

The live collection of sedentary nematodes of the Laboratory of plant parasitic nematodes of the CP IPEE RAS: the purpose of creation and the guidelines to maintenance

Natalia Butorina^{1,*}, Maria Plykina¹, Nikita Pustov¹, Elena Khudyakova², and Anton Ivanov²

¹The Center of parasitology of the A.N. Severtsov IPEE of the Russian Academy of Sciences, 33 Leninsky Prospekt, 119071, Moscow, Russia

²FGBU "VNIKR", 32 Pogranichnaya str., 140150, Bykovo, Russia

Abstract. The Collection of sedentary nematodes of the Laboratory of plant parasitic nematodes of the A.N.Severtsov IPEE of the Russian Academy of Sciences was established three years ago in order to maintain cultures of live nematodes, which are harmful agricultural pests. Currently, the Collection contains two species of root-knot nematodes: *Meloidogyne incognita* Chitwood, 1949 and *Meloidogyne hapla*, Chitwood, 1949, and four cyst-forming species: *Heterodera schachtii* Schmidt, 1871, *Heterodera filipjevi* Stelter, 1984, *Heterodera glycines* Ichinohe, 1952, *Globodera rostochiensis* Skarbilovich, 1959. The Collection is used as a repository of invasive material necessary for conducting research on plant parasitic nematodes. Materials of the Collection are used for demonstration during practical seminars for students, postgraduates and plant protection specialists. Also, according to the existing agreement, the collection material is intended for exchange with colleagues nematologists from specialized scientific research institutions of the Russian Federation. The present publication contains data on the technical equipment of the climatic room for plants, light and temperature conditions, materials used in the creation of the Collection, the species of nematodes maintained in the Collection and the maintenance guidelines.

1 Introduction

The history of the Helminthological laboratory of the USSR Academy of Sciences (Helminthological laboratory), organized by academician K.I.Scriabin, begins in 1942. In 1952, Professor A.A. Paramonov was invited to Helminthological laboratory and research on plant parasitic nematodes began under his leadership [1]. The Center of parasitology of the A.I. Severtsov Institute of Ecology and Evolution of the Russian Academy of Sciences (CP IPEE RAS) – is the legal successor and continuer of the traditions of Helminthological laboratory, and has extensive scientific contacts both in Russia and abroad.

* Corresponding author: nbut@list.ru

The main tasks that the Laboratory of plant parasitic nematodes of the CP IPEE RAS is working on are: studying the species diversity, phylogeny, taxonomy, morphology and biology of both plant parasitic nematodes and free-living nematodes of the Russian Federation; developing methods for their diagnostics; studying of the relationship of nematodes with host plants and other components of the biocenosis: bacteria, fungi and viruses.

The Laboratory of plant parasitic nematodes actively participates in educational programs. On the basis of the Laboratory, students of higher educational institutions of Russia undergo scientific and diploma practice. Interested students have an opportunity to continue in-depth study of nematodes within the framework of individual research projects or enroll in the graduate school of the IPEE RAS in the specialty "Parasitology".

In recent years, a request has been formed to conduct experiments on resistance to nematodes of new varieties of agricultural crops and to test the effectiveness of nematicide preparations.

To solve these problems, it was necessary to create a Collection of live nematodes. In 2021, the Laboratory staff began working on creating a Collection that would completely meet the definition of a "biological collection". According to the definition [2], this is a scientific collection that is formed by a set (fund) of zoological, botanical, microbiological, genetic, and other biological objects and can be used in scientific, scientific-technical, innovative, scientific-educational or educational activities, including those for the purpose of preserving biological diversity and using biological resources. Currently, the Collection has been created and being continually replenished.

The collection serves as a repository for invasive material of plant parasitic nematodes, which are necessary for conducting research experiments by the Laboratory, and for conducting practical classes with students, graduate students and plant protection specialists.

In addition, the Collection represents an exchange fund of invasive material with colleagues nematologists from specialized scientific institutions of the Russian Federation.

2 Materials and methods

Nematode-infected agricultural plants and samples of pre-root soil selected in different regions of Russia: from the Novgorod Region to the Khabarovsk Territory and abroad (Table 1) served as the initial material for the creation of the collection.

To establish the species diversity of nematodes in the source material, the staff of the Laboratory of plant parasitic nematodes of the CP IPEE RAS carried out a morphological analysis of larvae and females of nematodes isolated from the soil and roots of affected plants.

Preparations of perineal pattern plates of root-knot nematodes were made following a modified Taylor-Netcher technique [3, 4, 5]. The analysis was performed employing a ZEISS Primo Star microscope at 400x magnification. The preparations were photographed using a Leica DMC4500 camera mounted on a Leica DM2000 LED microscope.

To definitively confirm the species status of root-knot nematodes present in the collection, a molecular analysis of female nematodes using the direct universal primer JB3 (5' – TTTTTTGGGCATCCTGAGTTTAT – 3') and the reverse universal primer JB5 (5' – AGCACCTAAACTTAAAACATAATGAAAATG – 3') [6], was conducted on the basis of the "VNIKR" All-Russian Plant Quarantine Center".

Commercial universal soil and ceramic pots were used for planting. Prior to that, the soil in 7-liter pots was subjected to heat treatment in a thermostat for 5 hours at 70⁰ C for disinfection. The optimal volume of pots for planting was determined experimentally, in

accordance with the needs of the host plants and the duration of their cultivation period (Table 1).

Soil contamination in pots was carried out by introducing the roots of affected plants and the pre-root soil from the source of infection.

Three-week seedlings of tobacco and tomatoes were planted, and other host plants seeded, in pots with infected soil. Triplicates of each "nematode - host plant" variant were prepared.

The samples of the Collection were labeled and the data about them were entered in the Collection Log.

As the plants aged, new plants were planted or seeded in the same pots.

Watering was performed as needed, usually three times a week.

To estimate the number of invasive larvae and female nematodes in collection pots, an annual revision of the plants and soil of the Collection was carried out following generally accepted methods [7, 8, 9].



Fig. 1. Climatic room for plants of the Laboratory of plant parasitic nematodes of the CP IPEE RAS

To maintain the Collection, a climatic room was organized (Fig. 1). It was equipped with illumination racks with adjustable shelf height and four-channel illuminators based on high-power Edison LED: white (4000 K), blue (450-475 nm), red (650-670 nm) and Deep Pink (420-800 nm). The use of Ledil lenses provided IP67 protection and focusing of the entire light flux in the area of the plant location, which allowed saving up to 30% of electricity for illumination and reducing heat generation. Programming of day-night lighting cycles was provided, which makes it possible to normalize physiological processes in plants. The light mode was set for 8 hours of "daytime mode" from 8.00 to 16.00 and 16 hours and "night mode" from 16.00 to 8.00.

The forced air conditioning system the climatic room was equipped with ensured the air temperature maintained at 22⁰ C.

3 Results and discussion

When creating the Collection, the experience of the Federal State Budgetary Institution "All-Russia Scientific Research Institute of Phytopathology" and the Federal State

Budgetary Institution "VNIIEK" All-Russian Plant Quarantine Center" was taken into account [10, 11]. Greenhouses and lysimeters of these institutions were taken as an example when creating a Collection of sedentary nematodes of the Laboratory of plant parasitic nematodes.

Methods have been adjusted in relation to the conditions of the created Collection. This enabled to reduce the consumption of purchased commercial soil and optimal use of the area of illumination racks.

As a result of the experiments, the volume of soil necessary for growing plants and long-term maintenance of collection samples was optimized. For a number of host plants, both planted with seedlings and seeded: tobacco, tomatoes, a number of root and tuberous crops, 1.5 liters of soil were sufficient, whereas for others (sugar beet, potatoes, carrots, oats, soybeans, strawberries) not less than 5 liters of soil was required (Table. 1).

At the same time, the experience of creating the Collection has shown that despite precautions, there is always a risk of non-target objects, aphids and whiteflies entering the climatic room. Therefore, the climatic room was equipped with yellow glue traps. Contact insecticides were used when aphids and whiteflies appeared. The use of systemic insecticides Actara and Phytoverms for insect treatment, despite their effectiveness, had an extremely negative effect on the nematodes. According to the data obtained, after treatment with these chemicals, the number of nematodes on the roots of infected plants decreased by 70-80%. In some cases, there was a risk of complete elimination of the nematode population.

In order to prevent the complete loss of the living material of Collection of the sedentary nematode in case of force majeure, an exchange of invasive material with the "VNIIEK" All-Russian Plant Quarantine Center"(Bykovo) and the FNCBZR (Krasnodar) is provided.

Optimization of the Collection's maintenance conditions and pest control methods allowed the Collection to be maintained continuously for 3 years.

To date, the laboratory's Collection contains the culture of 4 species of cyst-forming and 2 species of root-knot nematodes (Table 1) - the most harmful plant parasitic nematodes, widespread both in Russia and abroad [12, 13, 14].

Table 1. List of nematode species in the collection of the Laboratory of plant parasitic nematodes.

Nematode species	Host plant	Pot volume, l	Sampling region
Cyst-forming			
<i>Heterodera schachtii</i> , Schmidt, 1871	Sugar beet (<i>Beta vulgaris</i> L.)	1,5	Voronezh region, Russia
<i>Heterodera filipjevi</i> , Stelzer, 1984	Oats (<i>Avena sativa</i> L.)	5	Chelyabinsk region, Russia
<i>Heterodera glycines</i> , Ichinohe, 1952	Soy (<i>Glycine max</i> Merr., 1917)	5	Khabarovsk Territory, Russia
<i>Globodera rostochiensis</i> , Skarbilovich, 1959	Potato (<i>Solanum tuberosum</i> L., 1753)	5	Moscow region, Russia
Root-knot			
<i>Meloidogyne incognita</i> , Chitwood, 1949	Tobacco (<i>Nicotiana tabacum</i> L.)	1,5	Philippines
	Tomato (<i>Solanum lycopersicum</i> L., 1753)	1.5	Philippines
	Celery (<i>Apium graveolens</i> L., 1753)	1.5	Philippines
	Parsley (<i>Petroselinum</i>	1.5	Moscow region, Russia

	<i>crispum</i> Fuss.,1866)		
<i>Meloidogyne hapla</i> , Chitwood,1949	Carrot (<i>Daucus carota</i> <i>subsp. sativus</i> Schubl. & Martens.,1834)	5	Novgorod region, Russia
	Strawberry (<i>Fragaria</i> × <i>ananassa</i> Duchesne & Rozier., 1785)	5	FNCBZR Krasnodar, Russia

At the time of writing this article, nematodes from the Collection were used in experiments and to provide training programs and training for students and specialists: conducting practical classes of the course "Harmful nematodes, ticks, rodents" at Russian State Agrarian University - Moscow Timiryazev Agricultural Academy, at the course "Parasitology" at Lomonosov Moscow State University and training courses for plant protection specialists.

4 Conclusions

Currently, the collection of the Laboratory of plant parasitic nematodes contains 6 species of sedentary plant parasitic nematodes, which are harmful pests of agricultural crops, both in Russian Federation and abroad: *H. schachtii*, *H. filipjevi*, *H. glycines*, *G. rostochiensis*, *M. incognita*, *M. hapla*.

The optimal light and temperature regimes of plant maintenance in the climatic room have been developed.

The optimal volumes of containers for various variants of "host - plant parasitic nematode" have been determined.

The collection of the Laboratory of plant parasitic nematodes serves as a resource for providing education and training programs for students and specialists at the Laboratory of plant parasitic nematodes of the A.N. Severtsov IPEE RAS.

The collection serves as a repository for the inoculum of plant parasitic nematodes necessary for conducting research experiments.

Annual revision, updating and expansion of the Collection is provided due to expedition fees and exchange with the "VNIIEKR" All-Russian Plant Quarantine Center"(Bykovo) and the FNCBZR (Krasnodar).

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