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Technical Information Bulletin 9

Potato Cyst Nematode *Globodera spp.*

Javier Franco

Developing females of potato cyst nematodes on potato roots (4x)

INTERNATIONAL POTATO CENTER (CIP)

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Technical Information Bulletin 9

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Potato Cyst Nematode *Globodera spp.*

Objectives. Study of this bulletin enables you to:

- discuss the damage caused by potato cyst nematodes,
- describe symptoms,
- point out taxonomic classification,
- describe morphology,
- explain life cycle and biology,
- discuss potato plant – cyst nematode relationship,
- explain methods to determine nematode population density,
- list and discuss methods of prevention and control.

Study materials

- Plants infested with developing females and cysts of potato cyst nematodes.
- Illustrations showing damage and symptoms.

Exercises

- Observe and discuss damage and symptoms of potato cyst nematodes in the field.
- Estimate field infestation with the two field methods described in section 7.
- Collect some cysts, place them on a glass slide, open them by squeezing with a needle, observe the content (eggs and juveniles) under a simple microscope or a good hand lens.

Questionnaire

1 What is the host range of potato cyst nematodes?

2 What is the distribution of potato cyst nematodes worldwide; in your country?

3 Why is potato cyst nematode damage often deceptively inconspicuous?

4 What symptoms are caused by potato cyst nematodes?

5 Name the two potato cyst nematode species.

6 What is the most obvious difference between the two species?

7 Describe roughly the morphology of a second stage juvenile, and of a maturing female.

8 Which nematode stage hatches from the eggs?

9 How do potato roots stimulate nematode hatching?

10 How do females develop?

11 How long may eggs inside the cysts remain viable?

12 How much may a potato cyst nematode pupulation increase per year?

13 Describe two principles that demonstrate the adaptation of potato cyst nematodes to the potato host.

14 How is resistance determined?

15 To which two processes are the mechanisms of resistance attributed?

16 What is the principle of tolerance?

17 What are pathotypes?

18 Describe two simple field methods to roughly estimate nematode infestation.

19 Describe the "bioassay" method to determine soil infestation.

20 Which components should an integrated nematode management program include?

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Potato Cyst Nematode *Globodera spp.*

1 Importance
2 Symptoms
3 Taxonomy
4 Morphology
5 Life cycle and biology
6 Potato plant – cyst nematode relationship
7 Determination of nematode population density
8 Prevention and control
9 Additional study

Nematodes are microscopic animals (also known as roundworms, threadworms or eelworms) that occur in a wide variety of habitats, mostly in soil and water. Most live saprophytically on dead or decaying organic matter although a few are parasitic on animals and plants. Two species of potato cyst nematode, *Globodera rostochiensis* and *G. pallida* damage roots of potatoes and cause serious yield losses in some potato producing areas. Other areas are so far free of the pest. Control of the pest requires knowledge about its life cycle and proper crop management.

1 IMPORTANCE

The principal economic crops affected by potato cyst nematodes are potato, tomato, and eggplant. A few additional plants of *Solanaceae* and some other families which include certain weeds may serve as hosts. Thus, the host range is relatively narrow.

Potato cyst nematodes probably originated in the Andean highlands of South America where they evolved along with their principal host, the potato. During the second half of the 19th century they were introduced into Europe. From there they spread to northern temperate countries as well as to high elevations in tropical latitudes.

Potato cyst nematode damage is often deceptively inconspicuous and many times population levels of the pest are masked. Nematode populations in soil may increase 10-fold yearly while damage only becomes obvious at a certain infestation level, depending on local conditions, such as soil fertility and adequate water supply. Under low soil fertility, damage may become visible when infestation is 10 to 20 eggs per gram of soil. High soil fertility with adequate water content may mask a far higher soil infestation.

Two types of losses related to nematode infestation are:

- direct, due to yield reduction,
- indirect, from expenses of control and quarantine.

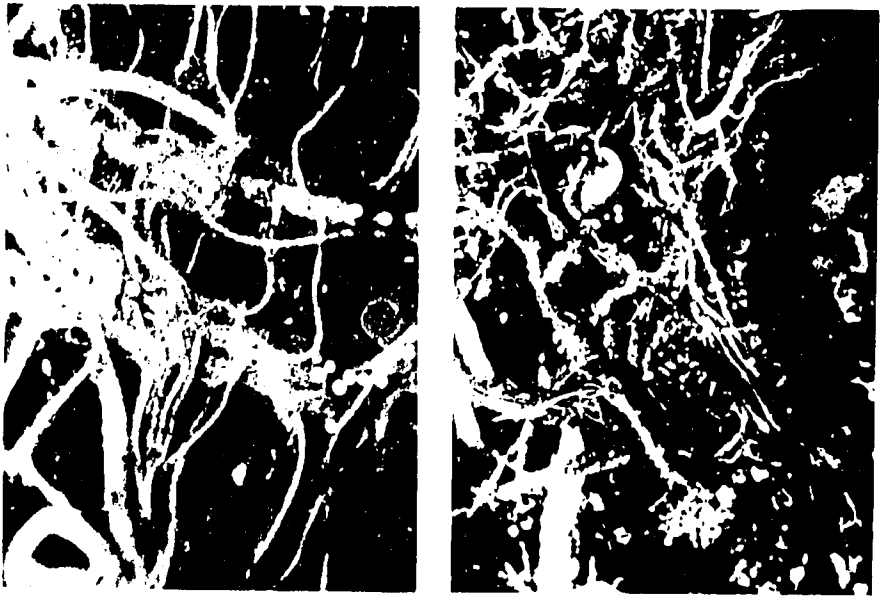
Direct losses. Even without obvious signs of a potato cyst nematode infestation, yield loss can be high. Production losses of up to 15 % may occur without any above-ground symptoms showing in the potato field. Yield may decrease by 2 tons per hectare when infestation increases by 20 eggs per gram of soil. Yield may be less than the amount of seed tubers planted.

Indirect losses. Once established in an area potato cyst nematodes are extremely difficult to eradicate. Lengthy crop rotations to reduce nematode populations mean that potatoes cannot be planted for many years in the same field. Chemical control is expensive, dangerous and not completely effective. National quarantine regulations to control distribution of the pest often restrict production of consumer and seed potatoes.

2 SYMPTOMS

Potato cyst nematodes do not cause immediate above-ground symptoms. The nematodes can remain undetected for years in the soil. First symptom is poor growth of plants in one or more spots in a field. These spots enlarge each year that potatoes are planted. Root growth may be reduced. Plants are usually off-color, stunted, appear unhealthy and wilt readily during the hot, dry part of the day. Lack of competition by these infested potato plants results in more rapid development of weeds. Tubers are smaller and yield is often reduced.

Careful examination of roots reveals tiny round bodies about 0.5 to 1 mm in diameter, and colored white, yellow or brown. Color depends upon nematode species and degree of maturity of the females which form the cysts. Cyst are easily detached from the roots.



Careful examination of roots reveal tiny spherical bodies about 0.5 to 1 mm in diameter, and colored white, yellow or brown, depending on female maturity and species (left *G. pallida*, right *G. rostochiensis*).

3 TAXONOMY

Potato cyst nematodes belong to the class *Nematoda*. They have been recently assigned to the genus *Globodera* because of the round, globular shape of their cysts. (Previously they belonged to the genus *Heterodera*, which characteristically have lemon-shaped cysts). Eleven species of *Globodera* affect plants of several families. Two species, *G. rostochiensis* and *G. pallida* attack potatoes. Common names for either one or both species are golden nematodes, potato root eelworms or potato cyst nematodes.

The most obvious difference between the two species is the color of immature females. *G. rostochiensis* forms yellow or golden immature females hence the name "golden nematode". Females of *G. pallida* are white or cream colored. Females of both species later convert into brown cysts, but *G. pallida* females do not pass through the "golden" stage.

Other taxonomic characteristics include the mouth spear (*stylet*) with its posterior knobs.

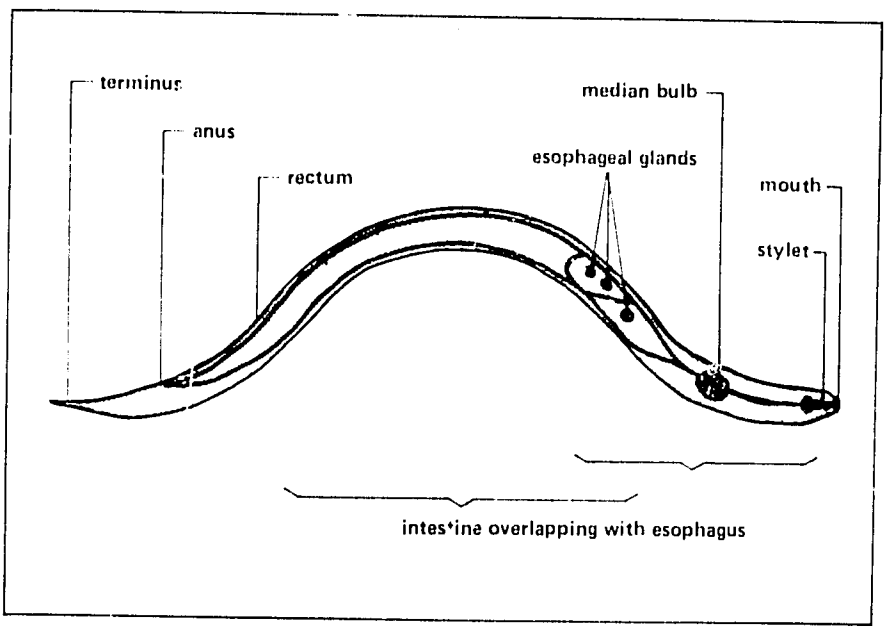
G. rostochiensis	stylet 19-21 μm long, knobs pointing backwards.
G. pallida	stylet 23-24 μm long, knobs pointing forward.

Both species occur in the Andean countries, while *G. rostochiensis* predominates in countries outside South America.

4 MORPHOLOGY

The second juvenile stage of potato cyst nematodes is characteristic for nematode morphology. This stage, that can only be studied with the aid of a microscope, resembles a typical elongate roundworm. The alimentary canal consists of mouth, esophagus, intestine, rectum, and anus.

Most obvious is the stylet within the mouth. This is a hard, hollow and motile mouth spear. At its posterior end, muscles are attached to three characteristic knobs. The stylet serves to perforate root cells and to absorb food. It leads to the esophageal tube containing the median bulb.

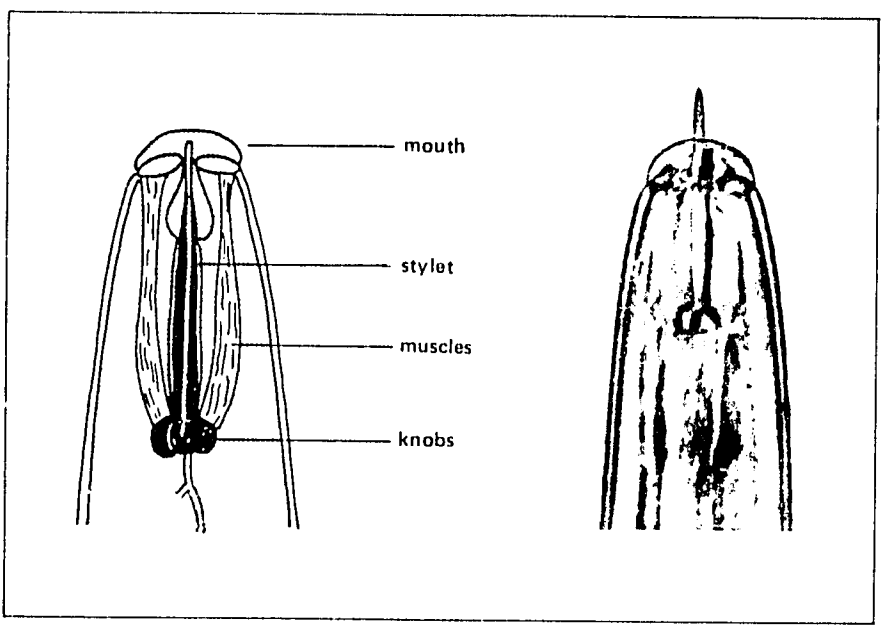


The second juvenile stage is characteristic for potato cyst nematode morphology.

With the aid of muscles and a "valve", the median bulb functions as a pump, forcing the food into the intestine. Following the median bulb, three esophageal glands form a terminal bulb continuous with the intestine. The intestine as a storage organ is usually filled with globules of an apparently fatty substance. It narrows to a rectum terminating at the anus.

Males preserve this elongate roundworm-like shape. When mature, they are about 1 mm long.

The female body, upon maturity, swells and after death converts into a tough and leathery cyst. Cysts are spherical (globular) and about 0.5 to 1 mm in diameter. A protruding "pin" corresponds to the former head, that was attached to the potato root.



Under microscopic examination an obvious organ is the stylet within the mouth of the potato cyst nematode. It is a hard, hollow and motile mouth spear. Muscles attached to the posterior knobs move the stylet backward and forward to perforate root cells.

5 LIFE CYCLE AND BIOLOGY

Unlike insects, nematode larvae pass through their various development stages without changes in external appearance. Since larvae resemble adults, they are called *juveniles*.

The life cycle begins with second stage juveniles that emerge from eggs inside the cysts. Hatching of the second juvenile stage is stimulated by a substance exuded from growing potato roots. Some eggs remain within the cyst and hatch during the following seasons.

Attracted by root exudates, second stage juveniles puncture and then penetrate into the roots, feeding and developing during two additional molts.

During the third juvenile stage, the sex of potato cyst nematodes is determined by the amount of nutrition available. With only a few nematodes and an abundant food supply, the population is predominantly female. If population is high and food supply limited, males predominate.

Females become sedentary and attach within the potato root surface. Their body swells, ruptures the potato root cells and becomes visible outside the root. Head and neck remain attached, buried inside the root.

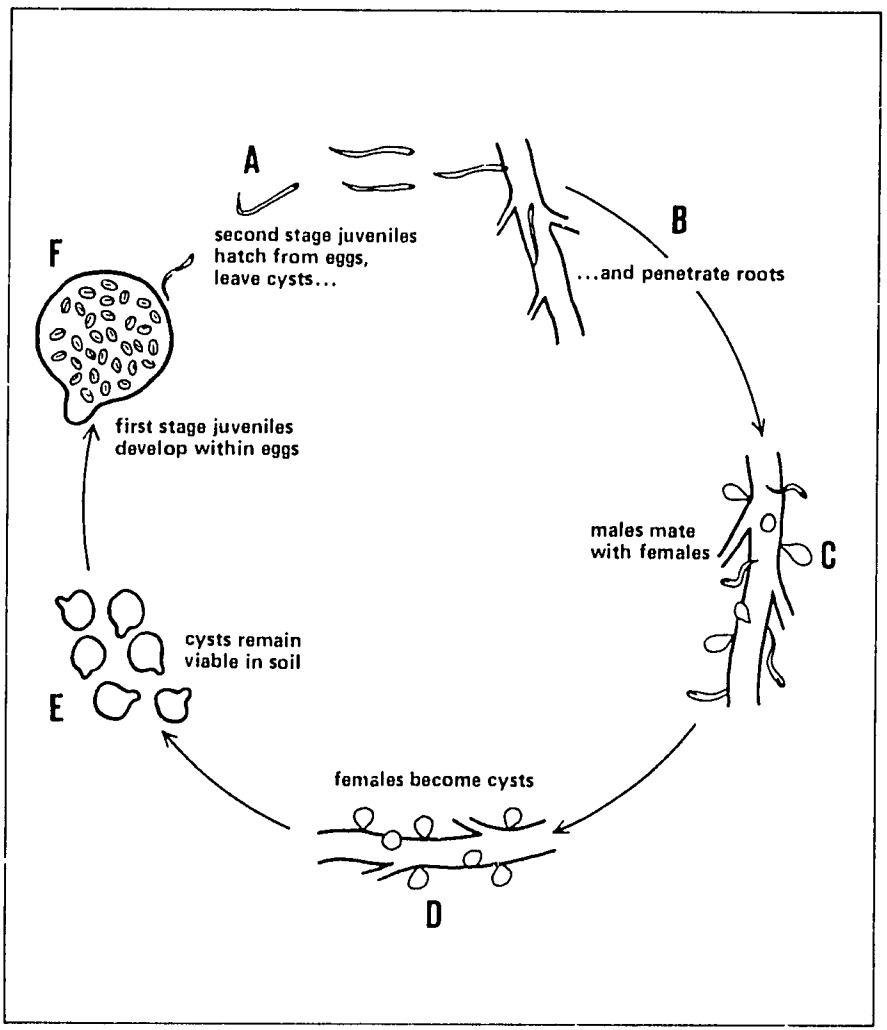
Males retain their original slender eelworm-like shape. They leave the roots, locate and mate with females that just rupture the root surface.

After the female dies, the cuticle of the originally white or yellow and spherical body changes chemically. It "tans". The dead female becomes a brown, hard cyst, resistant to unfavorable environmental conditions.

Cysts are easily detached from roots. Each may contain and protect from a few to up to 600 eggs. Eggs are additionally protected by individual egg shells and remain viable up to 20 years or more. The eggs may become active whenever potatoes are planted.

Still protected by the egg shell and cyst wall, the first juvenile stage develops within the eggs. The second juvenile stage emerges upon stimulation by root exudates.

One life cycle, that is, one generation, occurs per season, taking 6 to 10 weeks. During this cycle, when there is no competition for food, a population may increase up to 50-fold.



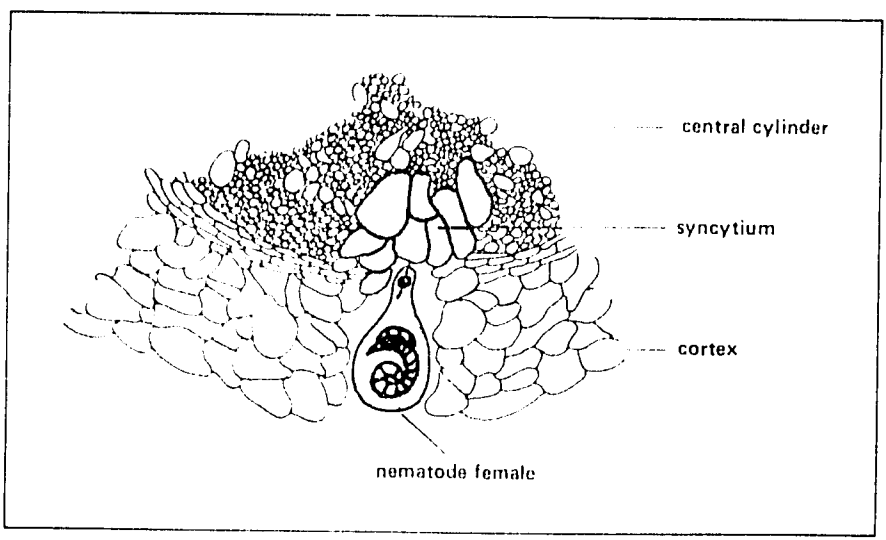
One life cycle of potato cyst nematodes that is one generation occurs per season, taking 6 to 10 weeks. Stimulated by root exudates, second stage juveniles hatch from eggs inside cysts (A). Juveniles penetrate roots (B). Female bodies protrude outside the roots. Males leave the roots and mate with the females (C). Dead female bodies become cysts (D). Cysts are easily detached from roots and remain viable in soil for more than 20 years (E). First juvenile stage develops within eggs, protected by egg shell and cyst wall (F).

6 POTATO PLANT – CYST NEMATODE RELATIONSHIP

Potato cyst nematodes are well adapted root parasites. The stimulating effect of the root exudate of the host assures that nematodes emerge only under favorable conditions where they can find potato roots. Second stage juveniles cut through the cell walls using the mouth spear and enter roots leaving a trail of ruptured cells.

Saliva excreted by the esophageal glands causes cells near the female's head to enlarge and coalesce. These enlarged cells, called *syncytia* or *transfer cells*, provide females with a permanent food supply and are necessary for nematode development. Development and maintenance of these transfer cells competes with plant growth.

Additionally, nematode damage causes water stress and disturbance of nutrient metabolism.



Enlarged cells, called *syncytia* or *transfer cells*, provide females with a permanent food supply.

Relationship of the potato host and the potato cyst nematode is controlled by:

- resistance of a potato variety,
- tolerance of a potato variety,
- pathogenicity of the nematode.

Environmental factors, such as soil fertility and other growth conditions, may alter this host-nematode interrelationship.

Resistance. A potato plant may or may not support multiplication of nematodes, depending on its degree of resistance. Resistance is determined by the relationship between initial nematode population density before planting and final nematode population density at the end of a potato growing season. This relation allows to calculate the nematode multiplication rate in the following form:

$$\text{nematode multiplication rate} = \frac{\text{final nematode population density}}{\text{initial nematode population density}}$$

where generally

nematode multiplication rate $>$ 1 indicates susceptibility,
nematode multiplication rate $<$ 1 indicates resistance.

Resistance results in reduction of a nematode population. The degree of resistance for specific cases depends on the local situation and on breeding material available.

The mechanisms of resistance are attributed to two processes. In the first process, roots may fail to stimulate emergence of second stage juveniles resulting in reduced hatching.

The second process restricts or inhibits formation of *transfer cells* that provide females with food. Restricted development of transfer cells does not prevent hatching. Hatched juveniles invade the roots and cause damage. However, restricted food supply disrupts the nematode life cycle. Juveniles either die or develop into males. This leads to a more rapid decrease of nematode population and is more effective than reduced hatching.

Any type of resistance may break down. When resistant varieties are planted continuously under conditions of high nematode population density, compatible pathotypes may expand due to natural selection or genetic adaptation.

Tolerance. Tolerance is ability of a potato variety to yield despite nematode infestation. It may occur in resistant as well as in susceptible varieties; thus it is independent from resistance. Non-tolerant plants suffer yield loss. Tolerant varieties are able to compensate for nematode damage.

Tolerance is independent from pathotype of the nematode. It appears more in the andigena varieties of potato (*Solanum tuberosum* ssp. *andigena*) than in tuberosum varieties (*S. tuberosum* ssp. *tuberosum*) and may have evolved because in the Andean regions both the (andigenous) potatoes and the nematodes coevolved. To survive cyst nematodes the potatoes developed tolerance. Tuberosum varieties have been developed in other areas where the potato cyst nematodes were not native.

Pathogenicity. In both species of *Globodera* (*G. rostochiensis* and *G. pallida*) several pathotypes occur. Pathotypes are physiological races that can be distinguished by their ability to multiply on so-called differential potato plants. These plants have different genes for resistance. Thus a certain differential plant may become increasingly infested by certain pathotypes of *Globodera*, but not by others.

The same is true for potato varieties. A supposedly resistant potato variety may become infested by increasing numbers of nematode populations due to selection and multiplication of other *Globodera* pathotypes.

Usually, pathotypes within each *Globodera* species interbreed freely. However, interbreeding between species is restricted.

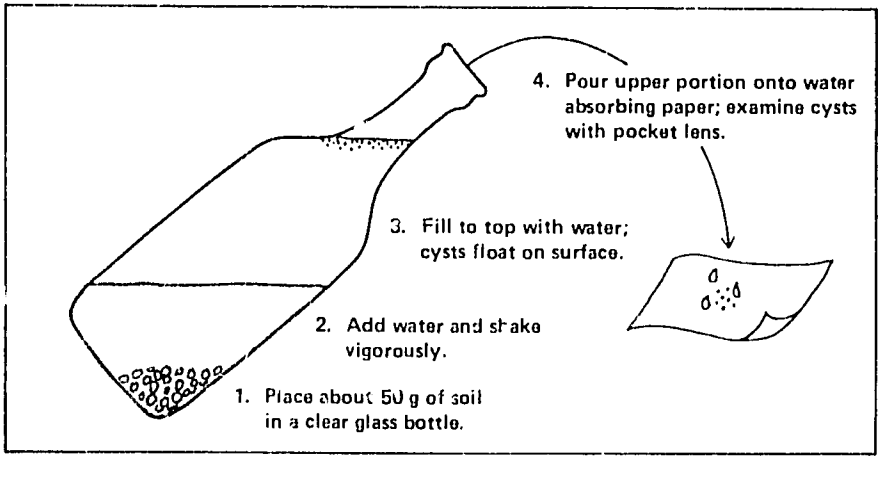
Several systems for differentiation of pathotypes exist.

7 DETERMINATION OF NEMATODE POPULATION DENSITY

Knowing population density of potato cyst nematodes in the soil is important for successful management control procedures. The most common methods of determining nematode population density are soil analysis and root observation.

Soil analysis. Soil is analyzed by a nematological laboratory using soil samples the farmer has furnished from his fields. The laboratory analyzes the samples by suspending the dried soil in a container of water and counting the number of cysts that float on the surface. A sample of cysts is opened and number of eggs and juveniles counted to determine total viability.

A simple field method adapted from soil analysis provides a rough estimate of nematode infestation. Place a handful (about 50 g) of well mixed soil from different parts of the field in a clear glass bottle. Add water enough to thoroughly moisten the soil and shake bottle vigorously. Fill the bottle to near the top with water. Cysts in the sample will float on the water surface indicating the amount of cysts in the sample. Pour this upper portion onto water-absorbing paper so that cysts can be examined more closely or counted by using an ordinary pocket-lens.



Simple method to estimate nematode infestation.

Root observation. A *bioassay* method is used at experiment stations to determine soil infestation. Some 2 or 3 months before planting, sprouted tubers are placed in pots containing samples of soil from the field being checked. If the field is infested, the round bodies of developing females of potato cyst nematodes can be detected visually on the ball of roots after 8 weeks.

A farmer may roughly estimate field infestation by examining roots of plants shortly before flowering time. At this stage of plant development, females have ruptured the root cortex and the round ball-like bodies are visible to the unaided eye (see cover photograph).

Critical infestation levels depend on local situations. Practical experiments, comparing untreated plots with nematicide treated plots may help to determine at which infestation level potato yield begins to decline.

8 PREVENTION AND CONTROL

When symptoms do become visible nematodes are already present in great numbers. Once established, potato cyst nematodes are difficult if not impossible to eradicate. Methods are available to minimize damage. Successful nematode management combines prevention and control in an integrated program. Integrated nematode management includes also components of proper crop production (irrigation and fertilization, for example) to

- prevent spread of potato cyst nematodes in new areas, and
- keep nematode population density below damaging levels in infested areas.

Integrated nematode management can reduce a high nematode population density to levels that permit successful and profitable potato production.

Quarantine regulations. Spread of potato cyst nematode occurs mainly through potato tubers contamined with cysts or soil containing cysts. Soil adhering to other plant material and on implements or soil transported by water and wind may also be a means of spreading the pest. Many countries apply strict quarantine regulations to prevent introduction of the pest. As a further means of limiting in-country spread, potato seed should not be produced in and disseminated from infested areas.

Sanitary principles. Plant seed tubers only from uninfested areas. Prevent spread from one field to another through cleaning of containers, tools and machinery by washing and brushing, or by treatment with nematicides. Cultivate clean fields first, prior to entering infested areas.

Crop rotation. Crop rotation is the most widely practiced control measure. It is effective because the host range of potato cyst nematodes is limited. Population density may decrease each year by about 30 % when host plants are not available. However, eggs may survive for more than 20 years. The usual rotation is for 5 or 6 years without potatoes or other host plants.

Early or rapidly developing potato crops may mature before the nematode life cycle is completed. These crops are not good nematode hosts and properly used may help shorten the crop rotation cycle.

Physical control. Several physical control methods – such as heat treatment – are possible under experiment station or greenhouse conditions. Exposure of the plowed field to sun during dry and hot seasons helps to reduce nematode populations.

Use of resistant varieties. Because they stimulate nematode hatching without providing a subsequent sufficient food supply, some resistant potato varieties may reduce nematode density more rapidly than a non-host crop. Resistance, however, is only effective against certain pathotypes. Potato clones or varieties that are resistant to pathotypes of *G. rostochiensis* predominate; resistance against *G. pallida* pathotypes is less frequent.

Repeated use of resistant varieties may result in loss of effectiveness due to increase of other pathotypes. Use of resistance depends on the pathotype spectrum prevailing in a certain production area. For an experiment station it is easy to evaluate resistance of potato varieties or breeding material. Also in the field, the resistance of varieties can be easily compared.

New promising sources of resistance, mainly from *Solanum vernei* and *S. tuberosum* ssp. *andigena*, are under investigation at CIP and elsewhere.

Chemical control. Chemicals to control nematodes, called "nematicides", are not very effective and last only a short time. They are expensive and toxic to men and environment.

Two general types of nematicides are used, *fumigants* and *non-fumigants*.

Fumigants include halogenated aliphatic hydrocarbons and methyl isothiocyanate liberators. Their use requires special applicators. Most of them are phytotoxic. Toxicity is efficient against many soil organisms, such as fungi and bacteria, as well as nematodes.

Non-fumigants include organophosphates and organocarbamates. Granular formulations are easily applied. Phytotoxicity is rare, but toxicity to man and animals (mammals) is high. Non-fumigants are less efective against soil fungi and bacteria, but they also control insects.

For specific recommendations consult local experts.

9 ADDITIONAL STUDY

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