CONTRIBUTS CONCERNING GLOBODERA SPECIES STUDY (CYST NEMATODES) IN POTATO CROPS FROM SUCEAVA DISTRICT

CONTRIBUȚII PRIVIND STUDIUL SPECIILOR DE GLOBODERA (NEMATOZII CU CHIȘTI) ÎN CULTURILE DE CARTOF DIN JUDEȚUL SUCEAVA

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Abstract. *Globodera rostochiensis and Globodera pallida are the two species of potato cyst nematodes which cause major losses in potato crops (van Riel and Mulder, 1998). The infective juvenile nematodes only move a maximum of about 1 m in the soil. Most movement to new localities is by passive transport. The main routes of spread are infested seed potatoes and movement of soil (e.g. on farm machinery) from infested land to other areas. Infection occurs when the second-stage juvenile hatches from the egg and enters the root near the growing tip by puncturing the epidermal cell walls, and then internal cells in the pericycle, cortex or endodermis. The nematode induces an enlargement of root cells and breakdown of their walls to form a large, syncytial transfer cell. This syncytium provides nutrients for the nematode. Infested potato plants have a reduced root system and, because of the decreased water uptake, plant death can eventually occur.*

Rezumat. *Globodera rostochiensis şi Globodera pallida sunt două specii de nematozi cu chişti ai cartofului care determină pierderi importante culturilor de cartof (van Riel şi Mulder, 1998). Nematodul juvenil infectiv se deplasează maxim aproximativ 1 m prin sol. Deplasarea către noi locații se face prin intermediul transporturilor pasive. Principala cale de răspândire o constituie cartoful de sămânță infestat precum şi solul (ex. deplasarea solului cu maşinile de lucru) din parcelele infestate în alte suprafețe. Infecția are loc când al doilea stadiu juvenil eclozează din ou şi intră în rădăcină lângă punctul de creştere prin înțeparea pereților celulelor epidermei şi apoi a celulelor interne cu ajutorul stiletului. Eventual el începe să se hrănească pe celulele periciclului cortexului sau endodermului. Nematodul induce o mărire a celulelor rădăcinii şi ruperea pereților celulelor pentru a forma o celulă mare de transfer numită sinciţiu. Acest sinciţiu furnizează nutrienții necesari nematodului. Plantele de cartof infestate prezintă un sistem radicular redus, şi datorită conținutului scăzut al apei plantele pot chiar să moară.*

MATERIAL AND RESEARCH METHOD

The fields which I made the Globodera species study is located in Calafindeşti village near the Suceava district. The period for this study was included between 2001 and 2004 years. The research purpose was an possibly detection of potato cyst nematodes because in the past near this region it was recorded this quarantine fitosanitary nematodes. The basic requirements of soil sampling to detect or estimate potato cyst nematodes in the soil are that:

1. the final sample examined in the laboratory is large enough to achieve the required accuracy and/or sensitivity;

2. the sample is derived from sufficient points to ensure that it is representative of the area sampled, i.e. as far as possible heterogeneity (patchiness) in the nematode distribution is overcome;

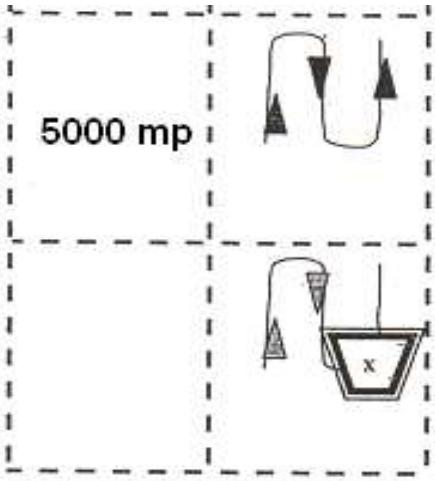
3. the laboratory processing procedures are as efficient and free from operator error as possible, so as to give accurate and consistent results.

The method which I made to take a sample of soil was: take a sample of soil abaut 400 ml (500 g) from 5000 m² area (plough) and 5 cm depth. The sample of soil was analysed in the Suceava Nematology Laboratory and it was made from 100 cores and each of thease cores had abaut 4-5 ml.

The materials which I made to take samples of soils was:

- un auger with 2-3 cm in diameter;
- polietinen bags, permanent markers;
- staks for measures 5000 m² areas;
- water and disinfectant to clean footwears and equipment before to take samples from anather areas;

In 2001 from 2 ha areas which I studied, I took 4 samples of soils. The samples was takes from all the surfaces, like in this sketch (fig.1):



All the informations about the surfaces and the dates of soil samples which I took was recorded on a piece of sheet. On the same sheet I made a sketch of the surfaces which present the distribution of subareas, the place which I took the samples and some characteristic points (the trees, the pillar, the road, the hedge etc.). The bags with samples of soil was closed to forestall some possibly crossed contaminate. This is a method which gave firmness concerning with the manner of took the samples.

Fig. 1. The sketch to takes samples of soil

For extractions of potato cyst nematodes I use Centrifugal method and Arvo can.

This is a semi-automatic extraction method for soil samples developed by J. Schuiling. I made about 250 dried-soil which was sift by an sieve with 4 mm eyes in diameter. The soil-dried is added to a transparent cylindrical container half-filled with water. The contents are swirled with a rotating two-pronged fork at 450-500 rev/min., creating a vortex and causing cysts and similar- sized floating particles to be forced to the centre through a wire-mesh cylinder with 1,5 mm aperture in diameter. The mesh cylinder is fixed above a tube of the same diameter leading down to an autlet. While swirling, more water is added around the inside of the main container washing off any adhering debris and cysts which are channeled to the outlet with the rest. The apparatus cleans itself after each sample processing. The apparatus requires only about 6 l of water per sample. After that the contents was pass through a siev (250 µ) in a funnel in which I put a filter paper about 30 cm in diameter and I filled the filter paper from funnel with water until 2 cm from the top of filter.

The potato cyst nematodes was retrieve from filter paper by examine it at Leica MZ 9,5 stereomicroscope. After that I obtain the next results, like in table 1 and table 2.

RESULTS AND DISCUSSIONS

After extractions and retrieved the potato cyst nematodes from the filter paper, the next step was identification of the species which I found. By this way, I used an optic microscope, Leica DMLB2 respectively, with an DFC 290 foto camera. To see the morfhologycal details, I cut the potato cyst nematodes with an scalpel and I made an perineal patern of vulval zone with all the identification feature: the fenestra, the anus and the cuticular ridges.

Table 1

Extractions results for potato cyst nematodes from 1 and 2 fields in 2001 year

Field	Surface	Year	Sample	Cysts/Sample	Cysts
1	1,5 ha	2001	1	1	1
			2	0	
			3	0	
			4	0	
2	2 ha	2001	5	1	29
			6	25	
			7	3	
			8	0	

Table 2

Extractions results for potato cyst nematodes from 1 and 2 fields in 2004 year

Field	Surface	Year	Sample	Cysts/Sample	Cysts
1	1,5 ha	2004	1	0	0
			2	0	
			3	0	
			4	0	
2	2 ha	2004	5	0	2
			6	2	
			7	0	
			8	0	

Identificaton of these species of potato cyst nematodes I made with micrometric measurements like in table3, table 4 and table 5.

Table 3

Globodera species identification from field 1 in 2001 year

Cyst	No. of cuticular ridges	Anus/ fenestra distance	Fenestra diameter	Granek`s ratio	Viability %	Species
1	19	74,4	21,6	3,4	50	*Globodera rostochiensis*

No. of land: 1
Year : 2001
Surface: 1,5 ha
No. of cysts examined: 1

Table 4

Globodera species identification from field 2 in 2001 year

Cyst	No. Of cuticular ridges	Anus/ fenestra distance	Fenestra diameter	Granek`s ratio	Viability %	Species
1	18	74,4	24	3,1	50	*Globodera rostochiensis*
2	19	64,8	14,4	4,5	50	*Globodera rostochiensis*
3	14	57,6	21,6	2,6	10	*Globodera pallida*
4	25	86,4	19,2	4,5	Non-viable	*Globodera rostochiensis*
5	15	64,8	21,6	3,0	10	*Globodera rostochiensis*
6	20	76,8	19,2	4,0	10	*Globodera rostochiensis*
7	13	52,8	16,8	3,1	Non-viable	*Globodera rostochiensis*
8	20	67,2	16,8	4,0	Non-viable	*Globodera rostochiensis*
9	24	69,6	19,2	3,6	Non-viable	*Globodera rostochiensis*
10	15	57,6	16,8	3,4	Non-viable	*Globogera rostochiensis*

No. of land: 2;
Year: 2001
Surface: 2 ha
No. of cysts examined: 10

Table 5

Globodera species identification from field 2 in 2004 year

Cyst	No. of cuticular ridges	Anus/ fenestra distance	Fenestra diameter	Granek`s ratio	Viability %	Species
1	13	76,8	24,0	3,2	Non-viable	Globodera rostochiensis
2	14	48,0	21,6	2,2	Non-viable	Globodera pallida

No. of land: 2;
Year: 2004
Surface: 2 ha
No of cysts examined: 2

On the slide of microscop I fixed the perineal patern (like in foto 1 and 2) with glycerol and after that I examine it on the microscope at x 100 magnification using the immersion oil.

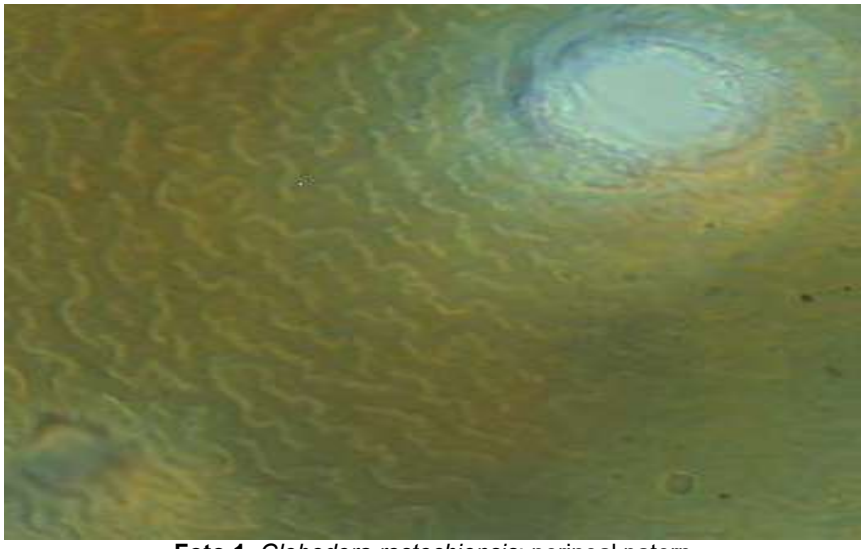


Foto 1. *Globodera rostochiensis*: perineal patern

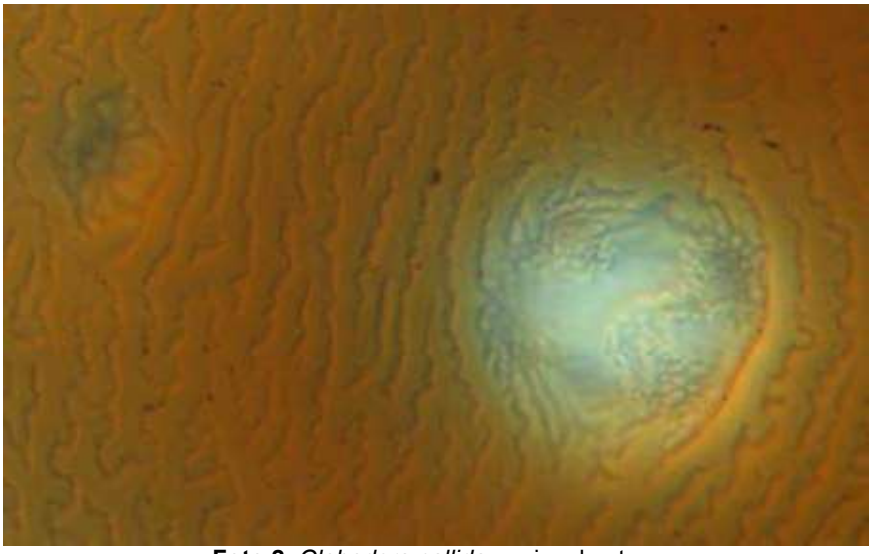


Foto 2. *Globodera pallida*: perineal petern

CONCLUSIONS

1. No sampling method is 100% efficient at detecting potato cyst nematodes in agricultural land. Ultimatly, the chosen method will be a compromise between an acceptable detection level for the type of sample taken and resources available.
2. There is a positive linear relation between potato cyst nematodes sampling intensity and detection exists. For each additional sample taken per given area potato cyst nematodes detection increased by over 7%. In practice is recommended that each additional sample of soil to be taken at higher risk location, e.g. inside gates of lands etc.

3. By the time a potato cyst nematodes is detectable by normal sampling methods, it is already widely distributed throughout the field.

4. However, potato cyst nematodes extraction is stil enough difficult and require a long time and often is conditional by the type of soil and athers factors that reduce the efficiency of this process.

5. In case of fields which I studied for 4 years we can see that if in 2001 the number of potato cyst nematodes was big in field 2 (29 respectively), in 2004 in absence of potato, after 4 years, the number of cyst was only 2.

6. In case of field 1, if in 2001 I detected only 1 cyst, in 2004 in absence of potato, after 4 years we do not detected the potato cyst nematodes.

7. The populations of potato cyst nematodes which I found was mixture and consist of *Globodera pallida* and *Globodera rostochiensis* species, but the *Globodera rostochiensis* population was prevail.

8. In absence of potato, I observed that the number of potato cyst nematodes populations and the viability of these populations was at a low level.

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