

PRELIMINARY ANALYSIS OF PLANT PARASITIC NEMATODES ASSOCIATED WITH STRAWBERRY AND RASPBERRY CROPS IN THE REPUBLIC OF MOLDOVA

**POIRAS Larisa, Cernet Alexandr, BIVOL Alexei,
POIRAS Nadejda, IURCU-STRAISTRARU Elena**

Abstract. Forty six species of plant parasitic nematodes were found in soils and roots of raspberry (27 species) and strawberry (35 species) crops in the fields of the central regions of R. Moldova. Among plant parasitic species, ectoparasites dominated by species diversity followed by semi-endoparasites and migratory endoparasites. Most damages to the root system were caused by root lesion endoparasite nematodes *Pratylenchus penetrans*, *P. pratensis*, *P. subpenetrans* and *P. neglectus*, stem migratory endoparasite *Ditylenchus dipsaci*, partly spiral nematode *Rotylenchus agnetis*, *R. robustus* and ectoparasites vectors of nepo-viruses *Longidorus elongatus*, *X. diversicaudatum*, *Xiphinema brevicolle*, *X. index*, *X. vuittenezi*, *X. rivesi*. They are spread by the use of infected planting materials and once established in the long term strawberry and raspberry plantations there are any methods of control other than eradication. In new plantations, the most effective control method is to assay soils for plant parasitic nematodes including nepovirus vectors to prevent secondary infection of berry plants.

Keywords: plant parasitic nematodes, species diversity, vectors of nepoviruses, strawberry and raspberry, Republic of Moldova.

Rezumat. Analiza preliminară de nematode fitoparazite asociate cu culturile de căpsuni și zmeură în Republica Moldova. S-au depistat patruzece și sase specii de nematode fitoparazite în plantații productive de zmeură (27 specii) și fragă domestică (35 de specii) în raioanele zonei Centru a R. Moldova. Conform diversității și clasificării fitonematozelor parazite, după spectrul ecologo-trophic predomină speciile ectoparazite, următe de speciile semi-endoparazite și endoparazite migratoare. Grave afectații ale sistemului radicular provoacă speciile de fitonematoze endoparazită tulpinelor *Ditylenchus dipsaci*, parțial însotite de speciile spiralate *Rotylenchus agnetis*, *R. robustus* și speciile ectoparazite-vectori de nepo-virusuri *Longidorus elongatus*, *X. diversicaudatum*, *Xiphinema brevicolle*, *X. index*, *X. vuittenezi*, *X. rivesi*. Aceste specii se extind și se stabilesc vertiginos pe materialul plantat de zmeură și fragă și infectează rădăcinile, formând timp îndelungat populații specifice, care necesită frecvent monitoringul biologic și metode radicale de investigație de prevenire și combatere. În plantații horticole de formare și productive cele mai eficiente metode de evidență sunt analiza solurilor în dinamică sezonieră și anuală, pentru a depista speciile fitoparazite și vectorii de nepo-virusuri, pentru a omite infestările secundare multiple ale plantelor de zmeură și fragă.

Cuvinte cheie: nematode fitoparazite, diversitatea speciilor, vectori de nepo-virusuri, fragă și zmeură, Republica Moldova.

INTRODUCTION

Plant parasitic nematodes destroy the plant tissue during their feeding activity, open the gates for second damage caused by soil-borne fungal and bacterial pathogens (SIKORA & CARTER, 1978) and also the high density of their populations can cause the depression of plants, as well as lead to a qualitative-quantitative yield losses of berry crops. The species of nematodes which have been found associated with strawberry and raspberry are: lesion, dagger, needle, pin, stunt, ring and spiral. The most commonly encountered nematode genera are *Pratylenchus*, *Helicotylenchus* and *Tylenchorhynchus*; however, numerous populations are created by the species *Criconemooides xenoplax* and *Ditylenchus dipsaci* (COEV et al., 1971; NESTEROV & COEV, 1972; ZEHR et al., 1986; GRECO et al., 1991; SAMALIEV & MOHAMEDOVA, 2011).

Moreover, some species fam. *Xiphinemidae* and *Longidoridae* are able to transmit the plant virus diseases (BROWN, 1989; BROWN et al., 1995). Feeding by longidorids causes the necrosis and discoloration of cortical tissues and also galling containing enlarges, modified cells on root tips. Some of longidorids are vectors of plant pathogen viruses. Virus particles are ingested from infected plants during feeding and some become attached at specific sites of retention on the wall of the food canal (TRUDGILL & BROWN, 1978). Viruses may be retained in longidorid bodies from 2.5 to 12 – 24 months (TAYLOR & BROWN, 1997). In continuous cultivation system the plant parasitic nematodes, especially nematodes vector nepoviruses and soil-borne diseases, become an important constraint in strawberry and raspberry production. The economically threshold of damage for *Longidorus elongatus* on strawberry is 15 – 20 individuals on 100 cm³ soil, for *Xiphinema bakeri* on raspberry is 20 - 25 individuals on 100 cm³ soil and *Xiphinema index* 4 individuals on 100 cm³ soil (MCELROY, 1972).

The problem of plant virus diseases and their vectors occurs in R.Moldova now because of the intensive development of agriculture and the increasing number of new and replanted berry plantations. Presently, the private farms want to be engaged in berry crop production and want to use ecological pure techniques. It is necessary to grow healthy plant material from virus-free mother plants and propagate it on soil free from virus-vector nematodes (STEGARESCU, 1972; CERNET et al., 2003; CALASEAN & RAPCEA, 2004; POIRAS & CERNET, 2004; POIRAS et al., 2004; POIRAS, 2012). In R. Moldova, the raspberry and strawberry crop losses due to plant parasitic nematodes and virus diseases were studied by LEMANOVA (1970), VERDEREVSKAYA, LEMANOVA, NESTEROV (1970), POLINKOVSKY (1979), VERDEREVSKAYA (1985), VERDEREVSKAYA, CALASEAN & CERNET (1989), also in neighbouring countries ROMANENKO (1993), etc.

In the last years, the changes in the farming practices, change of cultivar assortment and new varieties, and uncontrolled import of planting material enhanced the need for testing the phytosanitary state of berry crops in R. Moldova, as

well as the areas earmarked for planting with certified virus-free planting materials. The importance of this nematode-virus complex is more stringent in case of replantation, which often occurs in R. Moldova. Therefore, synergistically survey of species diversity and densities of plant parasitic nematodes, especially vectors of virus diseases is highly necessary.

MATERIALS AND METHODS

Strawberry (*Fragaria x ananassa*) and raspberry (*Rubus idaeus*) plantations were surveyed in the central regions of the Republic of Moldova during several years. At each berry plantation, root and soil samples were collected from 1 ha in the soil profiles 0 – 20 cm down to 50 cm (BROWN et al., 1990). The criteria used for selecting materials for samples are: 1) visual symptoms of present plant parasitic nematodes and nepovirus diseases at plants, 2) debilitation in plants. Stem nematodes (*Ditylenchus dipsaci*) and virus vector nematodes (fam. *Longidoridae*) were tested by the certification scheme for strawberry (TRUDGILL et al., 1983; TAYLOR & BROWN, 1997; OEPP/EPPO Bulletin 38, 2008; OEPP/EPPO BULLETIN 39, 2009). All soil and root samples have been packed into plastic bags and stored at a temperature of 10 to 15°C.

Plant parasitic nematodes were extracted alive by sieving and decanting using standard methods of brass screens (60, 100, 325 and 500 mesh) and Baermann funnels from a 100 cm³ soil (BROWN & BOAG, 1988; BOAG et al., 1989). Extracted nematodes were fixed on hot 4% formalin (60°C) (BEZOOJEN, 2006). The specimens of plant parasitic nematodes were also picked out and processed to glycerine by method Seinhorst, mounted on permanent slides, labelled with collection data and reference numbers, identified and deposited in a managed collection of nematodes.

The plant parasitic species of nematodes were identified by taxonomic keys (SANTOS et al. 1997; TAYLOR & BROWN, 1997; NICKLE, 1991; SIDDIQI, 2000 etc.) and arranged by nematode classification based on the SSU DNA data (DE LEY & BLAXTER, 2002; PERRY & MOENS, 2006). The plant parasitic nematodes were classified by trophic groups (YEATS et al., 1993) such as feeders of algal and moss, epidermal cells, ectoparasites, semi-endoparasites, endoparasites and ectoparasites vector nepovirus.

According to the European certification scheme, the testing of strawberries and raspberries for the presence of viruses was directly performed using herbaceous and woody indicators of immunological ELISA (OEPP/EPPO Bulletin 38, 2008).

RESULTS

Forty six species of plant parasitic nematodes were found in the soils and roots of raspberry (27 species) and strawberry (35 species) plantations, in the fields of the districts Criuleni and Orhei, R. Moldova. The species of plant parasitic nematodes are grouped into five trophic subgroups: algal, moss and epidermal cells feeders (Tylenchidae), semi-endoparasites (Hoplolaimidae), migratory endoparasites (Pratylenchidae, Anguinidae), ectoparasites (Telotylenchidae, Criconematidae, Paratylenchidae, Nardiidae) including vector of nepoviruses (Longidoridae, Xiphinemaatidae) (Table 1, Photo 1).

Table 1. Species diversity and feeding types of plant parasitic nematodes of raspberry and strawberry in some plantations of the central area of the Republic of Moldova.

No.	Species	Feeding type	Raspberry	Strawberry
class Chromadorea Pearse 1942, order Rhabditida Chitwood 1933, suborder Tylenchina Thorne 1949				
1	fam. Tylenchidae Orley 1880 <i>Tylenchus davainei</i> Bastian 1986	algal, moss	+	+
2	<i>T. minutus</i> Cobb 1983	algal, moss	-	+
3	<i>T. striatus</i> Das 1960	algal, moss	+	-
4	<i>Aglenchus agricola</i> (De Man 1884)	epidermal cells	-	+
5	<i>Coslenchus costatus</i> (De Man 1921)	epidermal cells	+	-
6	<i>Filenchus filiformis</i> (Butschli 1873)	epidermal cells	+	-
7	<i>F. misellus</i> (Andrassy 1958)	epidermal cells	+	-
8	<i>F. thornei</i> (Andrassy 1954)	epidermal cells	-	+
9	<i>Boleodorus impar</i> Khan, Basir 1964	epidermal cells	-	+
10	<i>B. thylacrus</i> Thorne 1941	epidermal cells	+	+
11	<i>Malenchus exiguus</i> (Massey 1969)	epidermal cells	+	+
12	<i>M. fusiformis</i> (Thorne, Malek 1968)	epidermal cells	-	+
13	<i>Lelenchus leptosoma</i> (De Man 1880)	epidermal cells	-	+
14	fam. Dolichodoridae Chitwood 1950 <i>Bitylenchus dubius</i> (Butschli 1873)	ectoparasite	+	+
15	<i>Tylenchorhynchus cylindricus</i> (Cobb 1913) Filipjev 1934	ectoparasite	+	-
16	<i>T. elegans</i> De Man 1876	ectoparasite	-	+
17	<i>Merlinius brevidens</i> (Allen 1955)	ectoparasite	-	+
18	fam. Hoplolaimidae Filipjev 1934 <i>Helicotylenchus digonicus</i> Perry 1959	semi-endoparasite	+	-
19	<i>H. dihydystera</i> (Cobb 1893) Sher 1961	semi-endoparasite	+	+
20	<i>H. erythrinae</i> (Zimmermann 1904)	semi-endoparasite	-	+
21	<i>H. multicinctus</i> (Cobb 1893) Golden 1956	semi-endoparasite	+	+
22	<i>H. vulgaris</i> Yuen 1964	semi-endoparasite	+	+
23	<i>Rotylenchus agnetis</i> Szczygiel 1968	semi-endoparasite	+	+

24	<i>R. robustus</i> De Man 1876	semi-endoparasite	-	+
25	fam. Pratylenchidae Thorne 1949 <i>Pratylenchus crenatus</i> Loof 1960	endoparasite	-	+
26	<i>P. neglectus</i> (Rensch 1924)	endoparasite	-	+
27	<i>P. penetrans</i> (Cobb 1917)	endoparasite	+	+
28	<i>P. pratensis</i> (De Man 1988)	endoparasite	+	+
29	<i>P. subpenetrans</i> (Taylor, Jenkins 1957)	endoparasite	-	+
30	<i>Pratylenchoides crenicauda</i> (Winslow 1958)	endoparasite	-	+
31	fam. Criconematidae Taylor 1936 <i>Xenocriconemella macrodora</i> (Taylor 1936) de Grisse, Loof 1965	ectoparasite	-	+
32	<i>Criconemodes xenoplax</i> (Micoletzky 1922)	ectoparasite	+	-
33	<i>Mesocriconema rusticum</i> (Micoletzky 1922)	ectoparasite	+	-
34	fam. Tylenchulidae Skarbilovich 1947 <i>Paratylenchus crenatus</i> Corbett 1966	ectoparasite	+	+
35	<i>P. hamatus</i> Thorne, Allen 1950	ectoparasite	+	-
36	<i>P. microdorus</i> Andrássy 1959	ectoparasite	-	+
37	fam. Anguinidae Nicoll 1935 <i>Ditylenchus brevicauda</i> (Micoletzky 1925)	ectoparasite	-	+
38	<i>D. dipsaci</i> (Kuhn 1857)	migratory endoparasite	+	+
39	<i>D. myceliophagus</i> Goodey 1958	ectoparasite	+	+
class Enopla Inglis 1983, order Dorylaimida Pearse 1942, family Longidoridae Thorne 1935				
40	fam. Nordiidae Jairajpuri, Siddiqi 1964 <i>Longidorella parva</i> Thorne 1939	ectoparasite	+	+
41	fam. Longidoridae Thorne 1935 <i>Longidorus elongatus</i> (De Man 1876)	ectoparasite, vector nepovirus	+	+
42	<i>Xiphinema brevicolle</i> Lordello, DaCosta 1961	ectoparasite, vector nepovirus	-	+
43	<i>X. diversicaudatum</i> (Micoletzky 1927)	ectoparasite, vector nepovirus	+	-
44	<i>X. pachtaicum</i> (Tulaganov 1938) Kirjanova 1951	ectoparasite	+	+
45	<i>X. rivesi</i> Dalmasso 1969	ectoparasite, vector nepovirus	+	-
46	<i>X. vuittenezi</i> Luc, Lima, Weischer, Flegg 1964	ectoparasite, vector nepovirus	-	+

Soil survey of berry plantations revealed uneven distribution of plant parasitic nematodes depending on the structures of the root system of plants. The greatest number of nematodes was found in strawberries (840 – 1,380 ex/100 cm³ soil) at a depth of 0 - 20 cm of soil profile and raspberries (650 – 1,180 cm soil ex/100 cm³ soil) at a depth of 20 - 40 cm. Species of plant parasitic nematodes vector viruses (*Xiphinema* and *Longidorus*) were mostly marked at a depth of about 40 cm and deeper to 50 - 60 cm for raspberry. In the observed berry plantations the species *X. diversicaudatum* and *X. rivesi* vector of nepoviruses were rare. A single specimen of nematode vector tabrovirus *Trichodorus primitivus* was found in rhizosphere of raspberry (Photo 1).

The ratio of sub trophic groups (YEATES et al., 1993) of the plant parasitic nematodes of surveyed berry plantations has revealed the predominance of ectoparasitic species for raspberry (42% species of the total plant parasitic species) and strawberry (39%), followed by endoparasitic species (raspberry - 30%, strawberry – 37%), epidermal cells feeders (raspberry - 20%, strawberry – 19%), algal and moss feeders (raspberry - 5%, strawberry – 8%) (Fig. 1 A, B).

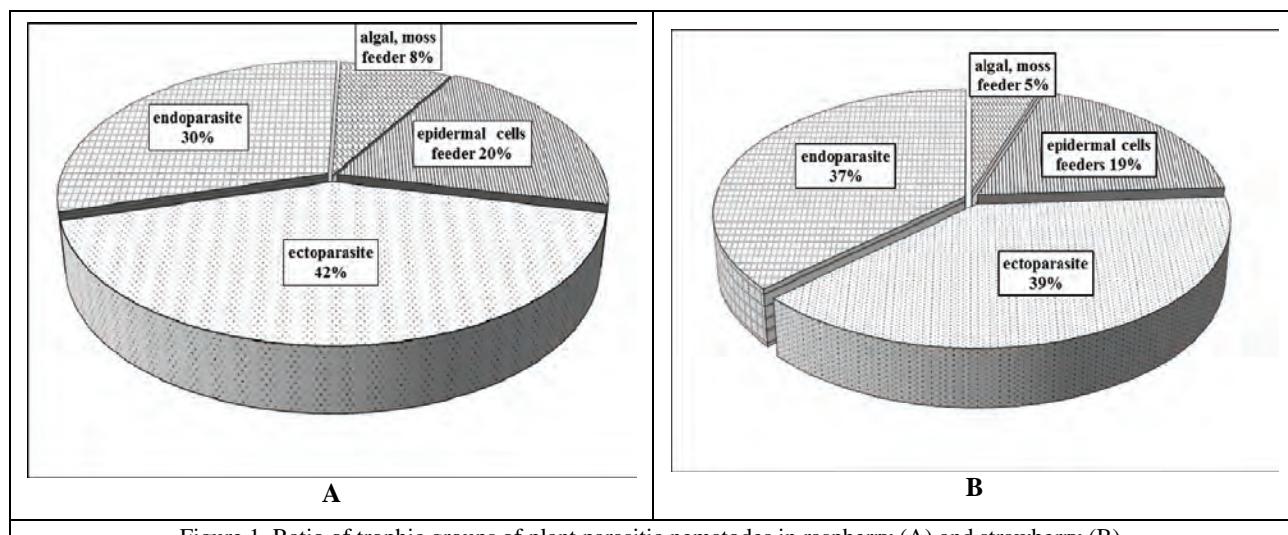


Figure 1. Ratio of trophic groups of plant parasitic nematodes in raspberry (A) and strawberry (B).

In all surveyed strawberry and raspberry plantations the plant parasitic nematode species diversity varied and consisted in about 48 - 50% species in strawberry and 38 - 42% in raspberry among all nematode communities including also free-living species. The species diversity of plant parasitic nematodes was about 28 (district Criuleni) and 35 (district Orhei) in the rhizosphere of strawberry. This is due to the fact that the loamy soil, high humidity, creates favourable conditions for the

growth and creation of high-density populations of plant-parasitic nematodes, and the development of specific pathogenesis at the level of adventitious roots in the zone of root hairs, causing the premature death of their root tissues.

The severe losses, limited growth and development of strawberry and raspberry plants are mostly induced by large population of dangerous root lesion endoparasite nematodes as *Pratylenchus penetrans*, *P. pratensis*, *P. subpenetrans* and *P. neglectus* that migrates through and feeders in the root cortex (LA MONDIA, 2002), which become visible as discrete necrotic lesion (the damage threshold is more than 30 specimens/100 cm³ soil), ectoparasites such as *Criconemoides xenoplax* (more than 250/100 cm³ soil) as well as partially spiral nematodes as *Rotylenchus agnetis*, *R. robustus* and semi-endoparasites *Helicotylenchus dihystera*, *H. vulgaris* (more than 300 specimens/100 cm³ soil) (WALTER et al., 2008).

The plant parasitic nematodes can also enable the penetration of pathogenic bacteria and fungi, which produce secondary infections (AGRIOS, 2004). It is noted that the severe damage caused by *Ditylenchus dipsaci* affects not only the roots but also the stems, leaves and fruits causing the specific nematode disease; the foci of severe lesions and high density of infective larvae and adults exceeds the threshold, which can reduce the plant productivity and thus make the crop unprofitable in the second or the third year.

DISCUSSION

Plant parasitic nematodes and virus diseases individually can cause serious losses to crop production; however, in combination, they can be very destructive in long-term crops such as berries. The responses of plants to virus infection are very diverse, ranging from symptomless infection to ring-spotting, chlorotic mottling, vein banding, vein yellowing, leaf distortion and the production of elations, discoloration and distortion of the leaves, stunting and death (BROWN, 1989; BROWN et al., 1995). Nematodes from the family Longidoridae (order Dorylaimida, phylum Nematoda) are ectoparasites of roots on a very wide host range among cultivated and wild plants, especially the perennial crops including most of berries cultures.

Moreover, some ectoparasitic nematodes from the family Longidoridae such as *Longidorus elongatus*, *L. macrosoma*, *L. euonymus* (raspberry ringspot virus RRSV), *Xiphinema brevicolle* (tomato ringspot virus TomRSV), *X. rivesi* (tomato ringspot virus TomRSV and cherry leaf roll virus CLRV) are considered potential virus vectors (POLINKOVSKY, 1980; TAYLOR & BROWN, 1997; WALTER et al., 2008; POIRAS, 2012). Species of vectors nepoviruses including *Xiphinema diversicaudatum* and *Longidorus elongatus* have been suspected of transmitting strains of RRSV (TRUDGILL et al., 1983). Both larvae and adults of *L. elongatus* transmit viruses, but the adult does not pass the virus to its progeny, nor it is retained when the nematode molts. *L. elongatus* is able to transmit also tomato black ring nepovirus - TomRSV (EPPO/CABI, 1996), which produces identical symptoms and is often found together with RRSV (OEPP/EPPO, 1983).

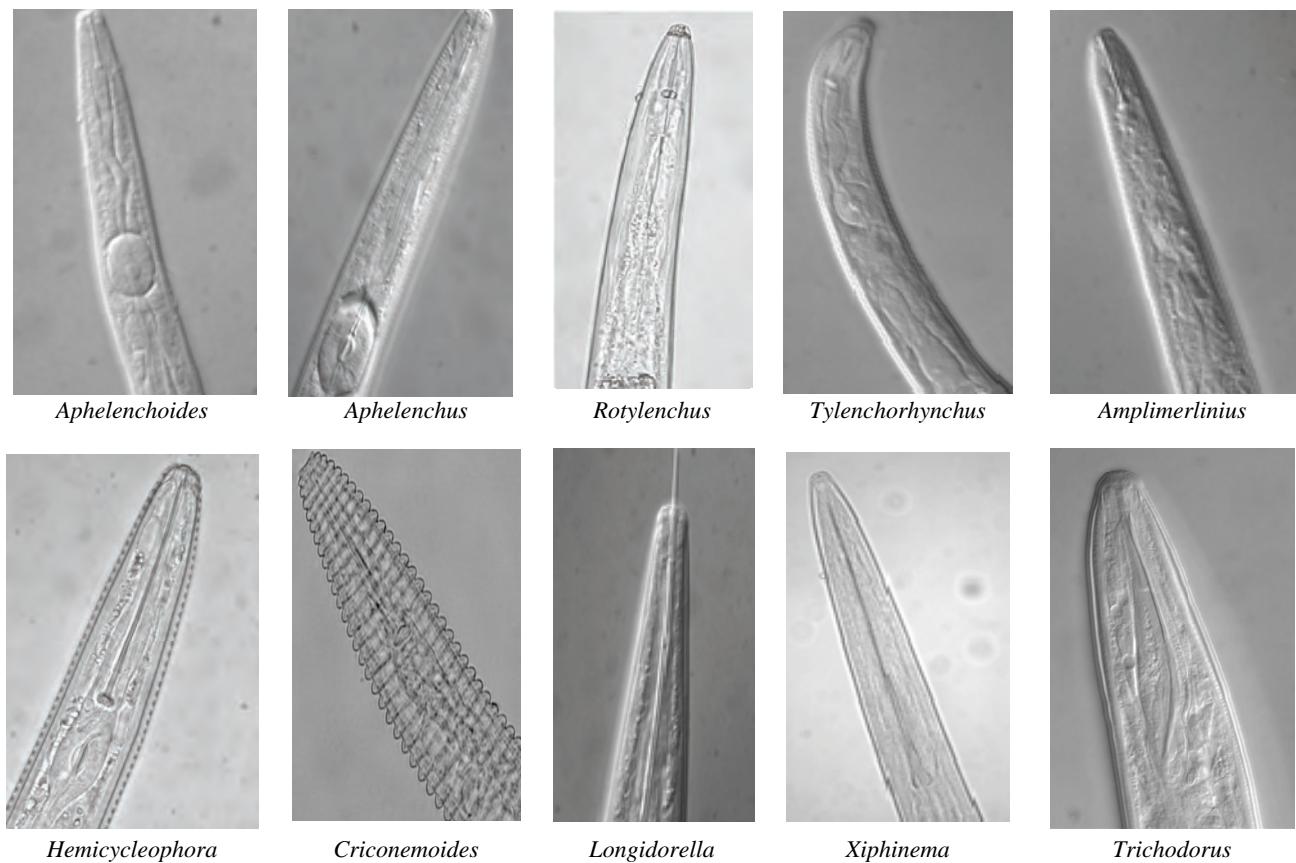


Photo 1 (original). Structure head and stylet (*Tylenchina*), odontostyle (*Dorylaimina*) and onchiostyle (*Diphterophorina*) of plant parasitic nematodes.

Currently, there are no methods to efficiently combat nepovirus diseases once the virus infects the plants. the recognition of the ability of several longidorid nematodes to transmit virus diseases to susceptible crops has resulted in the adaptation of new approaches to examine epidemiology and control of such viruses. Factors such as: previous cropping history of the land and cultural practices; distribution, activity and number of virus-vector nematodes; degree and type of weed infestation; transmission efficiency of species present; and susceptibility of host plants to viruses; each had to be taken into account in formulating appropriate control methods. In the past, the approach for controlling the numbers of the nematodes in replant situations was to apply chemical agents (LEMOS et al., 1997). However, they are expensive, toxic to humans and may have long term environmental consequences in current ecological agricultural practices in R. Moldova. Therefore, it is important to develop new reliable ecological data for indexing soils for nematode virus vectors and for indexing berries for the presence of nepoviruses in plantations to ensure high levels of berry production (COEV & POLINKOVSKIY, 1977).

According the European Union (EU) general rules for the multiplication and commercialization of propagated materials is the selection and growing of planting materials free of viruses. In the Institute of Horticulture and Food Technology ASM was evaluated the efficacy of the methods of cultivation on artificial nutrient medium apical and lateral meristem for recovery of raspberry virus promising varieties. The distribution and use of infected planting material is probably the most important way in which nematode-transmitted viruses are originally spread to new plantings. Once established in the field the nematodes can spread the virus to adjacent plants. For obtaining virus-free varieties of plants infected by viruses, penetrating into the meristem tissue, requires a combination of the method of tissue culture with chemotherapy in some cases with thermotherapy. To check the raspberry and strawberry plants for viruses, it is recommended a combination of ELISA test, PCR and biological tests on plant indicators. Therefore, "Certification schemes for strawberry and raspberries" (Schemes for production of healthy plants for planting, European and Mediterranean Plant Protection Organization, OEPP/EPPO, 2008, 2009) are one of the most important and effective means to control the viruses transmitted by nematodes.

CONCLUSIONS

Nematological analysis of berry plantations (plants and soil) revealed 46 species of plant parasitic nematodes on raspberry (25 species) and strawberry (38 species) in the central districts of R. Moldova during the last years. Among plant parasitic nematodes, there prevailed species of ectoparasites (39 – 42%) and endoparasites (30 - 35%) in studied berry plantations. Among ectoparasites, there were revealed species of potential vectors of nepoviruses such as *Longidorus elongatus*, *Xiphinema brevicolle*, *X. diversicaudatum* and *X. rivesi*.

According to the degree of damage, the root system development and pathogenesis of berry crops, there are also important the endoparasitic species of the genus *Pratylenchus* (*P. pratensis*, *P. penetrans*), ectoparasites such as *Criconemoides xenoplax* forming sometimes large populations in the root systems and *Ditylenchus dipsaci* causing the inhibition of plant growth in varying degrees also in aboveground parts of plants as well as *Rotylenchus agnetis*, *R. robustus* and semi-endoparasites *Helicotylenchus dihystera*, *H. vulgaris*. To reduce the losses caused by plant parasitic nematodes on the plantations of strawberry and raspberry, it is required a prior testing of the soil with regard to the presence of dangerous species.

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Poiras Larisa, Bivol Alexei, Poiras Nadejda
Institute of Zoology, Academy of Science of Moldova, Academiei Str. 1, 2028,
Chișinău, Republic of Moldova.
E-mail: poiras@gmail.com; poiras.nadejda@gmail.com

Iurcu-Straistraru Elena
University of State of Tiraspol
Chișinău, Republic of Moldova.
E-mail: iurcuelena@mail.ru

Cernet Alexandr
Institute of Horticulture and Food Technology,
Academy of Sciences of Moldova,
Chișinău, Republic of Moldova.
E-mail: chernetsa@rambler.ru

Received: March 24, 2014
Accepted: April 29, 2014