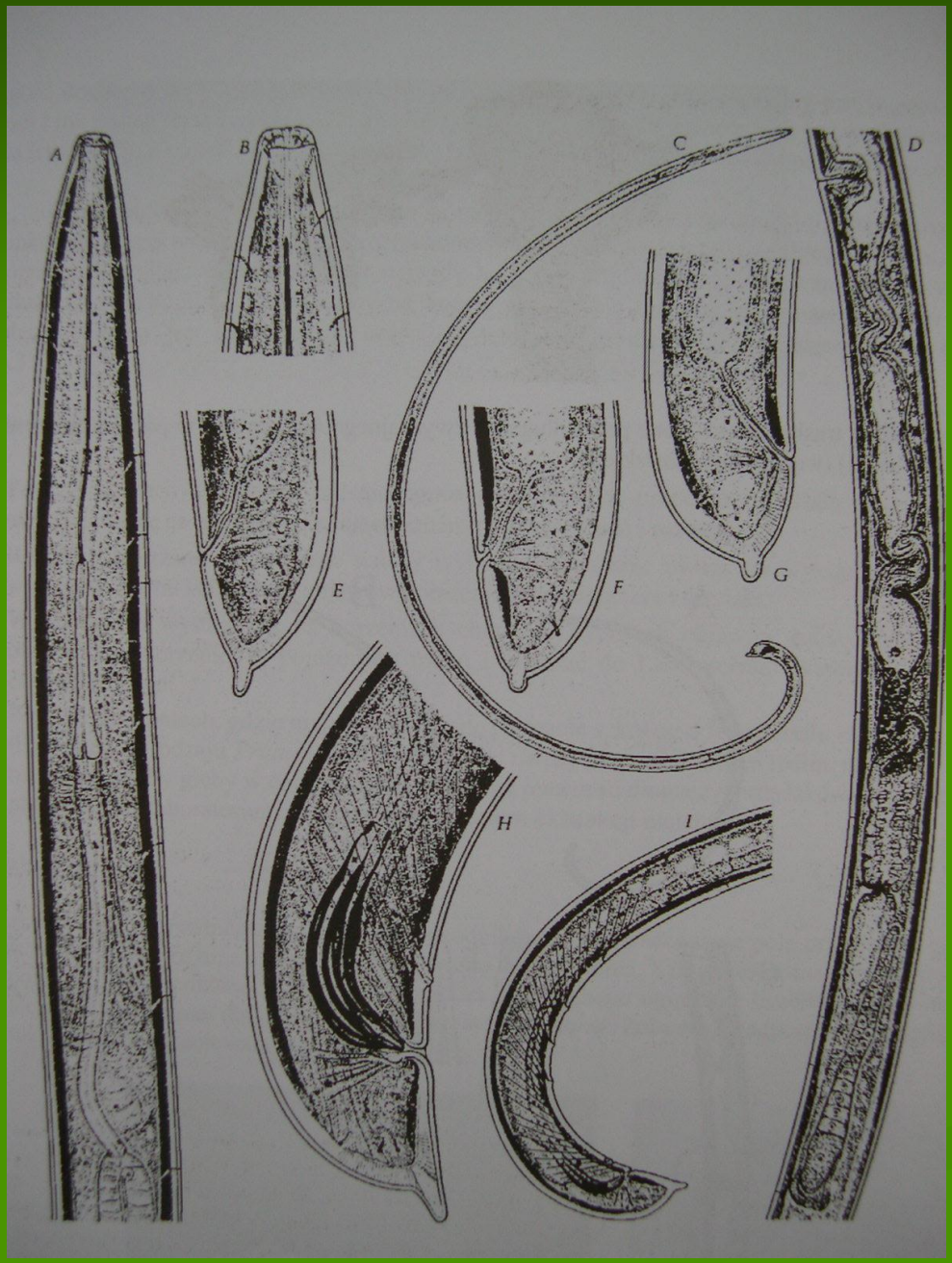
No particular symptoms on the plants

Virus: Tomato ringspot virus, Cherry rasp leaf virus, Tobacco ringspot virus

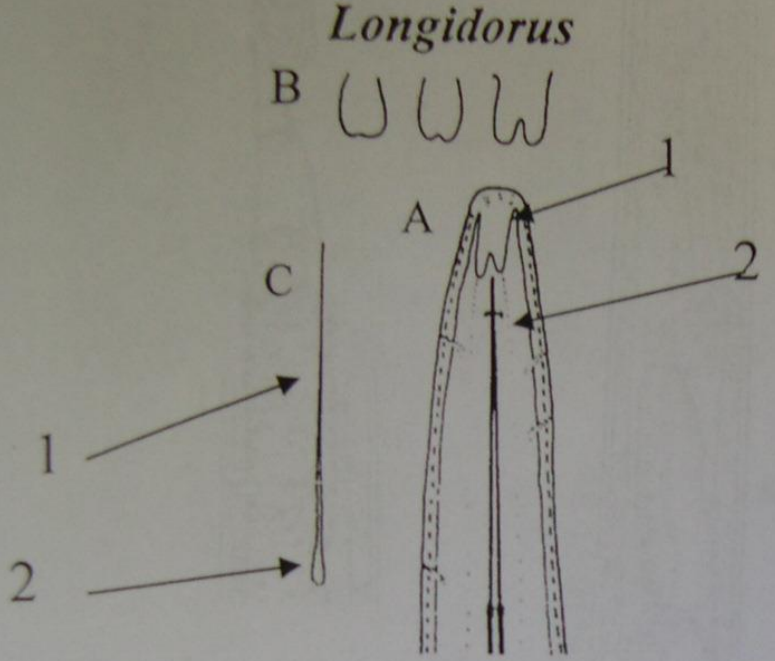
Extraction technique is the same as for *X. americanum sl*

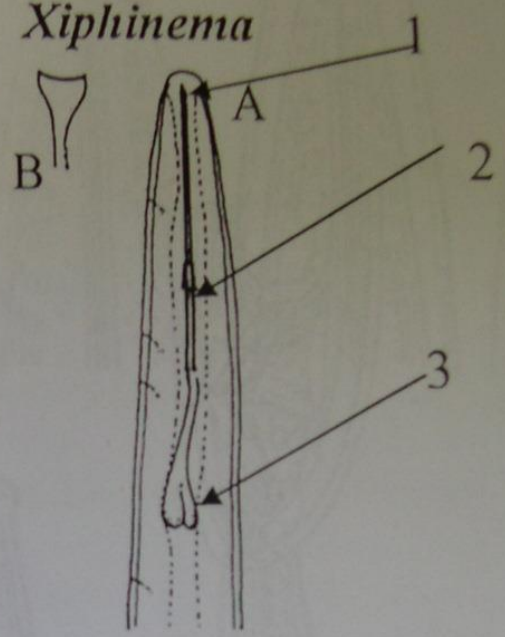


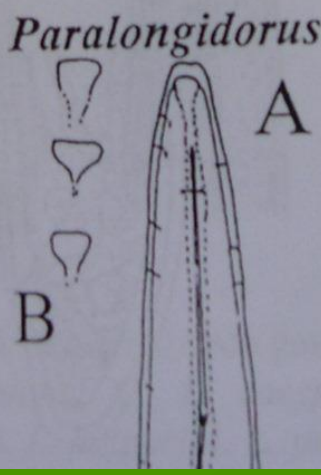

s. 6. *Xiphinema californicum*

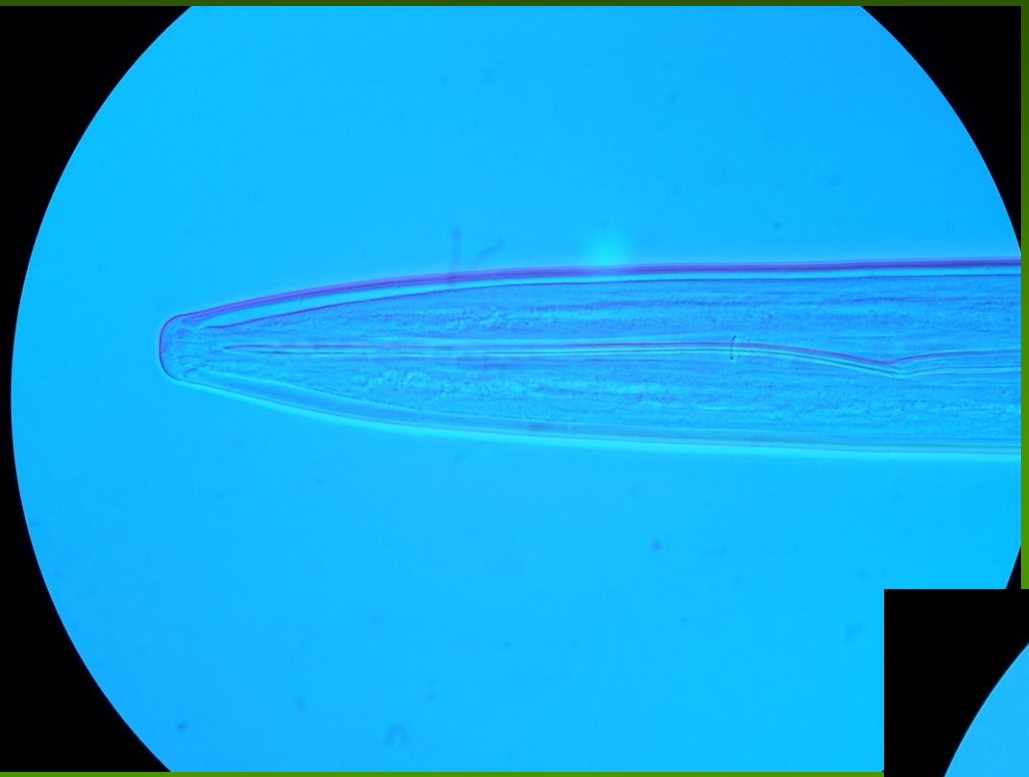


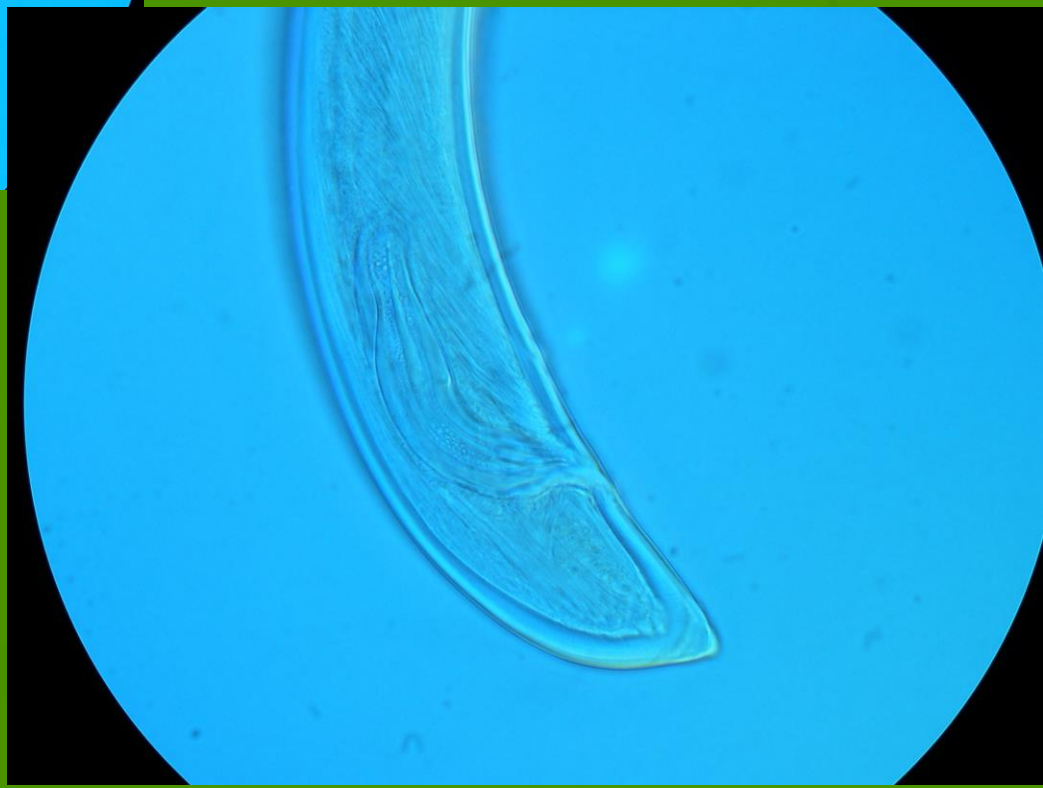

# *X. diversicaudatum*

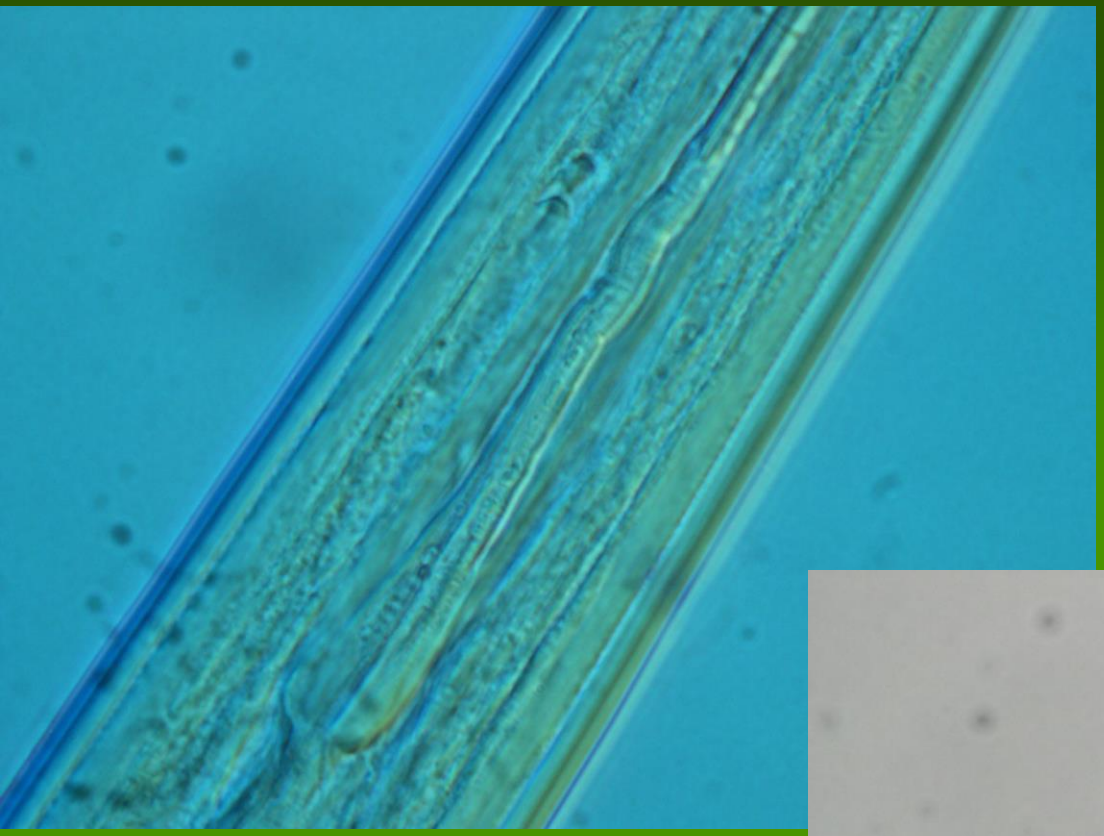

Longidorus
B
A
1
2
C
1
2


Xiphinema
1
A
B
2
3


Paralongidorus
A
B







# Insect vectors

## *Bursaphelenchus xylophilus*

(Pine wilt nematode)

Pine tree death

# Name: ***Bursaphelenchus xylophilus***

Common Name: **Pine wood nematode**
**Pine wilt disease**

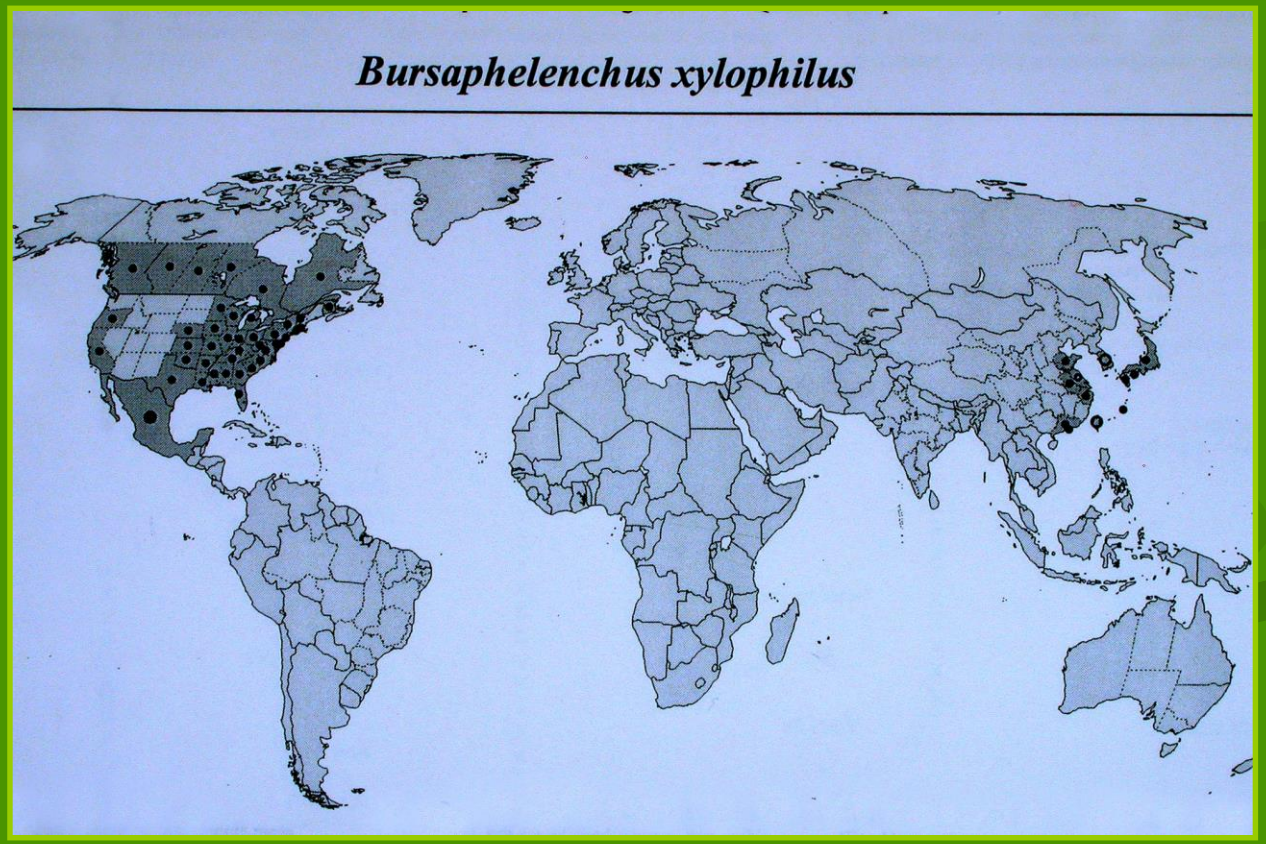
Hosts: Conifers - main found on *Pinus* spp.
(also *Larix, Abies, Picea, Pseudotsuga*)

Vector: *Monochamus* spp. (occurs only in northern hemisphere)

Origins: North America

Distribution: Japan, China, Korea, Taiwan

EPPO region: Present in Portugal. In other countries vector intercepted in imported timber products.



# Symptoms:

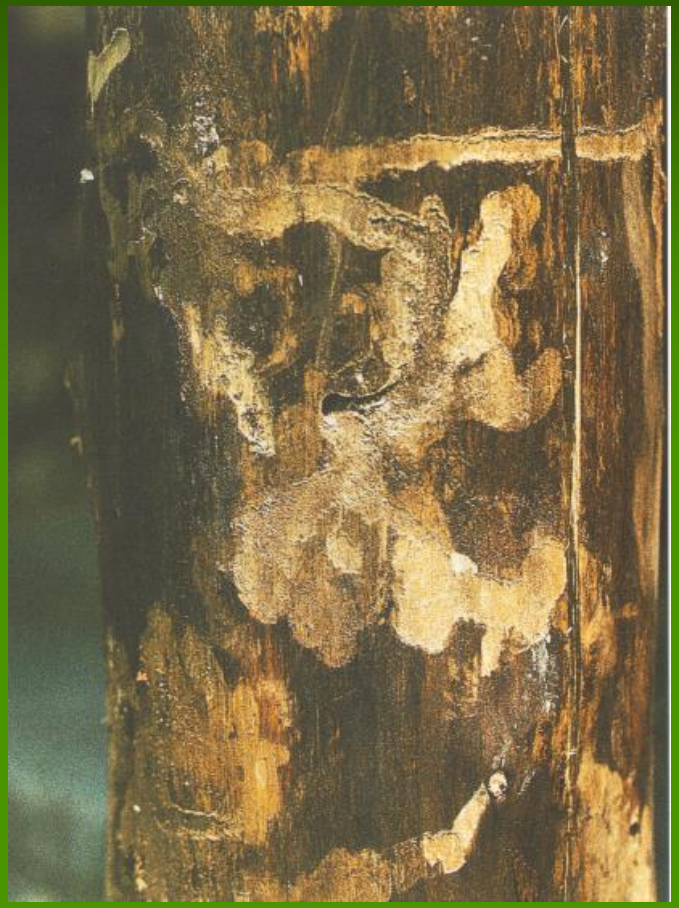
- nematode multiplies & blocks resin canals
- reducing oleroresin production, defence mechanism & transpiration from leaves
- cause yellowing & wilting of needles & death of tree (40 days since infection)


Bus
Stop

*Monochamus alternatus* - main vector in Far East

Feeding area of *Monochamus alternatus* on young pine branch

B. xylophilus in insect tracheas



A

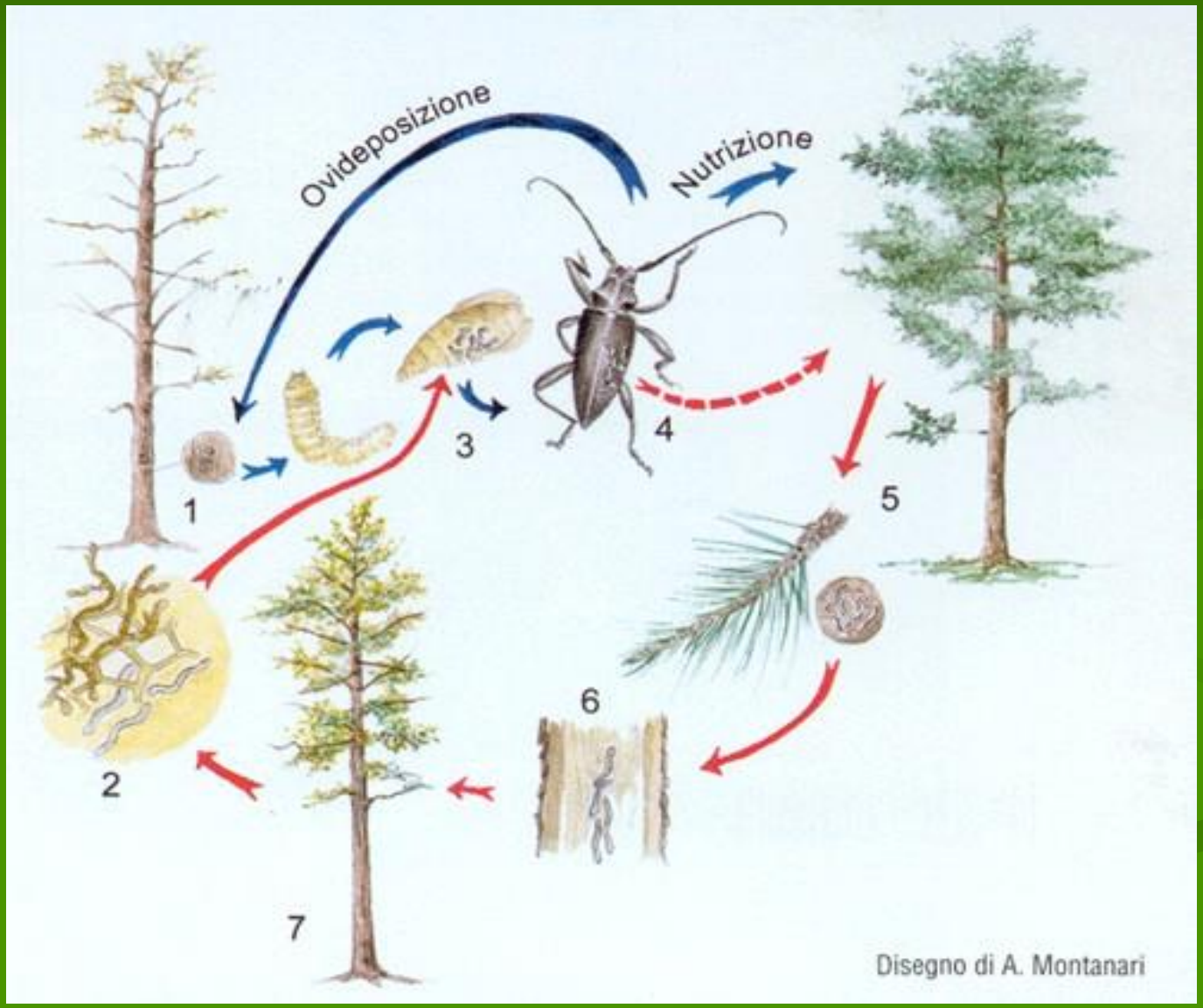
B

Feeding area of *Monochamus* spp.
A. under bark  B.  in wood


# Symptoms on wood

# Wood sample

# Life cycle


Ovideposizione
Nutrizione
1
2
3
4
5
6
7
Disegno di A. Montanari

*Monochamus* only oviposits on felled or trees under stress. Feeding larvae produces tracts in wood making it unsaleable.

Without vector *Bursaphelenchus* is incapable of dispersal to other host trees

Infested wood most probable means of international transport. Nematodes move actively from wood chips or sawdust

BURSAPHELENCHUS MUCRONATUS N. SP.

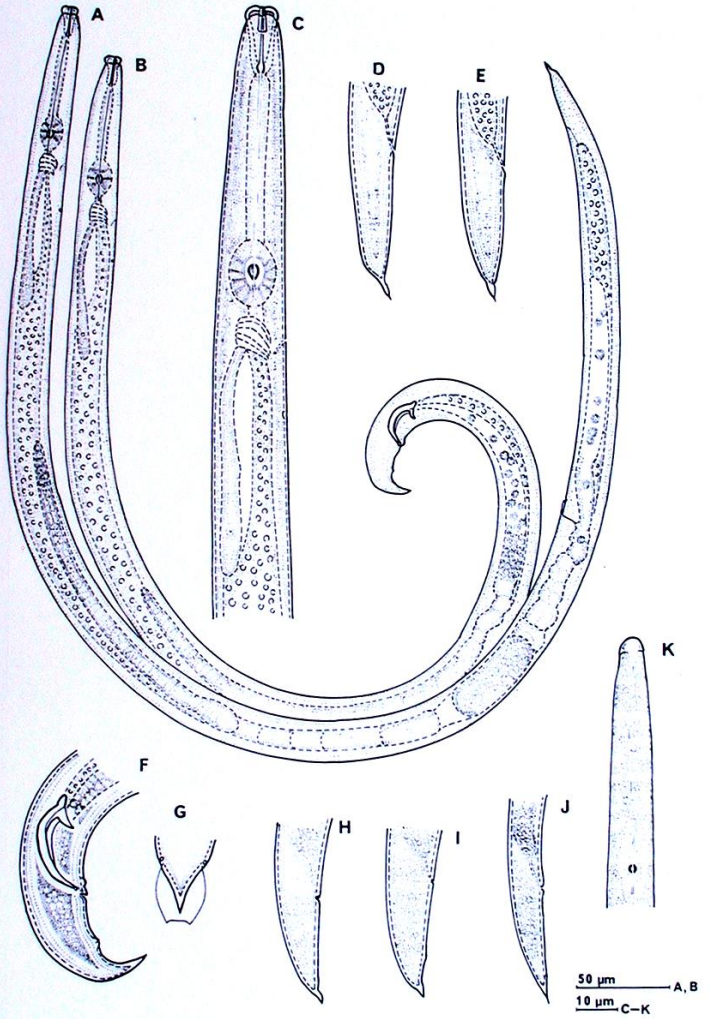



Fig. 1. *Bursaphelenchus mucronatus* n. sp. A. Female. B. Male. C. Female, anterior portion. D, E. Female tail. F. Male tail. G. Ventral view of male tail, tip with caudal alae. H, I. Tail of dispersal third stage larva. J. Tail of dauerlarva. K. Dauerlarva, anterior portion.



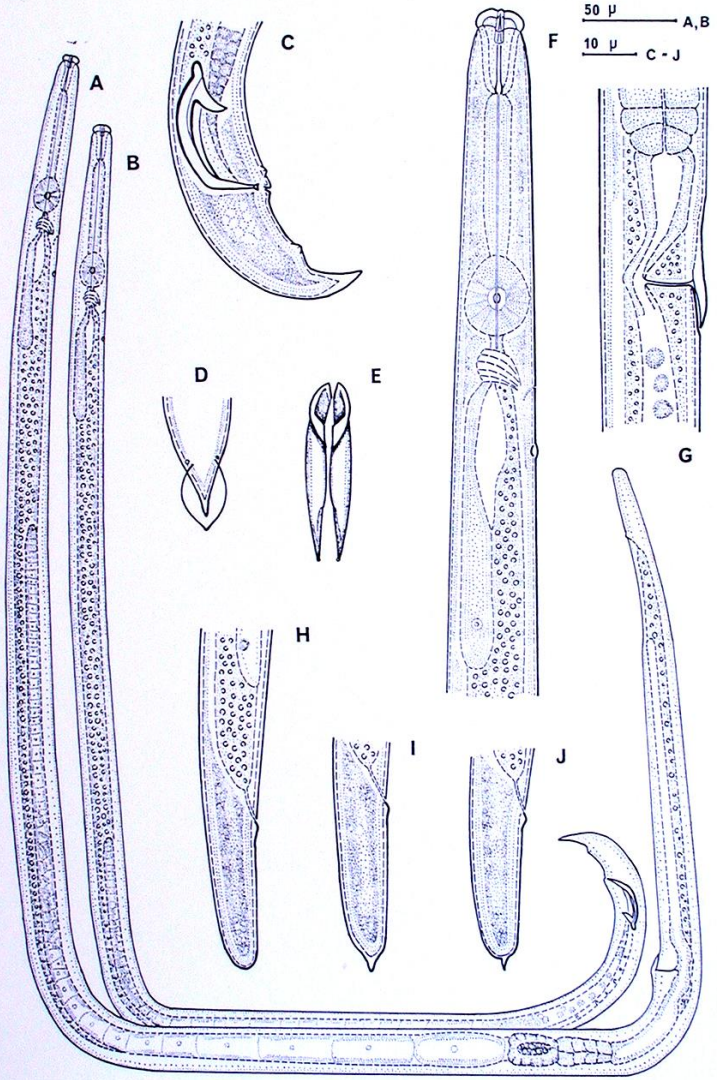
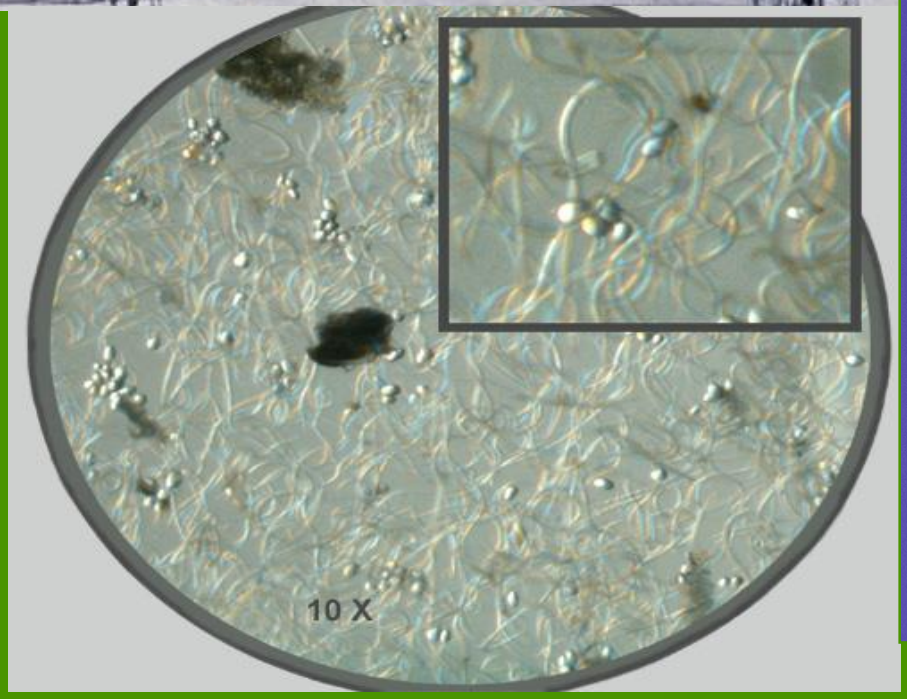


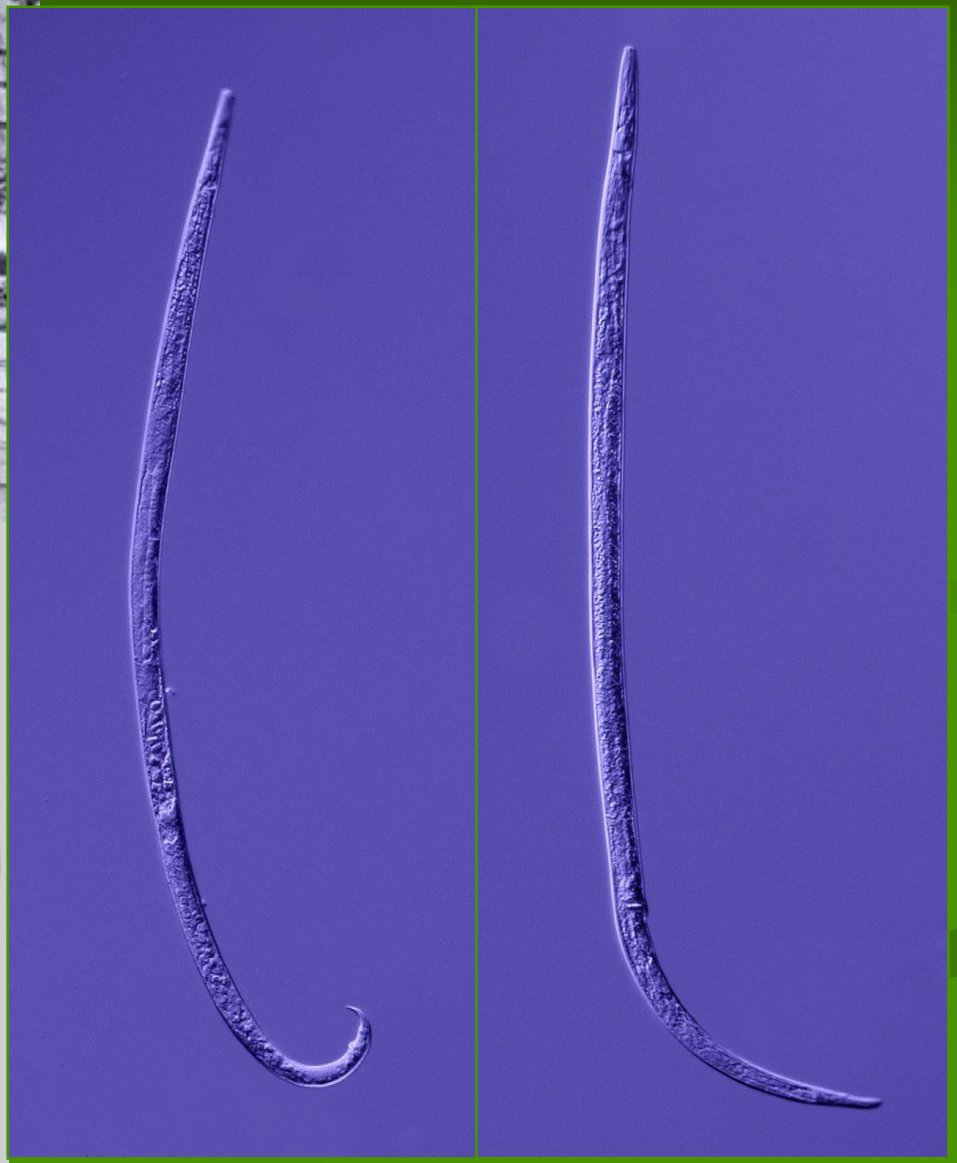

Fig. 1. *Bursaphelenchus lignicolus* n. sp. A. Female. B. Male. C. Male tail. D. Ventral view of male tail, tip with caudal alae. E. Ventral view of spicules. F. Female, anterior portion. G. Female. vulva. H-J. Female tail.

| *Bursaphelenchus* species | Spicules | Female tails |
|---|---|---|
| *B. leoni* |  |  |
| *B. silvestris* |  |  |
| *B. eidmanni* |  |  |
| *B. hofmanni* |  |  |
| *B. paracorneolus* |  |  |
| *B. hellenicus* |  |  |
| *B. sachsi* |  |  |
| *B. nuesslini* |  |  |
| *B. pinasteri* |  |  |
| *B. chitwoodi* |  |  |
| *B. abietinus* |  |  |
| *B. idius* |  |  |
| *B. cryphali* |  |  |
| *B. teratospicularis* |  |  |

| *Bursaphelenchus* species | Spicules | Female tails |
|---|---|---|
| *B. xylophilus* |  |  |
| *B. mucronatus* European genotype |  |  |
| *B. mucronatus* East Asian genotype |  |  |
| *B. fraudulentus* |  |  |
| *B. sexdentati* |  |  |
| *B. poligraphi* |  |  |
| *B. pinophilus* |  |  |
| *B. incurvus* |  |  |
| *B. naujaci* |  |  |
| *B. piniperdae* |  |  |
| *B. 'borealis'* |  |  |
| *B. fungivorus* |  |  |
| *B. eggersi* |  |  |
| *B. tusciae* |  |  |
| *B. glochis* |  |  |

# *B. xylophilus*



Control: Impossible to control *Bursaphelenchus* once introduced into a tree.

Phytosanitary measures: Coniferous plants should be prohibited from infested countries. If not, wood should be heat treated to a core temperature of 56 C for 30 minutes.