PLANT PARASITIC AND FREE-LIVING NEMATODES OF SOME ORCHARDS (PEACH, APPLE) IN THE REPUBLIC OF MOLDOVA

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Abstract. Species diversity of nematode communities of the orchards from two administrative districts (Criuleni, Soroca) of the Republic of Moldova has been studied during last years. Seventy five species of plant parasitic and free-living nematodes were revealed in soil and roots of peach and apple trees. In all studied orchards of fruit trees the non-pathogenic and pathogenic plant parasitic species were predominant (37-58%) followed by bacterivores (15-30%), omnivores-predators (11-27%) and fungivores (8-15%). Among plant parasitic species, the endoparasites *Pratylenchus pratensis*, *P. penetrans*, *P. thornei*, *Ditylenchus dipsaci* and ectoparasites *Rotylenchus robustus, Helicotylenchus dihystera, H. multicinctus*, *Paratylenchus hamatus, Criconemoides xenoplax* were identified. The maturity Index values varied between 2.2 and 3.02 due to the predominance of plant parasites (*cp* 3-5) including the vectors of nepoviruses *Longidorus elongates, L. euonymus*, *L. macrosoma, Xiphinema brevicolle, X. dentatum, X. rivesi, X. vuittenezi* and tabro-virus *Trichodorus primitivus*. Most bacterivores were represented by species from the fam. Cephalobidae, Panagrolaimidae and fungivores - fam. Aphelenchidae, Aphelenchoididae.

Keywords: nematode community, orchards (peach, apple), abundance, trophic groups, ecological parameters.

Rezumat. Fitonematode parasite şi libere la culturile pomicole (piersici şi mere) în Republica Moldova. Studiul biodiversităţii comunităţilor de nematode în agrocenoze pomicole au fost realizate în două raioane administrative (Criuleni, Soroca) al Republicii Moldova în perioda anilor precedenţi. S-a constat o varietate de şapte zeci şi cinci de specii de nematode fitoparazite şi libere în sol şi rizosfera pomilor de măr şi piersic. În toate sectoarele pomicole (măr şi piersic) investigate ale Republicii Moldova predomină speciile de nematode fitoparazite patogene şi nepatogene (37-58%), urmate de speciile bacteriofage (15-30%), omnivore-prădătoare (11-27%) şi micofage (8-15%). Semnificaţie majoră prezintă speciile de nematode fitoparazite cu efect patogen, unde au fost identificaţi endoparaziţii: *Pratylenchus pratensis*, *P. penetrans*, *P. thornei*, *Ditylenchus dipsaci* şi ectoparaziţii: *Rotylenchus robustus*, *Helicothlenchus dihystera*, *H. multicinctus*, *Paratylenchus hamatus*, *Criconemoides xenoplax*. Valorile Indexului de Maturitate oscilează de la 2.2-3.02 reprezentată de fitonematode libere şi parazite cu ciclul de viaţă lungă (*cp* 3-5), inclusiv speciile de nepo-virusuri *Longidorus elongates*, *L. euonymus*, *L. macrosoma*, *Xiphinema brevicolle*, *X. dentatum*, *X. rivesi*, *X. vuittenezi*. Nematodele bacteriofage mai frecvent sunt reprezentate de speciile din familiile Cephalobidae, Panagrolaimidae şi micofage - familiile Aphelenchidae, Aphelenchoididae.

Cuvinte cheie: comunităţi de fitonematode, livezi (piersic şi mere), abundenţă, grupe trofice, parametri ecologici.

INTRODUCTION

Horticulture is one of the perspective branches of agriculture in the Republic of Moldova providing fruit harvest. At present time, the plantations of apple cover about 17.5 thousand ha in central and northern regions and peach 7.7 thousand ha in central and southern regions. Mainly the horticultural plantations consist of many varieties, both local and imported from the western and eastern European countries. Sometimes the planting material is supplied to the country by a private companies without the pest control and certificates, which may contribute to the outbreak and spread of various plant parasites and diseases. Agro-climatic conditions of the Republic of Moldova are favourable for growing horticultural crops such as pome and stone fruits, as well as the formation and development of nematode communities including plant-parasitic and free-living species. The ecological and trophic relationship in the system "plant host - parasite - medium" is formed over a long period and forms the specialized population of plant-parasitic nematodes that are typical for fruit crops. The nematode communities of free-living and plant-parasitic species reflect the state of the soil and cultivated plants. When large nematode populations of some dangerous plant-parasitic nematodes feed on a plant root system, they interfere with the ability of the root to take up water, nutrients and transport nutrients causing the restricts of root growth, depression of plants and some losses of the quality and quantity of fruit production (SANTOS et al., 1997).

The important role of protection of fruit crops from plant parasites and their vector-borne diseases is a systematic phytosanitary control on the detection of the most economically dangerous and quarantine species, including some plant-parasitic nematodes vector of nepo- and tabro-viruses (SANTOS et al., 1997; TAYLOR & BROWN, 1997). Early estimates on economic threshold levels ranged from 4 nematodes per 100 cc soil for *Xiphinema index* on grapevine (KANKINA, 1981; ROMANENKO, 1993) to 20-25 specimens of *X. bekeri* per 100 cc soil on raspberry (MCELROY, 1972). We should be aware that most of the nematode diseases in fruit crops do not have specific symptoms or they do not appear immediately. The appearance and spread of nematode diseases is possible at the expansion of the areas under new plantations using lands that have recently cultivated with grapes, fruits or berries, as well as through the sick planting stocks. Nematode feeding also creates the open wounds, which provide entry to a wide variety of plant pathogenic fungi and bacteria. These microbial infections may result in greater losses than the damage from nematodes.

The most dangerous perennial plant parasitic nematodes are vectors of the viral diseases with the symptoms manifested for a long period, when control measures are no longer effective. Some species of plant parasitic nematodes from the genera *Longidorus* and *Xiphinema* are vectors of nepovirus diseases causing serious losses of long-term crops such as fruit trees, berries and grapevines. Viruses may be retained in longidorid bodies from 2.5 to 12-24 months (TAYLOR, BROWN, 1997). The economically threshold of damage for *Longidorus elongatus* on strawberry is 15-20 individuals on 100 cm^3 soil, for *Xiphinema bekeri* is 20-25 individuals on raspberry and *Xiphinema index* - 4 individuals on 100 $см^3$ soil (MCELROY, 1972; KANKINA, 1981; ROMANENKO, 1993). Crop productivity can be reduced from 10-30 % to 60 % (in some years); moreover, there may occur a reduction in the quality and quantity of the harvest (SANTOS et al., 1997). Soils testing on presence of virus-vector nematodes of fruit trees has been done by the Republic of Moldova (STEGARESCU, 1972; COEV & POLINKOVSKIY, 1977; POLINKOVSKIY, 1980; POIRAS & CERNET, 2004). The economically important and damaging nematodes of fruit trees belong to the genera *Xiphinema* (dagger nematode) and *Longidorus* (Longidoridae) (needle nematode), *Criconemoides* (Criconematidae) (ring nematode), *Pratylenchus* (Pratylenchidae) (lesion nematode), *Ditylenchus* (Anguinidae) (stem nematode), *Trichodorus* and *Paratrichodorus* (Trichodoridae) (stubby-root nematode) and are widely distributed throughout the world (NYCZEPIR & BECKER, 1998; WALTER et al., 2008). The studies regarding the nematodes associated with fruit trees are focused on different aspects, including their geographical distribution and occurrence (KUMARI, 2004), species diversity (LIŠKOVÁ et al., 2007; NIBLACK & BERNARD, 1985), population development and vertical distribution of *Xiphinema pachtaicum* on grapevine (POIRAS, TODERAS, RUSU, 2004**),** replant problems (NYCZEPIR & BECKER, 1998), virus transmission (TAYLOR & BROWN, 1997) and control strategies (KLUEPFEL et al., 2002). Plant parasitic nematodes from fam. Longidoridae are ectoparasites and vectors of nepovirus diseases, fam. Tobrilidae are vectors of tabravirus diseases. Furthermore, excessively high populations of *Criconemoides xenoplax* (>250 per 100 cm^3 soil) were associated with trees exhibiting peach decline symptoms (ZEHR et al., 1986; WALTERS et al., 2008).

Species diversity, trophic specialization and distribution of free-living and plant parasitic nematodes were studied on productive fruit orchards (peach and apple) in northern and central regions of the Republic of Moldova analyzing taxonomic-ecological characteristics of nematode communities. It elucidated a classification according to the functioning and diverse adaptation, as well as to the trophic level in the rhizosphere of fruit trees.

MATERIALS AND METHODS

Nearby 100 hectares of orchards (apple and peach) have been surveyed in the central (r. Criuleni) and northern (r. Soroca) regions of the Republic of Moldova during last years (photo 1 a-d). At each orchard ten soil samples were collected from at least 10 different trees selected by a zigzag pattern in the soil profiles 0 – 20 cm down to 50 cm in the drip line of each sampled tree and additional samples near trees with symptoms of nepovirus or debilitation (BOAG et al., 1989; SHURTLEFF & AVERRE, 2000). The soil samples were stored into plastic bags in a cooler at 4^oC until processed.

Nematodes were extracted from a 100 cm^3 soil by sieving and decanting standard methods of brass screens and modified Baermann funnels and fixed in hot 4% formaldehyde solution at 60^0 C (BEZOOIJEN, 2006); they were counted, transferred to glycerine by the modified method of Seinhorst and prepared for the mass slide collection. About a hundred nematodes from each sample were identified by taxonomic keys (NICKLE, 1991, SANTOS et al., 1997, TAYLOR & BROWN, 1997, SIDDIQI, 2000, PERRY & MOENS, 2006, ANDRASSY, 2007) and arranged by nematode classification based on the SSU DNA data (DE LEY & BLAXTER, 2002). The plant nematodes were classified by trophic groups (YEATS et al., 1991) such as bacterivores (Ba), fungivores (Fu), omnivores (Om), predators (Pr) and plant parasites (PP). The analysis of phytonematode communities has been done by functional guilds including trophic groups and life strategies from colonizer to persistence (*cp* value from *r*-strategy to *K*-strategy) and we calculated the Maturity Index $MI = \Sigma\ v(i) \times f(i)/ n$, where v (i) = colonizer-persister (c-p) value assigned to family, f (i) – frequency of family i in sample, n= total number of individuals in a sample (BONGERS & BONGER, 1998; FERRIS et al., 2001).

RESULTS AND DISSCUTIONS

Seventy five species of plant parasitic and free-living nematodes were found in rhizosphere and roots of peach and apple trees, in the orchards of the districts Criuleni and Soroca of R. Moldova (Table 1). The abundance of nematodes and their spatial distribution were predominant mostly in the level ground litter and around 30 - 50 cm trunk of tree; at the soil profiles 0 - 20 cm till 50 - 70 cm, there were 870 - 2640 ind./100 cm^3 soil in the orchards of apple trees and 570 - 1740 ind./100 cm^3 soil in peach trees (Table 1). The qualitative-quantitative analysis of nematode community shows that their numbers are lower at the base of a tree trunk than at a distance from it, where the soil is highly penetrated by young roots creating favourable conditions for the development of communities of plant-parasitic and free-living nematodes including the saprobiotic species. Thus, close to the base of a tree trunk the soil is more populated by free-living species and non-pathogenic plant parasitic nematodes. However, in the area of intensive growth of roots, there were more frequently observed plant-parasitic nematodes, including endo- and ectoparasitic species and vectors of viral diseases, also diverse free-living forms.

Table 1. Species diversity, population density, trophic groups and some community indices of plant parasitic and free-living nematodes of peach and apple productive trees in some districts of the Republic of Moldova.

Characteristics	Orchards			
	district Criuleni		District Soroca	
	Peach	Apple	Peach	Apple
Number of species in orchards	51	58	43	47
Trophic groups (%):	10	12	5	8
1 - plant parasites (PP) include:	4	7	2	6
-algal, moss and epidermal cells feeders	8	3	12	12
-semi-endoparasite	22	26	21	26
-migratory endoparasite	10	6	4	4
-ectoparasite including: a- vector nepovirus b- tobra-virus	2	-	-	-
1-bacterivores	22	21	30	20
3 - fungivores (Fu)	14	8	7	8
4 –omnivore-predators (Om-Pr)	20	23	23	20
Maturity index (MI)	2.6	3.0	2.3	2.4
Number ind./100 cm^3 soil (depth of 0-20 till 50 cm)	780 - 1740	1150 – 2640	570-1330	970 - 1970

The ratio of trophic groups of the nematode communities of surveyed orchards has revealed the predominance of plant parasitic species for peach (50% species of total) and apple (47%) trees, followed by bacterivores (20 - 21%), omnivores (15 - 16%), fungivores (8 - 12%) and predators (3 - 8%) (Figs. 1 a, c). Thirty five species of plant parasitic nematodes of peach and apple trees are grouped by 5 trophic subgroups: algal, moss and epidermal cells feeders (Tylenchidae, Anguinidae, Ecphyadophoridae, Psilenchidae), semi-endoparasites (Hoplolaimidae), migratory endoparasites (Pratylenchidae, Anguinidae), ectoparasites (Telotylenchidae, Criconematidae and Paratylenchidae), ectoparasites vector of nepoviruses (Longidoridae, Xiphinematidae) and tobravirus (Trichodoridae) (Fig. 1). Among plant parasitic species the ectoparasites dominated by species diversity (53 – 55%), followed by algal and moss feeders (18 - 26%), semi-endoparasites (11 – 18%) and migratory endoparasites (8 – 11%) in peach and apple orchards (Figs. 1 b, c).

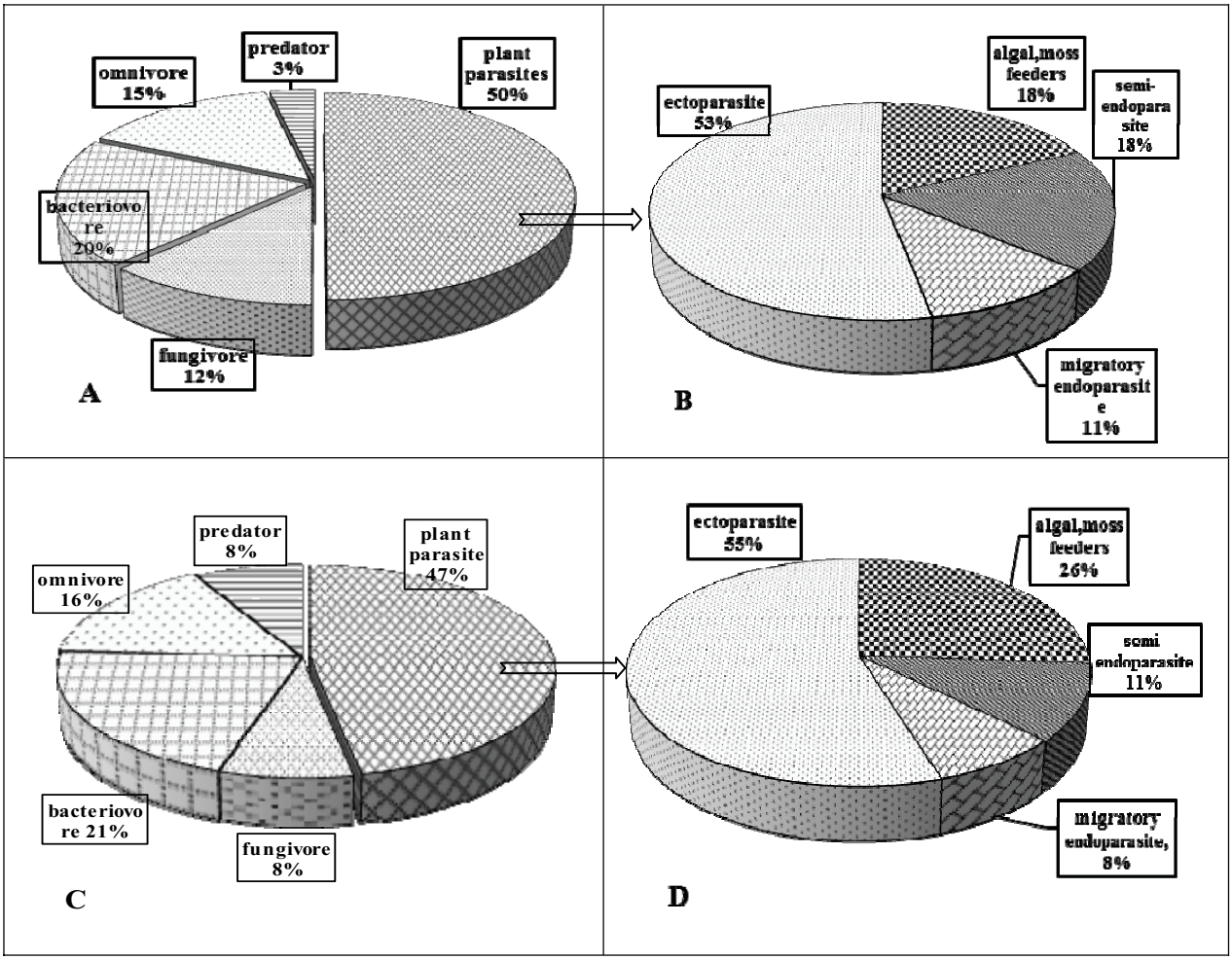

Figure 1. Ratio (%) of trophic groups of free-living and plant parasitic species of nematodes in peach (A), subgroups of plant parasitic species (C) and ratio of trophic groups of free-living and plant parasitic species of nematodes in peach trees (B),subgroups of plant parasitic species (D).

DISCUSSIONS

The area of intensive growth of tree roots is preferred by plant parasitic nematode species such as endoparasites *Pratylenchus brachyuris*, *P. penetrans*, *P. pratensis* (damage threshold >30 specimens per 100 cm^3 soil), semi-endoparasites *Helicotylenchus dihystera*, *H.vulgaris*, *Rotylenchus agnetis*, *R. robustus* (> 300 specimens per 100 cm^3 soil) and *Criconemoides insignis*, *C. zavadsky*, *C. xenoplax, Xenocriconemella macrodora*, *Ditylenchus dipsaci* (ectoparasites). Furthermore, high populations of *Criconemoides xenoplax* (>250 per 100 cm^3soil) are associated with tree exhibiting peach decline symptoms, causing in tertiary root destruction, stunted growth and general weakness limiting growth and productivity of plants (WALTER et al., 2008). *Pratylenchus* spp. (threshold > 30 specimens per 100 cm^3 soil) are often migratory endoparasites and feed within the cortex of the root; they were recovered from all orchards. Therefore, densities could be underestimated since only soil samples were examined (MAGGENTI, 1981). Among the ectoparasite species, there were revealed some vectors of nepoviruses from fam. Longidoridae such as *Longidorus elongatus, L. macrosoma, L. euonymus* (vectors TomRSV - tomato ringspot virus), *Xiphinema brevicolle* (TomRSV - tomato ringspot virus), *X. rivesi* (TomRSV - tomato ringspot virus, CRLV - cherry rasp leaf virus) (TAYLOR & BROWN, 1997; SANTOS et al., 1997; WALTER et al., 200; POIRAS et al., 2012).

The values MI (2.3 - 3.0) is higher enough for the monoculture of perennial crops (apple and peach) as most of the species of nematodes with long life span *cp* 3-5 were accumulated as well as the species with *cp* 1 were rare and represented by *Protorhabditis filiformis, Diplogaster rivalis, Mesorhabditis inarimensis* and *Panagrolaimus regius*. Bacterivores (20 - 30% of total nematode communities of peach and apple orchards) and fungivores (7 - 14%) were represented mostly by species with *cp* 2 belonging to the families Cephalobidae, Plectidae and Aphelenchidae, Aphelenchoididae. However, the most common species were *Aphelenchus avenae* and *Aphelenchoides parietinus.* Omnivores-predators (20 - 23%) were represented by species with *cp* 4 - 5 belonging to the families Qudsianematidae (*Eudorylaimus, Crassolabium, Epidorylaimus, Dorydorella, Ecumenicus*), Nordiidae (*Pungentus marietani*), Dorylaimidae (*Mesodorylaimus, Laimydorus*), Aporcelaimidae (*Aporcelaimellus, Paraxonchium*) and Mononchidae (*Clarcus papillatus*, *Prionchulus muscorum*) Mylonchulidae (*Mylonchulus brachyuris*, *M. curvicaudatus*).

Table 2. Taxonomic analysis of nematode communities of plant parasitic and free-living nematodes of some stone fruit orchards (peach and apple) in the Republic of Moldova.

Species of nematodes	Feeding type	Functional guild	Peach orchards	Apple orchards
PP				
Tylenchus davainei	algal, moss	PP2	+	+
Aglenchus agricola	epidermal cells	PP2	+	+
Coslenchus costatus	epidermal cells	PP2	-	+
Filenchus filiformis	epidermal cells	PP2	+	+
F. orbus	epidermal cells	PP2	-	+
Boleodorus thylactus	epidermal cells	PP2	+	+
Psilenchus hilarulus	epidermal cell, root hair	PP2	+	+
Helicotylenchus dihystera	semi-endoparasite	PP2	+	+
H. multicinctus	semi-endoparasite	PP2	-	+
Rotylenchus agnetis	semi-endoparasite	PP3	-	+
R. robustus	semi-endoparasite	PP3	+	+
Pratylenchoides leiocauda	migratory endoparasite	PP3	+	-
Pratylenchus penetrans	migratory endoparasite	PP3	+	+
P. pratensis	migratory endoparasite	PP3	+	+
Ditylenchus dipsaci	migratory endoparasite	PP3	+	-
Ecphyadophora leptosoma	ectoparasite	PP2	+	-
Merlinius brevidens	ectoparasite	PP2	-	+
Bitylenchus dubius	ectoparasite	PP2	+	+
Tylenchorynchus cylindricus	ectoparasite	PP2	+	+
Paratylenchus nanus	ectoparasite	PP2	-	+
Criconemoides insignis	ectoparasite	PP3	+	-
C. zavadskii	ectoparasite	PP3	-	+
C. xenoplax	ectoparasite	PP3	+	-
Mesocriconema rusticum	ectoparasite	PP3	+	+
Xenocriconemella macrodora	ectoparasite	PP3	-	+
Longidorella parva	ectoparasite	PP4	+	+
Trichodorus primitivus	ectoparasite, vector tobra-virus	PP4	+	-
Longidorus elongatus	ectoparasite, vector nepovirus	PP5	+	+
L. euonymus	ectoparasite, vector nepovirus	PP5	-	+
L. macrosoma	ectoparasite, vector nepovirus	PP5	+	+
Xiphinema brevicolle	ectoparasite, vector nepovirus	PP5	-	+
X. pachtaicum	ectoparasite	PP5	+	+
X. rivesi	ectoparasite, vector nepovirus	PP5	-	+
X. vuittenezi	ectoparasite, vector nepovirus	PP5	+	-
Fu				
Aphelenchus avenae	fungivore	Fu2	+	+

Paraphelenchus amblyurus	fungivore	Fu2	+	-
Aphelenchoides bicaudatus	fungivore	Fu2	+	-
A. parietinus	fungivore	Fu2	+	+
A.saprophilus	fungivore	Fu2	+	+
Deladenus durus	fungivore	Fu2	+	-
Ditylenchus miceliophagus	fungivore	Fu2	+	+
Ba				
Protorhabditis filiformis	bacterivore	Ba1	-	+
Diplogaster rivalis	bacterivore	Ba1	+	-
Mesorhabditis inarimensis	bacterivore	Ba1	+	-
Panagrolaimus regius	bacterivore	Ba1	-	+
Eucephalobus mucronatus	bacterivore	Ba2	+	+
E. oxyuroides	bacterivore	Ba2	-	+
Heterocephalobus elongatus	bacterivore	Ba2	+	+
H. latus	bacterivore	Ba2	-	+
Acrobeles ciliatus	bacterivore	Ba2	+	-
Acrobeloides buetschlii	bacterivore	Ba2	+	+
Chiloplacus lentus	bacterivore	Ba2	+	+
Ch. propinquus	bacterivore	Ba2	+	-
Plectus parietinus	bacterivore	Ba2	+	+
P. rizophilus	bacterivore	Ba2	-	+
Anaplectus granulosus	bacterivore	Ba2	+	+
Wilsonema agrarum	bacterivore	Ba2	-	+
Paramphidellus dolichurus	bacterivore	Ba4	+	-
Om-Pr				
Eudorylaimus acuticauda	omnivore	Om4	-	+
E. carteri	omnivore	Om4	+	+
Crassolabium ettersbergensis	omnivore	Om4	+	+
Epidorylaimus humilior	omnivore	Om4	+	-
Dorydorella pratensis	omnivore	Om4	+	-
Pungentus marietani	omnivore	Om4	-	+
Ecumenicus monohystera	omnivore	Om5	+	+
Mesodorylaimus bastiani	omnivore	Om5	-	+
Laimydorus filiformis	omnivore	Om5	+	-
Aporcelaimellus krygeri	omnivore	Om5	+	-
A. obscurus	omnivore	Om5	-	+
A. obtusicaudatus	omnivore	Om5	+	+
Paraxonchium laetificans	omnivore	Om5	-	+
Clarkus papillatus	predator	Pr4	+	+
Prionchulus muscorum	predator	Pr4	-	+
Mylonchulus brachyuris	predator	Pr4	+	+
M. curvicaudatus	predator	Pr4	-	+

CONCLUSIONS

Species diversity of nematode communities of the orchards from two administrative districts of the Republic of Moldova has been studied during last years. Seventy five species of plant parasitic and free-living nematodes were revealed in soil and roots of peach and apple trees. In all studied orchards of fruit trees the non-pathogenic and pathogenic plant parasitic species were predominant (47 - 50%) followed by bacterivores (20 - 21%), omnivore-carnivores (18 - 24%) and fungivores (8 - 12%). Among plant parasitic species with pathogenic effects, there were identified the endoparasites *Pratylenchus pratensis*, *P. penetrans*, *P. thornei*, *Ditylenchus dipsaci,* ectoparasites *Rotylenchus robustus, Helicotylenchus dihystera, H. multicinctus*, *Paratylenchus hamatus, Criconemoides xenoplax.* The maturity Index values were varied between 2.2 and 3.02 due to the predominance of plant parasites (cp 3-5) including the vectors of nepoviruses *Longidorus elongates, L. euonymus*, *L. macrosoma, Xiphinema brevicolle, X. dentatum, X. rivesi, X. vuittenezi* and tobra-virus *Trichodorus primitivus*. Most bacterivores were represented by species from the fam. Cephalobidae*,* Panagrolaimidae and fungivores - fam. Aphelenchidae, Aphelenchoididae*.*

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