Ukrainian Journal of Ecology

Ukrainian Journal of Ecology, 2021, 11(2), 372-377, doi: 10.15421/2021_125

ORIGINAL ARTICLE

Morphobiological development and distribution of oat (cereal) cyst-forming nematode (*Heterodera avenae* Woll.) in grain crops of Ukrainian southern steppe zone

I.I. Gulyaeva[1], A.I. Kryvenko[2], S.V. Stankevych[3], A.V. Matsyura[4]

[1]Odessa State Agrarian University, 13 Panteleimonivska Street, Odessa, Ukraine, 65012
[2]Odessa State Agricultural Research Station of the National Academy of Agrarian Sciences of Ukraine, 24 Mayatska Street, Khlibodarskoe village, Bilyaiv district, Odessa region, Ukraine, 67667
[3]V.V. Dokuchaiev Kharkiv National Agrarian University, v. Dokuchaevske, Kharkiv region, 62483, Ukraine
[4]Altai State University, 61 Lenin St, Barnaul, Russian Federation
**Corresponding author E-mail: inna_gulyaeva@ukr.net*
Received: 15.03.2021. Accepted 21.04.2021

Heterodera avenae Woll. affects grain crops and leads to severe economic losses in the areas of its distribution. The study was conducted to determine the species composition and distribution of *Heterodera avenae Woll*. in grain agrocenosis of the southern steppe zone of Ukraine. Nematological samples were taken according to standard and modified methods. Cysts from the soil were isolated by the decanting and sieving method. Production of temporary and permanent preparations determined the species composition of nematodes was carried out according to the generally accepted method. As a result of the conducted study, it was established that Heterodera avenae Woll significantly populated many grain crops in the Ovidiopolsky and Ivanovsky districts of the Odessa region. The number of *Heterodera avenae* in distribution areas significantly exceeded the economic threshold of harmfulness (ETH). At the same time, the symptoms of foci of suppressed plants are not always associated with infection with parasitic nematodes. One of the most pressing problems nowadays remains the improvement of existing and development of new mass nematological diagnostics of phytocenosis to identify foci of Heterodera avenae, its timely localization, and reduce the number population to an economically imperceptible level. It is necessary to have cartograms of the prevalence with the determination of the area of foci, the level of population of soil, and the species composition of cyst-forming nematodes, which should become the basis for differentiated selection and systematic application of various anti-nematode measures depending on their economic recoupment and environmental feasibility.
Keywords: agrocenosis, grain crops, oat (cereal) cyst-forming nematode, the economic threshold of harmfulness, nematological diagnostics, decline in number, cartogram.

Introduction

Cyst-forming nematodes are the most specialized obligate phytoparasites of the vast majority of crops. The world's fauna of cyst-forming nematodes, according to various scientists, has about 100 species. According to modern taxonomy, they belong to seven Heteroderidae genera: *Afenestrata (Heteroderinae), Betulodera, Cactodera, Dolichodera (Punctoderine), Globodera, Heterodera, and Punctodera* (Dekker, 1972; Zinovev & Volodchenko, 1972; Nikitin, 1976; Volodchenko, 1977; Polyakov et al., 1979; Sigaryova, 1988; Matveeva, 1989; Marks & Brodie, 1998; Subbotin, 2004).

Nematodes belonging to the Class Nematoda, type Nemathelminthes Schneide*r*, 1873, have an extensive distribution in nature, inhabiting both soil and aquatic biocenosis. In terms of species diversity and significance in natural and anthropogenic ecosystems, nematodes occupy second place after insects. They make up about 90% of the soil fauna in number, equal to 10% of the total biomass. The world's annual losses of plant production from phytoparasitic nematodes are 11-14 %, which cannot be ignored by plant protection specialists (Belyaev et al., 2017; Dekker, 1972; Matveeva, 1989; Yuan et al., 2010).

Wheat, soybean, rape, sugar beet, and potatoes should be mentioned among the most important crops for Ukraine. Obtaining high yields of these crops is associated with protective measures against phytopathogenic organisms in the technologies of their cultivation. Among these phytopathogenic organisms, the principal place is occupied by parasitic nematodes, which destroyed approximately 10% of annual plant production, and from which the entire world's losses are estimated at 100 billion US dollars. Among the complex of parasitic nematode species, the most dangerous for wheat, barley, oats, and soybean are representatives of cyst-forming nematodes belonging to the family of Heteroderidae, genus *Heterodera* and tend to accumulate in areas of intensive cultivation of host plants (Termeno, 1988; Hu et al., 2015).

Representatives of cyst-forming nematodes that belong to the Heterodridae family are probably the most dangerous pathogenes of different plants that damage the root system. *Heterodera avenae* has been found in more than 50% of the territory of Europe, which is the primary grain producer, as well as in a large area of Australia. However, despite such economic losses, agrotechnical control of cyst-forming nematodes is still a complex problem. The peculiarities of their biology play a significant role in this and, particularly, the presence of eggs protected by cysts in the development cycle, which are surprisingly resistant to environmental changes and can persist in the soil for many years (Sigarova & Kalatur, 2014).

One of the urgent today's problem is the improvement of existing and development of new methods of mass nematological diagnostics of phytocenosis for timely detection of foci of *Heterodera avenae*, slowing down the rate of its further settling, as well as reducing the number of population to an economically imperceptible level (Babich, 2013).
With a low initial population, signs of plant damage are not visually manifested, which allows the *Heterodera avenae* to stay in a latent state for a long time, and if the proportion of susceptible crops increases in crop rotations leads to mass reproduction and, as a result to deterioration of quality and significant crop losses (Babich, 2013; Sigarova & Kalatur, 2014).
Oat (cereal) cyst-forming nematode is a dangerous pest of grain crops. The first mention of it was known 164 years ago by the German scientist Kuhn on the root of oats, hence the species' oat nematode. However, as it became known later, this nematode turned out to be a parasite of oats and such crops as spring and winter wheat, barley, rye, corn, sorghum, millet, and other cereal grasses (Kiryanova & Krall, 1971).
The population of soil by oat nematode was first established in the Sumy region in 1957. In 1959 in the Ivano-Frankivsk region and 1961 in the Chernihiv regions. Oat cyst-forming nematode has been found in more than 50% of the territories of Europe, which are the leading producers of grain, as well as in a large area of Australia and India, where crop losses from grain reach 50% or higher. Its current area covers 18 regions: Odessa, Poltava, Kharkiv, Ternopil, Rivne, Sumy, Chernihiv, Cherkasy, Zhytomyr, Volyn, Chernivtsi, Ivano-Frankivsk, Kyiv, Transcarpathian, Kirovohrad, Vinnytsia, Mykolaiv, and Khmelnytskyi on crops of cereals. (Tihonova, 1971; Nikitin, 1983; Termeno, 1988). There are also several reports on the prevalence of phytoparasites in other areas (Kiryanova & Krall, 1971; Tihonova, 1971; Nikitin, 1983; Termeno, 1988). This species is distributed in the Western European countries (England, Germany, France, Holland, Belgium, Poland, Czech Republic, and Romania), USA, Canada, Japan, South Africa, India, Australia, Israel, Lithuania, Latvia, Estonia, Russia (Yan & Qiong, 2014).
Our research aimed at study of the oat cyst-forming nematode distribution in grain agrocenosis of the Southern steppe zone of Ukraine.

Materials and methods

The research material was cysts, eggs, larvae, and adults of the oat cyst-forming nematode *Heterodera avenae* Woll. (Tihonova, 1973; Volodchenko, 1977; Sigaryova, 1988). According to standard methods, the survey of agricultural lands was carried out (Kiryanova & Krall, 1969 Sigareva, 1986; Shesteperov, 1986). A hand drill and mechanical samplers were used to select nematological samples (Shesteperov & Shavrov, 1984).
The population density of the cyst-forming nematode in the soil was determined by the number of cysts and the average number of larvae and eggs in cysts isolated from 100 cm^3 of soil using the flotation funnel method (Kiryanova & Krall, 1969; Chen et al., 2015). The soil samples were thoroughly mixed, sifted through a sieve with a hole diameter of 2 mm, and dried in air to an air-dry state. Next, the batch of soil with a volume of 100 cm^3 was strewed into a chemical glass with a capacity of 1 liter and filled with 2/3 – 3/4 water. The soil was stirred with a glass stick for 2-3 minutes, then the contents of the glass were left to settle for 5 minutes until sediment appeared. The top layer of water with floating cysts and organic particles was drained into a sieve with a diameter of 0.1-0.2 mm. This procedure was repeated three times, adding water to the glass. The sediment from the sieves was washed off using a rubber bulb into a funnel with an inserted filter. After filtering, the filter was removed from the funnel and viewed under an MBS-9 microscope to count cysts. The cysts found on the filter were transferred to a drop of water on a slide. Using a preparation needle, 25-50 cysts were crushed, and the average number of larvae and eggs (l + e) per cyst was calculated. Further, their viability was evaluated.
Infection of agricultural lands with nematodes was determined by the number of eggs and larvae isolated from average batches of 100 cm^3 of soil (Kiryanova & Krall, 1969; Yang et al., 2017). Invasive larvae of the second age were isolated from plant tissues using the modified Baermann funnel method, while parasitic larvae of the third and fourth age were isolated by splitting roots in water in Petri dishes (Shesteperov & Shavrov, 1984; Sigareva, 1986). Production of temporary and permanent micro-preparations determined the species composition of nematodes was carried out according to generally accepted methods (Kiryanova & Krall, 1969; Kiryanova & Krall, 1971; Krall, 1978; Shesteperov & Shavrov, 1984; Chen et al., 2017).

Results

Morphology

Adult females are white and can be found on the roots of cereals in the tubing phase. The body is lemon-shaped with an indistinctly isolated, relatively blunt cone, 418-740 µm long, 285-534 µm wide, and the subcrystalline layer is well developed. The head is ringed, bears six small rounded lips, with the same number of small papillae, stiletto 22-32 µm (usually 26-32 µm). Cysts gradually change color from cream to dark brown as they mature; they are partially covered with a translucent white layer, have a length of 614-823 µm, a width of 382-627 µm, and a cone height of 34-46 µm. The anal-vulvar plate of a mature cyst is divided by a membrane (bridge) into two parts (bifenestra). The fenestra is 43-55 µm long and 14-32 µm wide, the vulvar bridge is 5.2-9.2 µm, the vulvar fissure is short – 7.0-13.0 µm, the distance between the anus and the fenestra is 19-35 µm. Number of eggs: on average 150-200, maximum – up to 900 (Kumari, 2017).

Fig. 1. Oat nematode *Heterodera avenae*

Differential diagnosis.

The body of males is vermiform, cylindrical in shape, 1070-1650 μm long and 28-43 μm wide, curved near the spicules, the latter – 32-38 μm in size, stiletto – 26-31 μm. Invasive larvae of the 2nd age: body length is 520-620 μm (usually 540-580 μm), and width is 19-24 μm, tapering to the tail, which has a length of 45-70 μm (usually 54-58 μm), the rounded hyaline part of the tail is more than half its length and is 34.5-48.0 μm. They can be found in the roots of plants by painting over. Young cysts are with a dense white subcrystalline layer. The color is brown without a yellow phase. Bifenestral type of vulva, vulvar fissure is 12 microns long, significantly shorter than the vulvar bridge. The semi-fenestra are almost rounded; the windows and vulva form an eight. Bulle is large and clear, drop-shaped. There is no vagina and lower bridge. The egg is slightly elongated on both sides. The size of larvae is 600 (540-680) microns. The transparent part of the tail is 1.0–1.5 times longer than the mouth stiletto.

Biology.

The oat nematode develops in a single generation. The development cycle lasts from 30 to 62 days, depending on the year's crop, variety, and weather conditions. Eggs and invasive larvae overwinter in cysts.

The coming out of larvae from cysts and their penetration into the roots of plants begins in spring at an average daily soil temperature of 7-8 °C and an air temperature of 9-11 °C, which coincides with the appearance of shoots of spring grain crops. In the roots, the larvae intensively feed on the contents of cells; they molt three times and turn into males and females (in a ratio of 1:1). The mass coming out of males and females from tissues of the roots occurs during the earing phase of plants and contributes to the development of root rot. Males die after the fertilization of females. Females acquire a lemon-shaped form with the front end of the body submerged in the root, where they continue to feed. With the naked eye, it is possible to see the "poppy seed" of white color. After a while, they turn into brown cysts that fall into the soil and remain viable for 10-12 years. High soil moisture in the spring period (40-60% of the absolute) contributes to embryonic development, the rebirth of larvae from eggs, and their mass penetration into the roots of plants. On the contrary, high temperature and insufficient moisture in the early spring period restrain the migration ability and vital activity of larvae. Larvae die when the soil humidity is below 3% and spring frosts (-3 °C). Nevertheless, eggs in cysts under such conditions remain viable.

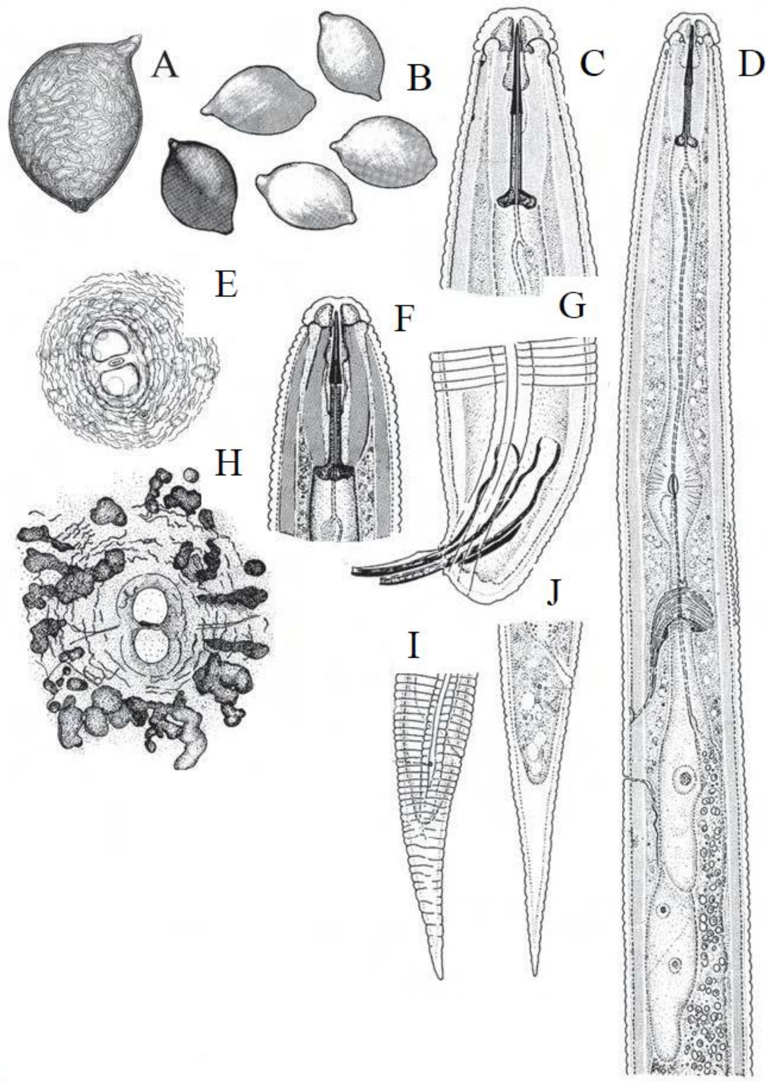

Fig. 2. Oat nematode *Heterodera avenae* (Zinovieva, 2012). A, B – cysts; C – the main end of the male; D – male esophagus; E, H – the structure of the anal-vulvar plate (Kiryanova & Krall, 1969, 1971; Williams, Siddiqi, 1972); F – the main end of the larva of the 2nd age, G – tail of the male; I, J – the tail of invasive larvae.

H. avenae is one of the most dangerous pathogens of cereals. The best host plants are wheat, oats, and barley. It also affects other cereals and herbs. It has several specialized physiological races that differ in aggressiveness towards the recommended varieties and host plant species.

As can be seen from the above data, examining agrocenosis for the spread of oat cyst-forming nematode continued until the end of the 90s of the last century when in the technologies of growing crops in the fields, preference was given to observance of scientifically based crop rotations. Over the past 20-30 years, there has been a significant violation of crop rotations, and therefore their saturation with grain and leguminous crops began to reach a critical 70-90%, which contributed to the accumulation of highly specialized pathogenic organisms in the soil –cyst – forming parasitic nematodes.

To detect oat cyst-forming nematode, we have conducted route surveys of cereals with foci of suppressed plants (winter barley, triticale, maize, amaranth, and saltbush), and clean soil was also selected in an untreated field (bogharic land). Sampling was carried out in the Ovidiopolsky, Ivanivsky, and Shyriaievsky districts of the Odessa region in 2018. In total, more than 20 medium-sized soil samples were analyzed (each of the ten initial samples), 7 of which were populated by the oat cyst-forming nematode, and 13 were free of it.

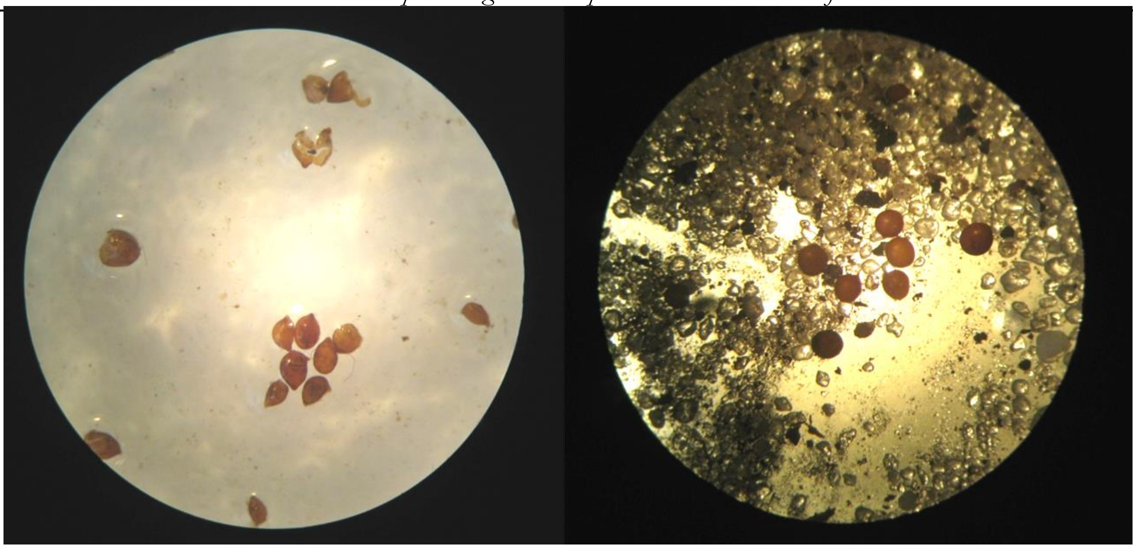

Fig. 3. Cysts of *Heterodera avenae* under the microscope.

Table 1. Soil contamination with the cereal cyst-forming nematode.

No	Survey area	Crop	The number of individuals per 100 cm^3 of soil	
			cysts	larvae+eggs
1	Odessa region Molodizhne village (field of 300 ha)	Barley	56	2380
2	-	-	49	2053
3	-	soil from under barley	15	374
4	-	Triticale	20	526
5	-	-	66	2726
6	-	-	31	983
7	-	maize (next to triticale)	25	703
8	-	maize (near bogharic land)	0	0
9	-	maize (undeveloped)	0	0
10	-	-	0	0
11	-	Saltbush	19	0
12	-	Bogharic land	0	0
13	-	-	0	0
14	Odessa region Ivanivsky district	Winter Barley	51	2575
15	-	-	60	5640
16	-	-	70	2142
17	Odessa region Shyriaievsky district Novoielyzavetivka village	Barley Field 1, with applied preparation	5	0
18	-	Field 1, control	10	0
19	--	Barley Field 2, with applied preparation	0	0
20	-	Field 2,control	0	0

According to the research results, many oat cyst-forming nematodes were established in the Ovidiopolsky and Ivanivsky districts of the Odessa region. In Ovidiopolsky district (Molodizhne village) we have examined suppressed crops of winter barley and triticale, which occupied small plots (about 20 hectares) in a field of 300 hectares, where maize was also sown apart from these crops. Soil samples from the rhizosphere of barley revealed a high number of oat cyst-forming nematodes, which reached 1602 (374-2380) larvae + eggs (l + e) in 100 cm^3 of soil in Ovidiopolsky district (Molodizhne village), 3452 (2142-5640) l + e in 100 cm^3 of soil in Ivanivsky district (Zhovte village). Besides, a cyst-forming nematode was found in the Ovidiopolsky district on triticale –1412 (526-2726) l + e in 100 cm^3 of soil and maize 703 l + e in 100 cm^3 of soil. These indicators are much higher than the thresholds of harmfulness, which reach 125-500 individuals per 100 cm^3 of soil.

Conclusions

Hence, it was established a significant distribution of the oat cyst-forming nematode. The absence of oat nematode was recorded in Ovidiopolsky district (Molodizhne village) on most maize crops (3 out of 4 samples), on bogharic lands radical soil of weeds of saltbush. This type of nematode was also absent in the barley field of Shyriaievsky district (Novosilky village), where four experimental areas of barley (4 samples) were examined. Thus, foci of suppressed plants on crops are not always associated with their infection with parasitic nematodes.

References

Babich, A.G. (2013). Vidovij sklad ta zonalna poshirenist cistoutvoryuyuchih nematod v Ukrayini. Naukovi dopovidi Nacionalnogo universitetu bioresursiv i prirodokoristuvannya Ukrayini, 6 (in Ukrainian).

Belyaev, V. I., Rudev, N. V., Maynel, T., Kozhanov, S.A., Sokolova, L.V., Matsyura, A.V. (2017). Effect of sowing aggregates for direct sowing, sowing seeding rates and doses of mineral fertilizers on spring wheat yield in the dry steppe of Altai Krai. Ukrainian Journal of Ecology, 7(4), 145-150. DOI: 10.15421/2017_98

Chen, C., Cui, L., Chen, Y. (2017). Transcriptional responses of wheat and the cereal cyst nematode *Heterodera avenae* during their early contact stage. Sci Rep, 7, 14471. https://doi.org/10.1038/s41598-017-14047-y

Chen, C., Liu, S., Liu, Q., Niu, J., Liu, P., Zhao, J., Jian, H. (2015). An ANNEXIN-like protein from the cereal cyst nematode *Heterodera avenae* suppresses plant defense. PLoS ONE, 10:e0122256

Dekker, X. (1972). Nematody rastenij i borba s nimi. Moscow, Kolos (in Russian).

Hu, X., Liang, X., Zhang, L., Chen, Y., Wang, X., Li, H.M. (2015). Phenotype characterization of interaction between different wheat cultivars and *Heterodera avenae.* Plan Protect, 41, 78-83.

Kiryanova, E.S. & Krall, E.L. (1969). Paraziticheskie nematody rastenij i mery borby s nimi. Part 1. Leningrad, Nauka (in Russian).

Kiryanova, E.S. & Krall, E.L. (1971). Paraziticheskie nematody rastenij i mery borby s nimi. Leningrad, Nauka (in Russian).

Krall, E.L. (1978). O sistematike cistoobrazuyushih nematod. Zashita rastenij, 10, 48–49 (in Russian).

Kumari, S. (2017). Morphological and molecular characterizations of cereal cyst nematode *Heterodera avenae* Wollenweber, 1924 from the Czech Republic. Journal of Integrative Agriculture, 16(3), 532-539. https://doi.org/10.1016/S2095-3119(16)61485-5

Marks, R.J. & Brodie, B.B. (1998). Potato cyst nematodes: Biology, distribution and control. Wallingford (U.K.): CAB International.

Matveeva, M.A. (1989). Cistoobrazuyushie kartofelnye nematody. Zashita rastenij ot nematod. Moscow: Nauka, 95–99 (in Russian).

Nikitin, B.C. (1976). Cistoobrazuyushie fitonematody Polesya Ukrainy. Thesis of Doctoral Dissertation. Moscow (in Russian).

Nikitin, V.S. (1983). Cistoutvoryuyuchi nematodi na zernovih kulturah. Kiyv (in Ukrainian).

Polyakov, N.Ya. et al. (1979). Rasprostranennost nematod-parazitov selskohozyajstvennyh kultur v SSSR. Moscow, VNIITEISH (in Russian).

Shesteperov, A.A. & Shavrov, G.N. (1984). Vyyavlenie i uchet fitogelmintozov. Voronezh (in Russian).

Shesteperov, A.A. (1986). Sposob fitogelmintologicheskoj diagnostiki Zashita rastenij, 12, 28–29 (in Russian).

Sigareva, D.D. (1986). Metodicheskie ukazaniya po vyyavleniyu i uchetu paraziticheskih nematod polevyh kultur. Kiyv. Urozhaj (in Russian).

Sigarova, D.D. & Kalatur, K.A. (2014). Vidovij sklad ta shkidlivist parazitichnih nematod v agrocenozah cukrovih buryakiv. Zahist i karantin roslin, 60, 303–317 (in Ukrainian).

Sigaryova, D.D. (1988). Paraziticheskie nematody osnovnyh kultur polevyh sveklovichnyh sevooborotov Lesostepi Ukrainy. Thesis of Doctoral Dissertation. Moscow (in Russian).

Subbotin, S.A. (2004). Molekulyarnaya filogeniya fitoparaziticheskih nematod. Paraziticheskie nematody rastenij i nasekomyh. Moscow, Nauka (in Russian).

Termeno, V.K. (1988). Ovsyanaya nematoda na zernovyh kulturah v Centralnom Polese Ukrainy i obosnovanie mer borby s nej. Thesis of Doctoral Dissertation. Moscow (in Russian).

Tihonova, L.V. (1971). Vozbuditeli naibolee opasnyh gelmintozov selskohozyajstvennyh kultur v SSSR. Moscow (in Russian).

Tihonova, L.V. (1973). Metodicheskie ukazaniya po vyyavleniyu i uchetu ovsyanoj (zlakovoj) cistoobrazuyushej nematody i mery borby s neyu. Moscow (in Russian).

Volodchenko, Z.G. (1977). Rasprostranenie geteroder na Ukraine. Zashita rastenij, 4(24) (in Russian).

Wouts, W.M. & Baldwin, J.G. (1998). Taxonomy and identification. In The cyst nematodes. S.B. Sharma (Ed.). Dordrecht. Kluwer, Pp. 83–122.

Yan, H., Qiong, W. (2014). He Occurrence and Development of the Cereal Cyst Nematode (*Heterodera avenae*) in Shandong, China. The American Phytopathological Society. Plant Disease, 98(12), https://doi.org/10.1094/PDIS-08-13-0830-RE

Yang, D., Chen, C., Liu, Q., Jian, H. (2017). Comparative analysis of pre and post-parasitic transcriptomes and mining pioneer effectors of *Heterodera avenae*. Cell Biosci, 7(11), https://doi.org/10.1186/s13578-017-0138-6

Yuan, Hx., Sun, Jw., Yang, Wx. (2010). New pathotypes of Heterodera avenae (cereal cyst nematode) from winter wheat in Zhengzhou, Henan, China. Australasian Plant Pathol, 39, 107–111. https://doi.org/10.1071/AP09050

Zinovev, V.G. & Volodchenko, Z.G. (1972). Novye svedeniya o rasprostranenii fitogelmintov na Ukraine. Nematody rastenij. Voronezh (in Russian).

Citation:
Gulyaeva, I.I., Kryvenko, A.I., Stankevych, S.V. (2021). Morphobiological development and distribution of oat (cereal) cyst-forming nematode (*Heterodera avenae* Woll.) in grain crops of Ukrainian southern steppe zone. *Ukrainian Journal of Ecology, 11* (2), 372-377.