INVASIVE NEMATOFAUNA AFFECTING PLUM UNDER THE ENVIRONMENTAL CONDITIONS OF THE REPUBLIC OF MOLDOVA

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Abstract

Parasitic nematodes and virus diseases individually can cause serious losses in plum production, however in combination, they can be very destructive to crops in the long-term. The result of the conducted investigation revealed that abundance of nematodes in the Northern area of the Republic of Moldova ranged from 50 to 250 specimens per 100 g of sampled soil. In the Central area, the values varied more comparative to Northern part, ranging from 80 to 300 specimens per 100 g of soil, due to temperature variations and humidity. Altogether, 32 species of free living and plant parasitic nematodes from fruit trees crops were revealed. The most frequent proved to be species from the orders *Thylenchida* and *Dorylaimida*, the genera *Pratylenchus*, *Rotylenchus*, *Ditylenchus* and *Criconemoides*. In addition, the species *Xiphinema index*, *X. brevicole*, *X. vuitennezi* and *Longidorus elongates* were identified as vectors of viral pathogens. According to the trophic specialization, 5 groups of nematodes were detected, the most abundant being the group of plant parasitic nematodes (18 species), which seriously affect absorbing bristles, followed by specialized endo- and ecto-parasitic adaptations and vectors of viral pathogens. These investigations present a major significance for developing netological management programs in plum orchards.

Key words: nematodes, plum crops, biological control, abundance, diversity, trophic specialization

Plum cultivation is a centuries-old tradition, surpassed only by apple, and the production of plum fruits obtained in the Republic of Moldova constitutes over 35%, with an important share of export, approximately 30%. (Balan V., 2010; Istrati L, 2018; Juraveli A., Terentii P., Cozmic R, 2020). Current plum plantations of intensive and super-intensive type are a capital investment, which can be exploited for 20-25 years, and the efficiency of exploitation depends on the correctness of the establishment of orchards, and the appropriate implementation of plant care technologies in the first 3-4 years after planting (Chira L., Honza D., 2010; Coroid A., 2020; www. statistic.md. 2021-2022).

Another significant priority in plum cultivation is the study of the evolutionary parasitic and vectorial impact of complexes of harmful organisms, associated with invasive nematode complexes that form trophic parasitic associations, with impact aggravated by adverse factors, triggered by the perennial monoculture of fruit trees (Bădărău S., 2012; Nesterov P., 1988; Stegărescu O., 1997; Poiras L., 2012).

According to the activity program, elaborated on the basis of the research project, our work is integrated in the strategic objectives of the development of fruit growing in the Republic of Moldova. This, research is conducted annually, on the biological control of invasive and vector nematode communities and on the parasitic and functional impact and the structure of populations in young and productive plum orchards, nurseries for the production of planting material, including other related species such as peach, apricot, cherry etc. Based on the current situation and the research program mentioned, the goal of our research has been: the phytosanitary helminthological control of some associations of parasitic nematodes and vectors of pathogenic viruses from the class *Secerneatae*, order *Thylenchida* and *Dorylaimida*, in plum, cultivated in intensive systems of orchards and nurseries, planted with various modern varieties, with diverse spectrum of precocity, in the Republic of Moldova. The main objective of our research has been to study, analyze and compare the diversity of the most dangerous complex of invasive nematodes and vectors of pathogenic viruses from the genera *Pratylenchus*, *Rotylenchus*,

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Ditylenchus and *Criconemoides*, families *Xiphinematidae, Trihodoridae, Longidoridae,* in plum, compared by zones, sectors and age, in the context of changes in climatic factors.

MATERIAL AND METHOD

Research was conducted in intensive plum orchards and nurseries for reproducing planting material from 6 administrative districts, on areas of over 500 hectares in the North, Center and South-East area of the Republic of Moldova. More than 300 samples of soil, productive trees, young trees and shoots attacked by viruses were collected in the field and laboratory and young saplings – from various orchard systems and nurseries. Samples were taken at a depth of 30-60 cm, in intervals of 15-20 days, covering the main phenological stages during the entire growing season. The surveys to establish the helminthic and viral parasitic impact were carried out by visual and optical observations to identify local damage, the severity of symptoms of specific viral diseases of trees. The results of the surveys are interpreted by the abundance and frequency indices of the species (individuals/100 cm^3 soil) and by the ratio of ecological-trophic specialization depending on the investigated plantations, environmental conditions, variety, soil, cultivation systems etc., indicated in pictures 1-6.

The analyzes of the nematofaunistic material collected were carried out in the Laboratory of Parasitology and Helminthology of the Institute of Zoology, by the *Baermann funnel* method, flotation-settling and fixation, to establish the numbers of individuals, the taxonomic identification in terms of genus and species, the trophic specialization and other biological peculiarities (Кириянова Е., 1971; Dekker M., 1972; Stegărescu O., 1972; Brown D., 1989; Siddiqi D., 2000; Baldwin J., 2004).

Figure 1-3 **Phytosanitary monitoring in productive plum orchards, Criuleni district, June, 2022**

Figure 4-6 **Plant nurseries investigated at the time of extracting and sorting the fruit tree saplings, Zaim village, Căușeni district, November, 2022**

The values of the damage indices of the degree of parasitic impact were established, such as: the values of the numerical density (*N.d.*) of the individuals, the intensity of the development of viral diseases (*I%*), estimation scales used in carrying out surveys of phytosanitary records, according to "Methodical guidelines for testing chemical and biological plant protection products against pests, diseases and weeds in the Republic of Moldova", 2022. In the laboratory, we used the available equipment to perform helminthological analyses, as well as auxiliary laboratory equipment. After the analyses on taxonomic determination, we frequently consulted the current bibliographic sources on helminthology, virology, as methodical support in the Laboratory of Parasitology and Helminthology: Деккер Х., 1972; Santos M. and col., 1997; Нестеров П., 1976-1988; Stegărescu O., 1970-1997; Aurel Maxim and col. 1994-2017; Decramer W., 2006; Poiras L. and col. 2004-2019.

RESULTS AND DISCUSSIONS

Damage and losses of fruit production despite favorable conditions for both trees and

harmful organisms, as parasitic agents and vectors, require maximum attention and particular research in productive, young orchards and nurseries for the production of seedlings. A lot of damage is also caused by the presence of invasive nematode complexes and vectors of pathogenic viruses, which also periodically trigger helminthoses with specific and non-specific pathogenic effects on the fruit material, associated with viral diseases.

Parasitic nematodes and vectors of pathogenic phytoviruses are among the most damaging and at the same time little studied as interactive pathogens, due to the complexity and specificity of the pathological process caused by them. In the case of infestation of plants with invasive nematodes and phytoviruses, trophic relationships are established between their genomes depending on the type of host's reaction to the respective pathogen. The negative economic impact caused by these pathogens varies between 10-70%, and sometimes it can reach 100%, where practically the level of infestation and inoculation is far above the economic threshold of damage (Haririson B., 1967; CalaseanI., Rapcea M., 2004; Decramer W., 2006; Poiras L. and col., 2013).

The research carried out initially resulted in the determination of the numerical abundance, recorded in average values per areas, according to the samples collected in the plum orchards indicated in Table 1. As a result of the comparative analyses, the numerical density of nematode populations (N.d.) was determined in average values per zones, from 19 to 130 individuals per 100 g soil. It was found out that the abundance was by 15-20% higher in the sectors of the North zone, being influenced by variations in temperature, humidity and more fertile soil, than in the Center and South-East zones. In the sectors of young orchards and nurseries of fruit trees, the numerical density was estimated in lower average values (7-15%) as compared with already productive orchards, aged 8-10 years, conditions caused by the formation of denser nematode complexes, depending on the environmental factors and the trophic reserve accumulated in the fruit monoculture. As compared with the North zone, the orchards in the Center and South-East zones were characterized by lower numerical density and low-to-moderate infestation (1-2 points, according to the estimation scale), which is also partly due to the more rigorous observance of technological management and the appropriate application of effective integrated protection measures.

Table 1

The numerical density of the parasitic nematode complexes, detected in plum orchards, in comparison, by new exploitation systems, 2021-2022

Zone, investigated district	Productive orchards, 8-10 years, (individuals/100cm^3 /soil)	Young orchards, 3-5 years, (individuals/100 cm^3 soil)	Nurseries 1-2 years, (individuals/100cm^3 soil)
North: Briceni d.	35-110	45-105	36-95
Soroca d.,	68-130	54-115	58-100
Center: Criuleni d.,	45-83	28-75	27-73
Nisporeni d.,	33-74	19-58	23-62
South-East: Căuşeni d., Zaim v.	57-98	32-74	42-80
Ştefan-Vodă d.	28-79	21-67	31-74
Average per zones:	28-130	19-115	23-100

Based on the previous and current investigations, according to the obtained results, we estimate that the complexes of invasive and vector nematodes detected in fruit orchards, including plum, restructured in new systems belong to the order *Tylenchida* and *Dorylaimida* (Dekker M., 1972, Siddiqi D., 2000). According to the establishment of the taxonomic aspects and the trophic group, the analyzed nematode samples found in plum orchards, are reported as ectoparasitic forms and vectors of pathogenic phytoviruses (Stegărescu O., 1972; Nesterov P.,1987; Poiras L., 2012).

The results of the comparative analysis on the abundance, frequency and diversity of detected species, which form invasive and vectorial complexes of pathogenic viruses belong only to the *Dorylaimida* order, detected in various areas, orchards and nurseries, which are presented in tables 2 and 3, where 15 more species are more dangerous and practically infests all fruit tree species, including plum, according to the results obtained by us, as well as those mentioned in the bibliographic sources. The abundance and frequency of species is obviously higher, by 30-40%, in the sectors of the Center and South-East zones, the reasons being, however, the higher average temperatures and the more humid microclimate, which are advantageous factors for

the proliferation and formation of specialized complexes.

The analyses of nematode species, as vectors of viruses in fruit crops, as well as different forms of relationships between viruses and nematodes, indicated their interspecificity, which determines the invasions that annually cause various forms of mixed parasitism (helminthic-viral), even from the first half of the growing season, favored also by the influence of environmental factors. The detected species infest and parasitize more actively on young trees recently planted in nurseries and orchards, they are polyphagous-oligophagous, thermo-hydrophilic pests of agro-economic importance, according to references noted and published a lot in the last decades by various specialists in nematology and virology, as well as in our own current research.

Table 2

Analysis of the comparative spatial frequency and structure of the complexes of parasitic nematodes and vectors of pathogenic viruses from the order *Dorylaimida* spp., detected in various fruit-growing areas, restructured productive orchards and nurseries

Nematode species	North zone		Center zone		South-East zone	
	Productive orchards	Nurseries	Productive orchards	Nurseries	Productive orchards	Nurseries
I.Gen. *Xiphinema,* Cobb, 1913, ***1.X. index,* Thorne et al., 1950**	++	+	++	+	++	+
***2.X. brevicolle,* Lordello et da Costra, 1961**	++	+	+	+	+	+
3.X. rivesi, Dalmasso, 1969	+	-	+	-	+	-
***4.X. vuittenezi,* Luc et Lima,1964**	+	-	++	+	++	+
***5. X. pachtaicum,*Tulganov,1938**	++	+	+	+	-	-
***6.X. diversicaudatum,*Thor. 1939**	+	+	++	++	+	+
***7.X. americanum,* Cobb,1913**	-	-	+	+	+	+
***8.X. italiae,* Meyl, 1953**	-	-	+	+	+	+
***9.X.turcicum,* Dalmasso, 1963**	-	-	+	-	+	+
II.Gen.*Longidorus, Micole.1922* ***10.L.elongatus,* de Man, 1876**	-	-	-	-	+	+
***11.L. macrosoma,* Hooper, 1961, Andrassy, 1956**	+	-	+	+	+	-
***12.L.eunymus,* Hooper, 1974**	-	-	-	-	+	+
III.G. *Trichodorus,* Cobb, 1931, ***13.T. primitivus,* de Man, 1880**	-	-	+	-	+	+
***14.T. simili,* Seinhorst, 1963**	-	-	+	+	+	+
***15.T.cylindricus,* Hoof, 1962**	-	-	+	-	+	+
Total species – 15 species	7	4	13	10	14	12

Legend: - no individuals **(0 points) ;** + from 50 to 100 individuals **(1 point);** ++ from 100 to 150 individuals **(2 points);** +++ from 150 to 200 individuals **(3 points);** ++++ over 200 individuals **(4 points).**

We would like to mention that the species of nematodes estimated in table 2, belong to the genera: *Xiphinema spp.*, *Longidorus spp.*, *Trichodorus spp.*, they are ectoparasites and specialized vectors of various forms of pathogenic viruses, which participate in triggering specific viral diseases in fruit tree plantations. They maintain particles of infectious viruses in their body from 8 weeks to 2 years (for example, tomato ring spot virus, detected on the nematode *Xiphinema riversi* (Bitterlin și Gonsales, 1986; Cerneț A., Poiras L. *et al* 2006-2016). Pathogenic viruses also detected in the Republic of Moldova in fruit-bearing species, transmissible through 15 species of nematodes according to the classification of Cadman (1986), are divided into 9 large groups, but only 2 groups are significant for fruit-growing in our area, according to table 3, which are involved in the transmission of the specific virus, equally contagious, disastrous in the process of pathogenesis and absolutely incurable. The specific symptoms also reported in plum, during the growing season, are: deformations, mosaic appearance, chlorosis, ring spots, deformations, dwarfing and proliferations. *Table 3* estimates the main species of viruses detected in the Republic of Moldova and transmitted by some species of nematode vectors to fruit trees, also detected on plum trees, associated with arthropod complexes. They are responsible for triggering severe viral diseases, as confirmed by the authors who detected the vector aspects of the nematode species and the pathogenic virus found in fruit trees in the Republic of Moldova (Verderevckaia *et al.* 1986; Aurel M. *et al*. 2002-2007; Cerneț A. *et al.*, 1995-2020; Poiras L. *et al*. 2006-2016).

The research results obtained from the phytosanitary surveys in intensive plum orchards

and nurseries revealed the degree of helminthic disease and the presence of viruses in the trees, especially on the roots, shoots and young leaves of plum, in average values of 15-30 % frequency (*F.%)* and 10-20% intensity (*I%*), detected practically in all the investigated agrocenoses.

Table 3

Nematode vectors of pathogenic viruses invasive to fruit trees, including plums, detected in the Republic of Moldova.

Virus	Vector – Nematodes	Authors of vector nematodes and phytoviruses
1. Cherry leaf roll *2. Prunus necrotic rihg spot* *3. Prunus dwarf virus* *4. Arabis mozaic* *5. Raspberry ring spot* *6.Tomato ring spot* *7.Tomato black ring* *8. Cherry mottle leaf* *9. Cherry necrotic rusty mottle,* *10. Cherry virus A* *11.Cherry leaf roll virus* *12.Prunus latent virus* *13. Apple cholorotic* *14.Perach mosaic leaf spot* *15.Grapevine fanleaf virus* *16.Strawberry latent ringspot* *17.Peach rosette mosaic virus* *18.Mulberry ringspot virus* 19.*Carnation ringspot virus* 20.*Cherry rosette disease virus,* **(Nepovirus**).	***1. Xiphinema index*** ***2. X. riversi*** ***3. X. brevicole*** ***4. X. diversicaudatum*** ***5. X.vuittenezei*** ***6. X. italia*** ***7. X. pachtaicum*** ***8. X. americanum*** ***9. X. turcicum*** ***10. Longidorus macrosoma*** ***11. L. elongatus*** ***12. L. eunymus***	Fritzsche 1972 Fritzsche & Kegler, 1964 Flegg, 1969 Fritzsche & Kegler, 1968 Bloom et al., 1972 Cameron et al.,1977 Harrison et al., 1971 Harrison & Cadman,1959 Thorne & Allen,1950 Dalmasso, 1963-1070 Stegărescu, 1966-1979; Fritzeche & Sehmelzer, 1967 Sol & Seinhorst, 1961 Harrison & colab.,1955 Verderevskaia et al.,1970 Nesterov et al.,1976 Brown et al., 1995 Taylor & Braun, 1997 Maxim A. et al.,1994-2010 Isac et al., 1998 Poiras et al., 2007-2016
21. Tobacco rattle virus-TVR *22. Pea early-browning- PEBV* *23.Pepper ring spot-virus-PRSV,* **(Tobravirus**).	***13.Trihodorus primitivus*** ***14.T. similis*** ***15.T. cylindricus***	Cobb. 1931, Thorne, 1931 Kramer & Coistra, 1964 Cadman, 1963, Hoof, H. A. Van 1963, Seinhorst, 1963 Harrison et al.,1974

Some ectoparasite-vector species of nepoviruses were detected more frequently, such as: *Xiphinema index, X. diversicaudatum, X. brevicole, X. vuitennezi, X. riversi, Longidorus elongatus* and specific viral diseases were identified in plum trees: *Prunus necrotic ring spot, Prunus dwarf virus, Arabis mozaic, Cherry leaf roll virus, Prunus latent virus, Prunus rosette disease virus,* listed in table 3. These nemato-virological investigations have theoretical-applicative significance and act as long-term bio-indicators in the evaluation of the phytosanitary status in fruit orchards, including plum, in the prevention and adjustment of some methods of controlling the nemato-parasitic and patho-viral impact.

CONCLUSIONS

As a result of the research carried out in the new type of intensive plum orchards, in production associations in the North, Center, South-East zones of the Republic of Moldova, on areas of more than 300 hectares, over 250 soil samples and plant organs affected by helminthes and viruses were collected and analyzed, determining the parasitic and viral impact, the numerical density values and the severity of damage, abundance, frequency and diversity of nematodes and viruses.

The abundance of the populations was from 20 to 130 individuals/100 g/soil and young roots in orchards and nurseries, analyzed comparatively in the investigated sectors and zones, where the abundance was by 15-20% higher in the sectors of the North zone than in the Center and South-East zones. However, in young orchards and nurseries, the numerical density was estimated in relatively lower average values (7-15%) as compared with productive orchards, but the frequency of species was by 30- 40% higher in the sectors of the Center and South-East zones than in the North.

There was a significant diversity, of 15 species, of invasive nematodes and vectors of pathogenic viruses of the order *Dorylaimida*, genera *Xiphinema* spp., and *Longidorus* spp., species*: Xiphinema index X. vuitennezi, X. riversi, X. brevicolle, X. diversicaudatum Longidorus elongatus,* with poly-oligophagous ectoparasitic trophic specialization, responsible for the transmission of 20 species of pathogenic viruses, causing symptomatic diseases in plum, detected practically in all investigated zones and sectors.

The results of the phytosanitary surveys conducted to establish the helminthological and viral impact of parasites, revealed nematological diseases on young roots in values of 5-20% and 7 specific viruses detected especially on young saplings, leaves and shoots of plum in values of 15- 30% frequency (F.%) and 10-20% intensity (I%). Viral diseases detected more frequently in current plum orchards are caused by the following specific forms of viruses: *Plum pox virus, Prunus necrotic ring spot, Prunus dwarf virus, Arabis mozaic virus, Prunus latent virus, Prunus rosette disease virus*.

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