ВЪЗРАСТОВА СТРУКТУРА НА ПОПУЛАЦИИ НА ЦИСТООБРАЗУВАЩИТЕ НЕМАТОДИ *HETERODERA FILIPJEVI* (HETERODERIDAE: NEMATODA) ПРИ РАЗЛИЧНИ СОРТОВЕ ПШЕНИЦА И ЕЧЕМИК
AGE STRUCTURE OF POPULATIONS OF THE CYST NEMATODE *HETERODERA FILIPJEVI* (HETERODERIDAE: NEMATODA) ON DIFFERENT WHEAT AND BARLEY VARIETIES

Женя Илиева[1]*, Илиян Илиев[2], Божан Зарков[3]
Zhenya Ilieva[1]*, Iliyan Iliev[2], Bozhan Zarkov[3]

[1]Институт по почвознание, агротехнологии и защита на растенията „Н. Пушкаров” – Костинброд
[2]Биологически факултет, Софийски университет „Св. Кл. Охридски”
[3]Институт по земеделие – Карнобат
[1]Institute of Soil Science, Agrotechnology and Plant Protection “N. Pushkarov” – Kostinbrod, Bulgaria
[2]Biological Faculty, Sofia University “St. Kl. Ohridski”
[3]Institute of agriculture – Karnobat

***E-mail: zhenya.i@abv.bg**

Abstract

In Bulgaria the plant parasitic nematode *Heterodera filipjevi* was firstreported in the region of Karnobat at the end of 20th century. Laboratory and field experiments on the seasonal dynamics of the species were conducted. The age structure and the population density of the nematodes were studied during the vegetation period. Delay inthe juvenile development and respectively emergency of white females was observed under both controlled and field conditions in barley for the varieties *Aheloy*, *Veslets*, and *Obzor* and under field conditions only in the *Milena* wheat variety.

The laboratory tests showed that fodder barley *Aheloy* was resistant and *Veslets* and *Obzor* were moderately susceptible to the nematode. The *Mirjana* wheat variety demonstrated high susceptibility, whiletherest of the wheat and barley varieties had intermediate position.

Key words*:* *Heterodera filipjevi*, wheat, barley, resistance, development.

INTRODUCTION

Cyst nematodes of the “avenae” group parasitize on wild and cultivated Poaceaeand some monocotyledonous plants. *Heterodera filipjevi* (Madzhidov) is one of the three most economically important cereal cyst nematodes (CCN) (McDonald and Nicol, 2005; Smiley & Nicol, 2009). It is a main pest in cereal production in North Europe, America, Middle East and Australia, along with *H. avenae* (Wollenweber) and *H. latipons* (Franklin).

These pests cannot be observed directly due to their small size (the second stage invasive juvenile is transparent, about 500 µm long and 20 µmwide) andcryptic development in the plant root. Moreover, the symptom sofin vaded plants are often attributed to local unevenness of the depth of the soil horizon, the soil structure, the water balance or other soil pathogens (e.g. barley yellowdwarfvirus - BYDV) (Smiley et al., 2011). Leaf symptom sarequite similar to those caused by *Rhizoctonia*. As a result not only farmers but even agronomists and scientists do not appreciate properly or do not detect nematode invasions on cereal crops. Recent data on yield evaluate the losses caused by CCN in the Pacific states Idaho, Oregon and Washington at about 4.3 millions per year (Smiley and Yan, 2015).

All three economically important species were found in Bulgaria but the latest record concerns *H. filipjevi* (Bekal et al., 1997). In further studies the species appeared to be much more common in cereal productive areas of the country than *H. avenae* and *H. latipons* – it was foundin 21 localities (regions of Vidin, Pleven, Velico Tarnovo, Shumen, Varna, Burgas, Sliven, Haskovo, Stara Zagora, Plovdiv and Pazardjik) of all 31 where cereal cysts nematodes were found (Ilieva and Iliev, 2013). Biology of the species resembles the life cycle of other species of “avenae” group and one generation per growing season (Hajihasani et al., 2010a; Seifi et al., 2013).

Anessentialelementin the control of CCNisthe useof resistant varieties. No experiments have been conducted onresistance of Bulgarian varieties of barley and wheat to *H. filipjevi.*

The aim of present study is to follow the population dynamics of *H. filipjevi* on six Bulgarian varieties of barley and three varieties of wheat and to determine their resistance to the parasite.

MATERIALS AND METHODS

Fieldexperiment. The experimental plots were on the territory of the Agricultural Institute in Karnobat situatedin anareaof 30-year monoculture cultivation of wheat and barley with no application of soil pesticides. GIScoordinates: N-42.6764E-26.9949, 174 ma.s.l. The climate belongs to the transitional continental region. Spring is long and cool, summer is hot and autumn is prolonged, warm and dry. Mean annual precipitation is a limiting factor for non-irrigated crops cultivated in the region (Zarkov and Atanassova, 2008). In the Köppen-Geiger classification the region belongs to the temperate zone with warm summer and no dry season (Peel et al., 2007). The experimental plot areas were 40 m^2 per variety. The soil type is pellikvertisol, slightly acid pH=6,2in water. The crops were sown in the period of 20-25 October, 2001. Seven samplings were performed: I – 15 Nov 2001; II – 27 March 2002; III – 2 April 2002; IV – 17 April 2002; V – 25 April 2002; VI – 10 May 2002; VII– 29 May 2002. Each sampling consisted of five bulk samples per plot/variety (20 subsamples taken from 0-20 cm depth with auger with d=25 mm) and 20 plants per plot. The cyst density in the samples was recalculated for dry soil after humidity correction.

Pot experiment: The experiment was conducted in a climate chamber with 16 h day regime and temperature19-21^{o}C. Germinated seeds were placed singly in pots(d=8 cm) with 0,5 kg sterilized growing mixture (soil and sand in proportion 2:8). A week after sowing each plant was infected with 2 ml solution containing about 300 juveniles II and eggs. Masterblend fertilizer (20:16:20)was applied three times during experiment. Sampling was made on the 4^{th}, 8^{th} and 12^{th} week after infestation in the period January - March 2007. Five replicates per variety per sampling were used. Resistance was evaluated with respect to the presence of white females and the number of cysts (Ireholm, 1994).

Varieties: *barley* – four brewing varieties: Aster – winter distichum; Korten – winter distichum; Obzor – winter-spring distichum; Panagon – winter polystichum and two forage varieties: Aheloy– winter polystichum; Veslets – winter-spring polystichum; *wheat* – three varieties of common winter wheat: Mirjana, Prjaspa and Milena.

Sample processing and nematode extraction: After a careful elutriation on 1000 and 250 µm sieves for white females catch. Plant roots were stained in Lugol's iodine for 2 minutes and juveniles III, IV and young females were counted directly in the plant tissue under a dissecting microscope Olympus SZ 61 (Bezoojen, 2006). Invasive juveniles II were not counted because they were inrecognizable from other endoparasitic and semi endoparasitic nematodes in the roots in field experiment (e.g. *Pratylenchus, Helicotylenchus*). The soil was elutriated separately with Fenuwik cane and white females and cysts were counted under a dissecting microscope.

The nematodes were initially identified using permanent slides of vulvarcones of cysts (Hooper, 1986) and glycerin slides of Juveniles II (Bezoojen, 2006). Several specimens recovered from plot No. 11 which is not included in the results and discussion probably belong to *H. latipons*. The morphometric identification was confirmed by sequencing of the ITSand SSU region (Ilieva and Iliev, 2013). The results of the molecular analysis were the reason to excludethe last two plots 10^{th} and 11^{th} (respectively barley Perun and Emon) from the experimental scheme.

Sample processing, nematode identification, pot experiment and data analysis were conducted in the Laboratory Unit for Testing and Diagnostic sat the Plant Protection Division of ISSAPP "N. Pushkarov".

Statistics and Program Products Used. Differences in varieties and each sampling were confirmed by nonparametric Kruskal-Wallis ANOVA tests. Multiple comparison by pairs was conducted by Tukey test at significance level α 0,05.The data and text were processed and formatted using Microsoft Office 2007 (Word, Excel) and Statistica 6.0.

RESULTS AND DISCUSSION

Field experiment. The initial density of nematodes in the experimental plots sampled before sowing was 23.16±5.96 (7–61) full new cysts in 200 g dry soil. The seasonal dynamics of the age structure of *H. filipjevi*on the wheat varieties are shown on Fig. 1 and on the barley varieties – on Fig. 2. The following common tendencies were observed both for wheat and barley: 1) no distinguishable stages (older than Juveniles II) in the first sampling (15 November); 2) Only stage Juvenile III was observed in the second sampling (27 March) and 3) The most abundant stage in the third sampling (April 2) was Juvenile III and the first Juveniles IV appeared.

The three studied wheat varieties are susceptibleto *H. filipjevi*. The age dynamics of Juveniles III and IV are similar. Juveniles III appeared at the end of March, reached the highest densities in the beginning of April and gradually decreased in number at the beginning of May. They were followed by Juveniles IV, respectively, which appeared as single specimens at the beginning of April and reached their maximum at the end of April – beginning of May. The first white females appeared at the beginning of May and consequently the first cysts were observed at the end of the same month in Mirjana and Prjaspa varieties. A delay in the parasite development in Milena wheat was observed. In the beginning of May there were still single Juveniles stage III and at the end of May only some Juveniles IV and white females were observed but no cysts were found.

All barley varieties were significantly less infested in comparison to the wheat varieties ($p \leq 0.05$). The three brewing varieties Aster, Panagon and Korten were relatively more susceptible and the observed age dynamics were very similar to the ones recorded for Mirjana and Prjaspa (Fig. 2), but with significantly lower overall degree of infestation. At the end of May the first cysts also appeared. The two forage varieties Veslets and Aheloy and the brewing variety Obzorshoweda relative delayin the development of Juveniles III and IV. White females appeared at the end of May and no cysts were found during the experimental period. The general population density *H. filipjevi* in Aheloy variety was very low during the whole study period suggesting resistance to the parasite.

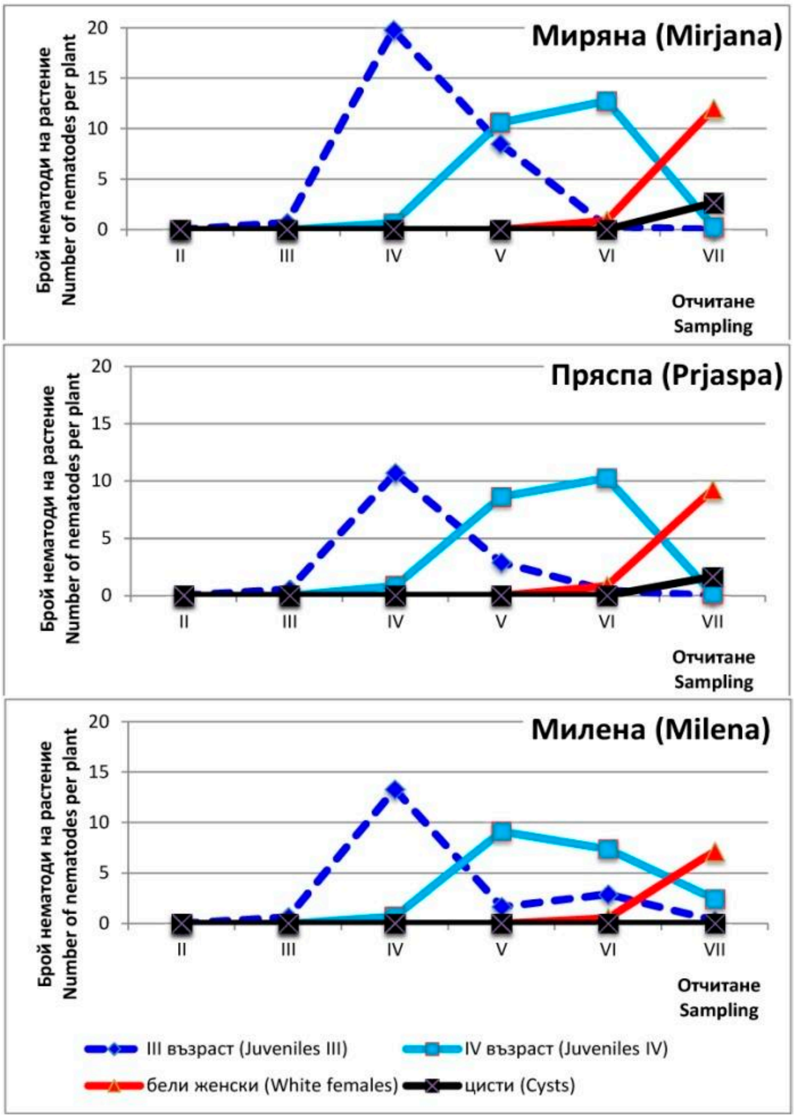

Fig. 1. *Age structure of the populations of H. filipjevi in three wheat varieties – open field experiment*

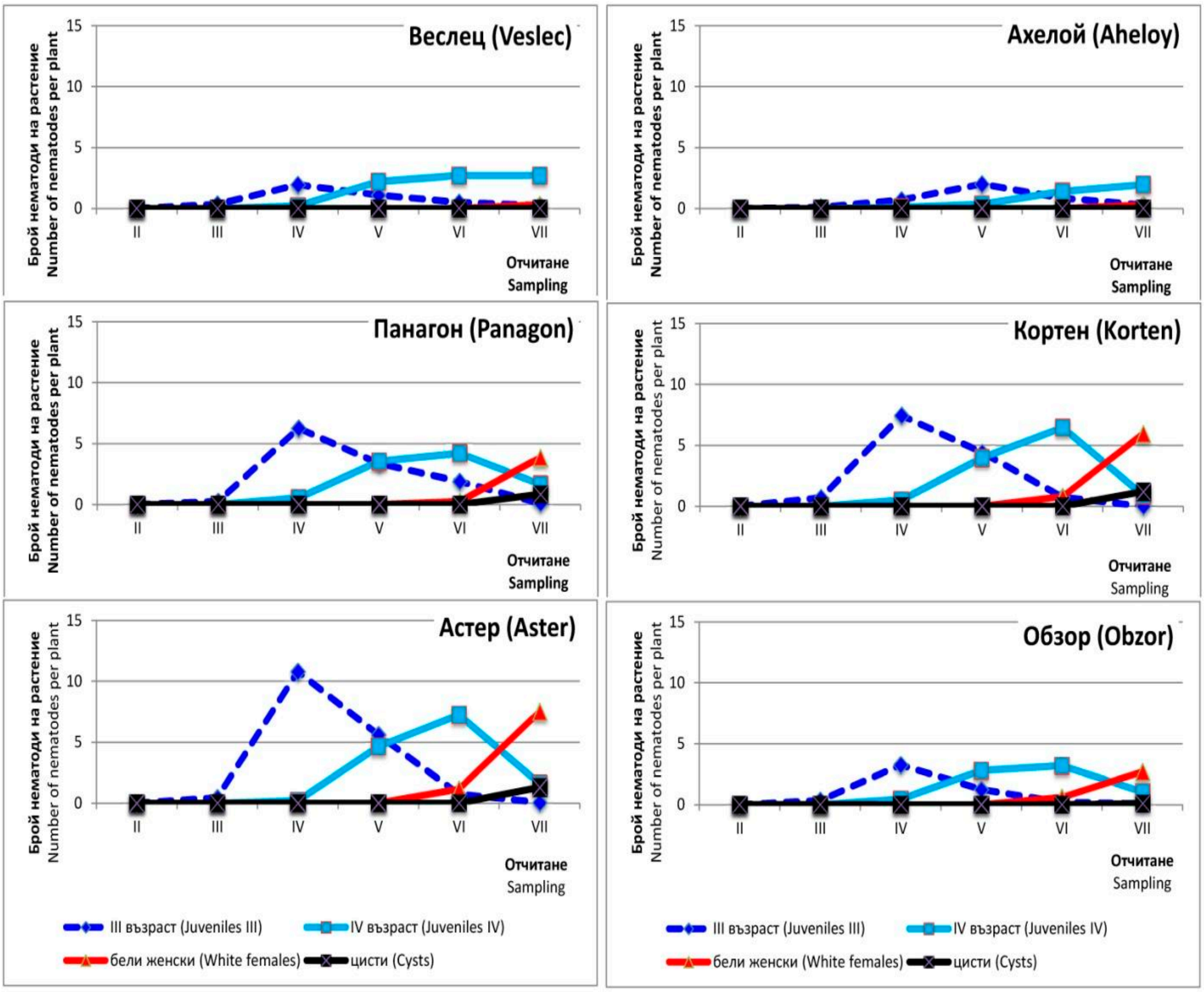

***Fig. 2.** Age structure of the populations of H. filipjevi on six barley varieties – open field experiment*

Pot experiment. Arelatively high degree of pathed distribution of the field population of *H. filipjevi* as well as findings of *H. latipons* in the immediate neighbourhood were the reasons to repeat the experiment undercontrolled conditions. The results of the experimentare presented in Table 1.

A delay in development was observed again in the parasite populations in wheat Milena and barley Aheloy and Veslec. In the other barley variety Obzor that developmental peculiarity was not so prominent. The comparison of the resistance of different varieties showed a significant positive result for forage barley Aheloy. A relative moderate susceptibility was observed in forage barley Veslets and brewing barley Obzor. The highest susceptibility was observed in Mirjana wheat and all other varieties of wheat showed intermediate susceptibility. The moderate susceptibility of wheat Milena observed in field experiment was not confirmed in controlled conditions and was probably a result of local factors in the field.

A similarmore pronounced aggressiveness of the parasiteon wheat in comparison to barley varieties was observed by Holgado et al. (2009) for "West *H. filipjevi"* populations. Differences between populations of *H. filipjevi* were also reported by other authors (Andersson, 2005; Lietal, 2012; Toktai et al., 2012; Imren et al., 2013). This could be explained by the existence of races within the species similar to those of*H. avenae.* The relatively wide distribution of *H. filipjevi* in cereal crop areas in Bulgaria (Ilieva and Iliev, 2013) and the observed mixed populations presuppose expansion of the investigations on CCN in our country.

Table 1. Age structure of *H. filipjevi* and resistance of 3 wheat and 6 barley varieties in a pot experiment (average number and standard deviation per sampling in five replicates). Different letters mark significant differences in pairs in each sampling estimated by Tukey test (α 0.05).

Сорт Variety	1 отчитане Sampling		2 отчитане Sampling		3 отчитане Sampling					Устой-чивост Resistance
	III*	IV**	III	IV	III	IV	БЖ# WF	Цисти Cysts	БЖ+Ц& WF+C	
Миряна (Mirjana)	32,6a ±12,30	1,33a ±0,58	18,4a ±5,98	16a ±4,74	0	2,4a ±3,29	30a ±5,61	2,2 ±1,64	32,20a ±6,91	**S**
Пряспа (Prjaspa)	19ab ±5,10	1a ±1,00	12,2ab ±2,95	7,6ab ±2,70	0	2,4a ±2,88	16,8b ±2,49	0,4 ±0,55	17,20b ±3,04	**S**
Милена (Milena)	34,2a ±6,06	0,4a ±0,55	24a ±4,85	8,8ab ±1,64	1 ±1,22	5,8a ±2,77	20,2ab ±3,56	0	20,20b ±3,56	**S**
Веслец (Veslets)	5,8c ±4,15	0,2a ±0,45	5c ±1,58	2c ±2,35	0	1a ±0,71	5,8c ±2,05	0	5,80c ±2,05	**(MS)**
Ахелой (Aheloy)	2,8c ±2,59	0,4a ±0,55	1c ±0,71	2,8bc ±3,03	0	1,8a ±1,64	1,8c ±1,48	0	1,80c ±1,48	**R**
Панагон (Panagon)	12,4b ±2,41	0,8a ±0,84	2,4c ±1,52	11ab ±3,08	0	0,6a ±0,55	12,2b ±3,03	0,6 ±0,89	12,80bc ±3,93	**S**
Астер (Aster)	21,2ab ±5,63	0,4a ±0,55	1,4c ±1,14	19,8a ±3,19	0	1,2a ±1,30	17b ±2,24	2,4 ±1,67	19,40b ±3,91	**S**
Кортен (Korten)	15b ±4,74	1,6a ±1,14	3,4c ±2,19	13,6ab ±2,70	0	1,2a ±1,30	14,4b ±4,98	1,2 ±1,10	15,60b ±6,08	**S**
Обзор (Obzor)	6c ±3,24	0,4a ±0,55	0,8c ±0,84	5,8bc ±2,28	0	0,4a ±0,55	5,4c ±1,52	0,2 ±0,45	5,60c ±1,96	**(MS)**

**III – трета възраст ларви (III stage juveniles)*
***IV – четвърта възраст ларви (IV stage juveniles)*
#БЖ – бели женски (WF – white females)
&БЖ+Ц – бели женски и цисти (WF+C – white females+cysts)
°S – чувствителен (Susceptible); R – устойчив (Resistant),
MS – умерено чувствителен (Moderately susceptible)

CONCLUSIONS

1. The study of the age structure and dynamics of the CCN species *H. filipjevi* on 3 wheat and 6 barley varieties revealed a delay in juvenile development and the emergence of white females in some varieties. This was observed both in the field and under controlled conditions on barley varieties Aheloy, Veslec and Obzor and only under field conditions in wheat variety Milena.

2. Forage barley Aheloy is resistant to *H. filipjevi*, while the varieties Veslets and Obzor are moderately susceptible. The wheat variety Mirjana showed high susceptibility. The relatively wide distribution of CNN and the existence of mixed populations and races are prerequisites for further studies with respect to the Bulgarian sort list of cereal varieties.

REFERENCES

Andersson, S., 2005. Susceptibility of cereal market cultivars to two pathotypes of Heterodera filipjevi. Journal of the Swedish Seed Association 115: 112–121.

Bekal, S., J.P. Gauthier and R. Rivoal, 1997. Genetic diversity among a complex of cereal cyst nematodes inferred from RFLP analysis of the ribosomal internal transcribed spacer region. Genome, 40: 479–486.

Bezooijen, van J., 2006. Methods and Techniques for Nematology. Edt. Brill, 108 pp.https://www.wageningenur.nl/upload_mm/4/e/3/f9618ac5-ac20-41e6-9cf1-c556b15b9fa7_MethodsandTechniquesforNematology.pdf.

Holgado, R., S. Andersson, J, Rowe, I. Clark, C. Magnusson, 2009. Management strategies for cereal cysts nematodes Heterodera spp. in Norway. In "Cereal cyst nematodes: status, research and outlook" Eds. I.T. Riley, J/:/ Nicol, A.A. Dadabat: pp. 154–159.

Hooper, D.J., 1986. Handling, fixing, staining and mounting nematodes. In: Southey, J.F. (ed.) Laboratory methods for work with plant and soil nematodes. MAFF, London: pp. 59–80.

Ilieva, Z. and I. Iliev, 2013. Distribution of several cereal cyst nematodes (Heteroderidae: Nematoda) in Bulgaria. IX National Conference of Parasitology, 18–21 September 2013, Plovdiv: 49–50.

Imren, M., H. Toktai, R. Bozbuga, A. Dababat and I.H. Elekcioglu, 2013. Pathotype determination of the cereal cyst nematode, Heterodera avenae (Wollenweber, 1924) in the Eastern Mediterranean Region in Turkey. Türk. entomol. derg., 37 (1): 13–19.

Ireholm, A., 1994. Characterization of Pathotypes of Cereal Cyst Nematodes, Heterodera Spp., inSweden. Nematologica 40(1-4): 399–411.

Li, H., L. Cui, H. Li, X. Wang, T.D. Murray, R.L. Conner, L. Wang, X. Gao, Y. Sun, S. Sun and W. Tang, 2012. Effective Resources in Wheat and Wheat–Thinopyrum Derivatives for Resistance to Heterodera filipjevi in China. Crop Science, 52: 1209–1217.

McDonald, A. and J.M. Nicol, 2005. Nematode Parasites of Cereals, pp. 131–191 In: "Plant Parasitic Nematodes in Subtropical and Tropical Agriculture" (Eds. M. Luc, R. A. Sikora, J. Bridge) C.A.B. International.

Peel, M. C., B. L. Finlayson, T. A. McMahon, 2007. *Updated world K¨oppen-Geiger climate classification map*. Hydrol. Earth Syst. Sci., 11: 1633–1644.

Smiley, R. W, J. M. Nicol, 2009. "Nematodes which challenge global wheat production": pp. 171–187. In: Carver, B. F. (Eds.) Wheat Science and Trade. Ames, Iowa, USA, Wiley-Blackwell.

Smiley, R.W., G.P. Yan, and J.N. Pinkerton, 2011. Resistance of wheat, barley and oat to Heteroderaavenaein the Pacific Northwest USA. Nematology 12(5): 539–552.

Smiley, R.W., G.P. Yan, 2015. Discovery of Heterodera filipjevi in Washington and comparative virulence with H. avenae on wheat. Plant Disease 99: 376–386.

Toktay, H., E. Yavuzaslanoglu, M. Imren, J.M. Nicol, I.H. Elekcioglu and A. Dababat, 2012. Screening for resistance to Heterodera filipjevi (Madzhidov) Stelter (Tylenchida: Heteroderidae) and Pratylenchus thornei (Sher&Allen) (Tylenchida: Pratylenchidae) sister lines of spring wheat. Türk. entomol. derg. 36 (4): 455–461.

Zarkov, B., D. Atanasova, 2008. Comperative investigation of sorghum, planted in Karnobat region. Inernational Scientific Conference 5–6 June 2008, Stara Zagorawww.sustz.com/Proceeding08/Content/Issues_in_plant_studies_09.html.