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Phytosanitary investigations on the invasive and vectorial impact on apple cultivation in the environmental conditions of the Republic of Moldova

Alexei Bivol, Ștefan Rusu, Elena Iurcu-Străistaru, and Viorelia Rusu

Abstract. In this study, bioecological and taxonomic investigations were conducted on the populations of invasive and vectorial parasitic nematodes associated with apple cultivation in intensive orchards. The purpose of the research was to establish a comparative analysis and assess the diversity of nematode complexes with parasitic and vectorial impacts on apple crops from various horticultural regions, aiming to identify the most dangerous species with harmful effects on productive apple orchards. The objectives of the study focused on highlighting the most invasive species of nematodes and virus vectors from the orders *Tylenchida* and *Dorylaimida* associated with apple cultivation. The research confirmed the presence of 15 species of invasive and vectorial nematodes with parasitic impacts, which cause viral diseases in the studied sectors. Additionally, comparative estimates of the nematode population density were made, and 13 associated viral diseases were identified, with symptomatic features and varying degrees of infection.
Keywords: nematodes, apple orchards, biologycal control, viral diseases, phytosanitary indices.

Investigații fitosanitare asupra impactului invaziv și vectorial la cultura de măr în condițiile de mediu ale Republicii Moldova

Rezumat. În cadrul acestei lucrări, au fost realizate investigații bioecologice și taxonomice asupra populațiilor de nematode invazive și vectoriale parazite asociate la cultura de măr în livezile de tip intensiv. Scopul cercetărilor a fost de a stabili comparativ și de a analiza diversitatea complexelor de nematode cu impact parazitar și vectorial asupra culturii de măr din diverse zone horticole, cu scopul de a identifica cele mai periculoase specii cu efecte nocive asupra livezilor productive de măr. Obiectivele cercetării au vizat evidențierea celor mai invazive specii de nematode și vectori ai virusurilor patogene din ordinele *Tylenchida* și *Dorylaimida*, asociate la cultura de măr. În urma cercetărilor, a fost confirmată prezența a 15 specii de nematode invazive și vectoriale, cu impact parazitar, care provoacă viroze în sectoarele investigate. De asemenea, au fost estimate valorile comparative ale efectivului numeric, iar 13 viroze provocate asociativ au fost semnalate, având manifestări simptomatice și un grad variat de afecțiune.
Cuvinte-cheie: nematode, livezi de măr, control biologic, boli virotice, indici fitosanitari.

1. Introduction

The main fruit species in temperate zones, widespread across all continents in terms of surface area and horticultural production, is the apple [1, 2, 3, 4, 5] In the Republic of Moldova, this fruit species ranks first among the horticultural species cultivated, in accordance with environmental conditions, being well represented in all agro-ecological zones of the country, in new technological formats, modern varieties, planted in smaller orchards, using intensive and super-intensive systems [2, 4, 18]

Phytosanitary biological control is a priority through seasonal and annual monitoring of reporting and remediation methods, using logistical and intellectual resources, which form the basis of the research program within the project – State Program, code 20.80009.7007.12, 2020-2023, followed by the research conducted in the current project's subprogram, code 010701, concerning the evaluation of the diversity and structure of invasive nematode species from natural and anthropized habitats, as well as the impact of phytoparasites on crop plants, including apple cultivation [8, 11, 16]

The research was conducted at the Parasitology and Helminthology Laboratory, Institute of Zoology, according to the annual research programs of the project mentioned above, in the years 2020-2024. The issues addressed above motivated the purpose of the investigations, which aimed to establish a comparative study of the diversity of invasive nematode complexes and the helminthological-parasitic vectorial impact on apple cultivation in various zones and fruit-growing sectors, identifying the most harmful species with a negative effect in productive apple orchards.

The estimated research objectives are as follows: Establishing the most invasive species of parasitic nematodes and vectors of pathogenic viruses from the orders Tylenchida and Dorylaimida, associated with apple cultivation, including other fruit species from various zones and productive orchards in the Republic of Moldova.

Analysis and assessment of the parasitic impact, with a comparative estimation of the most dangerous species of invasive nematodes, with specific pathogenic and vectorial effects in triggering helminthiasis and specific viral diseases in orchards cultivated under current systems (intensive, super-intensive, and nursery systems for reproductive planting material).

2. Materials and Methods

In order to achieve the goals and objectives set for the research, approved for the years 2020-2024, phytosanitary investigations were carried out through helminthic route surveys in intensive and super-intensive apple orchards, which had entered the bearing

stage, including in reproduction nurseries on areas exceeding 800 hectares. The pilot samples were apple orchards from the following areas: North – Briceni, Edineț, Drochia, and Soroca districts; Central – Criuleni, Orhei, Criuleni, Nisporeni districts; South-East – Căușeni, Ștefan-Vodă districts; and South – Comrat, Leova districts, where surveys for monitoring were conducted by collecting soil samples from the rhizosphere of apple trees at depths of 30-55 cm, as well as from surface root segments and shoots affected by viruses, at monthly and seasonal intervals, covering all development stages throughout the entire vegetation period.

The assessment of parasitic impact values was carried out through phenological visual and microscopic observations for identifying local diseases, extended to areas with localized virus symptoms on plant organs. The results were analyzed through helminthic and binocular analysis.

a) b) c)

Figure 1. Phytosanitary monitoring surveys conducted in the Central area, Criuleni and Nisporeni districts, productive intensive apple orchards, May-September, 2020-2024.

The study of the labeled faunistic material was conducted in the laboratory using classical and current methods adapted to the requirements of the Parasitology and Helminthology laboratory for performing the analyses. Initially, nematodes were extracted from soil and organs affected by helminths using the classical method with some modifications and adjustments, based on the "*Baermann funnel*" technique, which involves flotation and filtration through sets of sieves with various perforation sizes, specific to certain nematode taxa. The extracted nematodes were then fixed in hot 4% formalin at a temperature of 60°C [6, 7, 9].

The extracted helminths were fixed by preparing temporary mounts, with subsequent identification of genera and species using the identification keys and specialized bibliographic sources approved by the Parasitology and Helminthology laboratory: Dekker H., 1972; Nesterov P.I., 1988 [10, 12, 13, 14].

The results obtained and analyzed estimate the diversity and abundance of species associated in complexes, the density of the numeric population (D.n.) of individuals per 100 g of soil sampled, the frequency and intensity of viral disease development (I%), as well as the estimation scales used for classifying the infestation level through monitoring surveys, later classified into groups with trophic specializations depending on the investigated plantations, environmental conditions, and fruit-growing areas.

3. Results and Discussions

According to the analyzed bibliographic sources, it is estimated that in the structure of anthropized agroecosystems, globally, there are significant amounts of free and parasitic nematodes in the soil (approximately 7.8 million individuals per m^2). Due to their remarkable reproductive capacity, the population of invasive species included in the anthropized agrocenoses is much higher (65-85%) than that of the associated free forms. This phenomenon is influenced by the biotope and environmental factors, which are characteristic of all fruit-growing zones and are highly favorable for the development of harmful organisms, including invasive nematode complexes already reported in all cultivated fruit species [1, 2, 4, 5, 8, 15, 16].

According to previous helminthological studies and ongoing research based on national and international project programs, we estimate that all the populations of parasitic nematodes identified in fruit orchards, including apple cultivation, planted in new intensive systems in the Republic of Moldova, belong to the orders Tylenchida and Dorylaimida [6, 7, 8, 11, 16].

The results of previous analyses and the trophic classification place these species in various taxonomic families, most of the genera and species already being reported in fruit crops as invasive agents causing direct and indirect helminthic diseases in young trees [12, 14]. Among the most common families are: *Tylenchidae*, *Pratylenchidae*, *Hoplolaimidae*, *Heteroderidae*, *Longidoridae*, and *Trichoderidae* from the Tylenchida order. None of the representatives of this group are solely carriers of pathogenic viruses, but also ecto-endoparasitic pests, capable of infecting newly formed root zones. Additionally, representatives of the *Dorylaimida* order are recognized and currently estimated as specialized virus vectors, being exclusive members of the *Dorylaimidae* family, with genera *Xiphinema*, *Longidorus*, and *Trichodorus*. The species listed in Table 1 represent the structure of associated nematode complexes, consisting of 15 species that are frequently and abundantly found in apple orchards, and the bibliographic references indicate their presence in other fruit crops as well [9, 10, 11, 15, 16].

Table 1. Structure of parasitic nematode complexes detected in horticultural plantations, including apple orchards, with invasive and vectorial impact from the Tylenchida and Dorylaimida orders.

№	Parasitic species	Specific diseases caused	Affected species	Investigation methods	Diseases
Endoparasites					
1	*Pratylenchus pratensis*	Pratylenchosis	Phytotechnical crops, Horticultural crops, Baciferous crops	Surveys with sample collections	Affects the apical meristems
2	*P. penetrans*				
3	*P. pratensis*				
Semi – endoparazite polifage					
4	*Rotylenchus agnestis*	Rotylenchosis Helicotylen chosis	Young field horticultural crops, ornamental	Evidence in the early stages of vegetation	It affects the area of the absorbing roots
5	*R. robustus*				
6	*Helycotylenchus dihystera*				
7	*H. vulgaris*				
Migratory ectoparasites					
8	*Ditylenchus dipsaci*	Ditylenchosis	Technical, horticultural, floricultural crops	Periodic surveys with laboratory analyses	Pests of stems and young roots
9	*Mesocriconema xenoplax*	Criconemohel minthosis	Vine saplings, horticultural species, floricultural species	Field and laboratory investigations	Affects the new roots
Ectoparasites, vectors of nepoviruses – pathogenic viruses					
10	*Xiphinema diversicaudatum*	Helminthoses and vector of viral infections	Horticultural species, vine, forest, and ornamental species	Evidence in detecting viral diseases, soil analysis	Affects the roots severely, transmits viral infections
11	*X. index*				
12	*X. brevicolle*				
13	*X. vuittienzi*				
14	*Longidorus elongatus*				
Quarantine ectoparasites, vectors of tobamoviruses – pathogenic viruses					
15	*Trichodorus primitivus*	Tabro virus vector	Quarantine species, horticultural and decorative	Phytosanitary quarantine monitoring	Triggers viral diseases

Research on nematode vectors of viral diseases in fruit crops, as well as the relationships between viruses and nematodes, indicates that these interactions are largely responsible for the processes of helminthic and viral pathogenesis in cultivated plants and perennial plants from the spontaneous flora. Most species parasitize living plants, acting as invasive agents of agro-economic importance, and the findings from previous research by nematofaunist researchers, both domestic and international, are confirmed by our own current research [7, 8, 11, 17].

As a result of the research conducted, we observed the structure of diversity and nematode complexes, identifying the most dangerous species found in both productive and newly planted apple orchards. These were classified based on their parasitic trophic specialization into five groups, from the genera: *Pratylenchus*, *Rotylenchus*, *Ditylenchus*, *Criconemoides*, as presented in Table 1. Additionally, an assessment of the viral infection level was carried out in intensive apple orchards, where it was found that the young shoots exhibited a viral infection rate of 5-20%, detected across all the orchards studied.

Table 2. Diversity of viral diseases reported in apple orchards and other fruit species in intensive orchards during the growing season.

Culture	Disease	Transmission vector	Intermediate indicator plants
Apple	1. Apple mosaic (*Malys virus 2* Smith)	It is transmitted through grafting and insect vectors with a piercing-sucking mouthpart, nematodes from the genera *Xiphinema* and *Longidorus*	The varieties *Lord Lambourne* and *Malling IX (Malus pumila domestica)* are used
	2. Ring Mosaic		
	3. Nerve chlorosis		
	4. Leaf curl		
Plum	Ring mosaic of pear (*Pyrus virus 3* Christoff)	Transmission of the virus through grafting, insects, and nematode vectors from the genus *Xiphinema*	The pear varieties *Beurre Hardy* and *Nene Poiteau* are used
Peach	The banded leaf mosaic (*Prunus virus 9* Chris.), leaf curling, and nerval chlorosis	The virus is transmitted by aphids, through grafting, as well as insects with a piercing-sucking mouthpart and vectors from the genus *Xiphinema*	The varieties used are peach, almond, apricot, sour cherry, and plum (*Prunus genus*)
	Ring spot disease on peach, apple, sour cherry, and cherry leaves (*Anulus cerasi Hildebrand*)	Transmission occurs through grafting, seeds, insects, and nematode vectors from the genera *Xiphinema* and *Longidorus*	The varieties used are cherry, peach, and mahaleb

The bibliographic study and the results of comparative surveys conducted in intensive fruit orchards, on various fruit species across the mentioned regions, highlight the presence of viral diseases, with the most common being: *Apple Mosaic, Apple Viral Proliferation, Apple Leaf Chlorotic Spotting, Apple Red Ring, Small Fruit Production (Apple Chat Fruit), Leaf Curling* and *Twisting, Ring Mosaic, Fruit Deformation (Green Crinkle*, and *Star Cracking*, as shown in Figure 2 (a, b, c). The manifestation of these diseases varies depending on the variety, the viral agent, transmission vectors, and the mode of infection. The inoculation occurs through the piercing stylet, which penetrates the meristematic zones and the absorbing root hairs during the adaptation period after planting, both in nurseries and in apple orchards.

(a) (b) (c)

Figure 2. Diffuse chlorosis, mottling, and whitening symptoms observed on apple leaves during the shoot formation phase of early apple varieties, July 2023-2024, Nisporeni district.

The research conducted was supplemented by determining the abundance of the numerical population in average values for the areas observed in apple orchards, as per the collected samples, estimated in Figure 1. As a result of the soil and young root analyses carried out in the laboratory, the numerical density (D.n.) was calculated, with average values ranging from 20-100 individuals/100 g of soil in the apple orchards of the Northern region, compared to the Central region, where the amplitude of values was almost at the same level, influenced by temperature and humidity variations observed during the first.

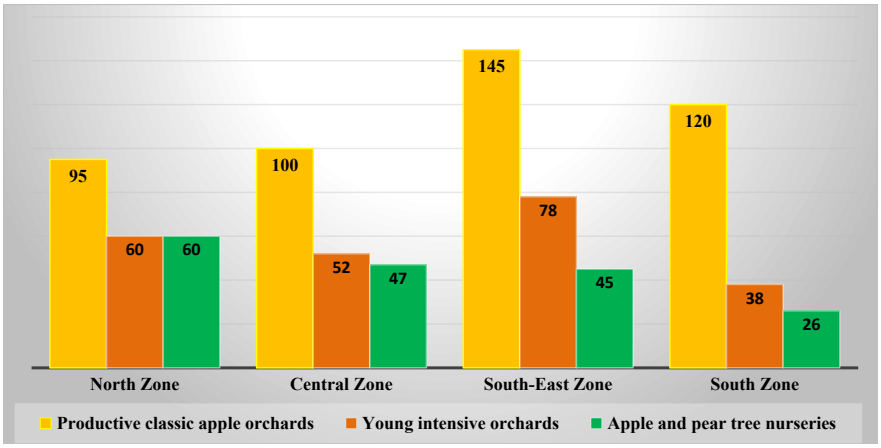

Figure 3. Results of the numerical population of parasitic nematode complexes and virus vectors, 2020-2024.

In the sectors of young orchards and seedling propagation nurseries, the numerical density was estimated at lower average values (20-30%) compared to the orchards investigated, as reflected in Figure 3.

The South-East region was characterized by higher numerical density values, ranging from 35 to 140 individuals/100 cm^3 of soil, compared to the South region, where lower values were recorded, 25% lower, particularly in newly planted orchards. The same situation was observed in the seedling propagation sectors, where the efficiency of agrotechnological and chemical protection management processes had a significant impact.

4. Conclusions

(1) As a result of the phytoparasitic impact assessment in apple orchards and nurseries for the propagation of planting material, as well as laboratory nematological analyses, an associative diversity of nematodes forming invasive complexes with specific helminthological and vectorial effects was identified in the apple crop. These nematodes belong to the orders *Tylenchida* and *Dorylaimida*. The identified species were classified according to their trophic spectrum, with endo-semi-ectoparasitic and vectorial specialization, playing a role in the onset of helminthic and viral diseases reported in all the investigated areas, both in productive orchards and in nurseries for apple planting material.

(2) The evidence and analysis of the contribution of ectoparasitic-vector species detected in the rhizosphere of apple trees highlighted their involvement in the contamination and development of viral diseases as well as helminthic disorders, both in intensive apple orchards and in nurseries for planting material. The presence of 15 species of pathogenic virus vectors was confirmed, with the most frequently found species being: *Longidorus elongatus*, *Xiphinema index*, *X. riversi*, *X. brevicolle*, *X. diversicaudatum*, and *Trichodorus primitivus*.

(3) Following the results of the surveys assessing the helminthological parasitic impact on apple orchard sectors and nurseries, a growth and development dynamic of the trees was observed, along with values of frequency and abundance of nematode species that are pathogenic virus vectors. These species were found to cause symptomatic viral diseases in bearing trees and young seedlings, on leaves and shoots, with values ranging from 5-20%, depending on the level of agrotechnical maintenance applied.

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(Alexei Bivol, Ștefan Rusu, Elena Iurcu-Străistaru, Viorelia Rusu) Institute of Zoology,
Moldova State University
E-mail address: `bivolalexei65@gmail.com, russus1974@yahoo.com, iurcuelena@mail.ru, viorelia1@yahoo.com`