Journal of Plant Protection Research ISSN 1427-4345

ORIGINAL ARTICLE

Plant parasitic nematodes in the soil and roots of winter wheat grown in crop rotation and long-term monoculture

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Vol. 58, No. 2: 184–191, 2018

DOI: 10.24425/122934

Received: December 27, 2017
Accepted: June 19, 2018

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Abstract

The species structure of plant parasitic nematode populations from the rhizosphere of winter wheat grown with crop rotation or in 48-year-old monoculture was analyzed and compared. Dominating species: *Bitylenchus dubius*, *Merlinius microdorus*, *Paratylenchus neglectus* and *Heterodera avenae,* in monoculture plots, had higher populations than in crop rotation plots. *Heterodera avenae* eggs and larvae were infected by pathogenic fungi in 68% of the monoculture crops (vs. 65–66% of the cysts from crop rotation), 12–20% of *Paratylenchus* sp. specimens were colonized by bacteria, mainly by *Bacillus penetrans*. This study shows nematological changes occurring in long-term wheat breeding, thus providing additional information necessary to fight dangerous viral vectors of the examined cereal.

Key words: nematodes, monoculture, pathogenic fungi, vector of cereal virus, winter wheat

Introduction

Winter wheat plantations in Poland in 2015 covered around 23.9 million ha and is the most valuable cereal in Polish agriculture. Soil nematodes are still serious pests of wheat. Karnkowski (2005) reported that *Anguina tritici* was found in seeds during quarantine examinations. Soil nematode species near wheat roots were surveyed in Poland by: Brzeski and Sandner (1974), Kornobis (1984), Głąba (1986), Wilski (1979), Wolny (1986, 1989), Wasilewska (1990), Witkowski (1992). In European countries surveys of grass nematodes made by Bezoijen (1979). Observations on *Verticillium chlamydosporum* pathogenicity to *Heterodera avenae* were published by Kerry *et al.* (1984), Kerry (2000) and Wronkowska and Janowicz (1986). The Bałcyny Station has grown winter wheat as a monoculture for 48 years. For the first time, the occurrence of nematodes in monocultured spring barley was examined by Skwiercz and Wolny (1988), Skwiercz and Zawiślak (1988). During all that time, nematode populations were subjected to interactions with fungi and bacteria (Hoestra 1994; Sosnowska 2004; Szczygieł and Zepp 2004) and toxic substances like fertilizers, pesticides, etc. Our research describes changes in nematode communities over the last 30 years. With intensification of cereal production and simplification of crop rotation, wheat production is exposed to the development of soil pests, including parasitic nematodes on the roots. By studying the densities of nematode populations, it should be possible to determine the scale of the threat and which species develop under monoculture

conditions. The increasing population density may be seen as the result in the neediness of nematicides' use.

Materials and Methods

The research was carried out in 2015 at the Experimental Station of the University of Warmia and Mazury in Bałcyny. A medium loamy area of 300 m^2 was divided into two plots: one plot with crop rotation and one plot from 48-year-old monoculture. In each 300 m^2 field four 1 m^2 plots, located at the corners of the monocultured and rotation wheat fields were designated for research. Each of the four 1 m^2 squares was a repeat sufficient to evaluate the statistical results in accordance with the adopted method. The samples were collected at periods given below, so that the dates were comparable throughout the entire cultivation experiment, since the development of the parasitic nematode population correlates with the development of the host plant. Four soil samples from each of the small 1 m^2 plots were taken with a pedestrian cane of a 3 cm section at a depth of 40 cm in the vicinity of the wheat roots at the start (shooting in the spike) and a day after harvest. Each soil sample (1 kg) consisted of 50 g of fresh roots and spikes. The samples from each of the four 1 m^2 were a repetition (and at the same time unity of the soil + roots + stems), hence the isolated nematode from each part of the sample was a set of nematodes associated with the host on 1 m^2. Four soil samples from each of the plots were taken at a depth of 40 cm in the vicinity of the wheat roots at the start (shooting in the spike) and a day after harvest. Each soil sample weighed 1 kg and consisted of 50 g of fresh roots and spikes. Isolation of nematodes was conducted in three steps. First, according to the Baermann (1917) method: incubation of 20 g of fresh wheat roots on sieves in water for 5 days for large virus vectors (*Longidorus*, *Trichodorus*, *Xiphinema* sp.) and for endoparasitic specimens belonging to the genus *Paratylenchus*. The same method was used for isolating foliar nematodes from the spikes of wheat. Second, the centrifugation method of Szczygieł (1971) to extract other mobile soil nematodes. Third, extraction of nematode cysts using the simple bottle method. All cysts were collected and washed four times in distilled water, crushed and centrifuged. Parasitic and dyed eggs (intense blue) were separated by dyeing the sediment with 0.05% lactophenol (Wronkowska 1986, 1990). For fungal parasite analysis the cysts were washed in water and used. Nematodes isolated from incubation and centrifuging were combined with water and killed with hot 6% formaldehyde. The number of living eggs and larvae or fungal pathogens obtained from cysts were checked separately. After processing with glycerin by Seinhorst's rapid method (1959), permanent slides of nematodes were made. Species of nematodes were identified using the keys of Brzeski (1998) and Andrassy (2007).

Fungal isolation

The nematode cysts were obtained from the soil while the wheat was growing. Spores and mycelium of different fungi taxa were found with microscopic examination. These were identified by characteristics of both saprophyte species and pathogens. A taxonomic affiliation to verify the fungi associated with *H. avenae* was prepared on potato dextrose agar (PDA) medium on which the periphery filtrate from the cysts and other forms of nematodes blurs. Sterile Petri dishes with sprouting fungi cultures were incubated for 7 days at 24°C. After using the single spore technique, transplanted fungi were assessed as a pure culture. Species of fungi were identified according to Skirgiełło (1991).

Statistical analysis

Due to the nature of the results (lack of normality of distribution and constancy of variance), a nonparametric version of the Kruskal-Wallis variance was made. The analyzes were performed using the computer program Statistica ver. 13

Results and Discussion

Eleven species of plant parasitic nematodes were identified from the soil and roots in plots with winter wheat growing either with crop rotation or long-term monoculture from the Bałcyny Research Station during 1968–2015: *Trichodorus viruliferus* (Hooper, 1963), *Bitylenchus dubius* (Butschli, 1873), *Sauertylenchus maximus* (Allen, 1955), *Merlinius brevidens* (Allen, 1955), *Merlinius microdorus* (Allen, 1855), *Scutylenchus tessellatus* (Goodey, 1952), *Paratylenchus neglectus* (Rench, 1924), *P. pseudopratensis* (Seinhorst, 1968), *Paratylenchus nanus* (Cobb, 1923), *Heterodera avenae* (Wollenweber, 1924) and *Ditylenchus dipsaci* (Kuhn, 1857). All these plant parasitic nematodes were classified and grouped according to the exact location where they were found and their impact on the plants. The population density was counted in spring (Pi – initial population density) and in autumn, after harvest (Pf – final population density) with coefficient of reproduction – R (Figs. 1–12).

Nematodes, vectors of plant viruses

There was only one species of the stubby root nematode *T. viruliferus* (Hooper 1963) in both soil samples, either from the plots with crop rotation or monoculture.

Maximal population density was 80 specimens/100 cm^3 of soil under 18 years of monocultured wheat and 40 specimens after 48 years of monocultured wheat (Fig. 1). All *Trichodoridae* in Polish soils are known as vectors of all *Tobraviruses* during feeding (as ectoparasites) on the roots of plants (Decreamer and Robbins 2007).

Migratory endoparasitic nematodes

There were two species of the genus *Paratylenchus*: *P. neglectus* (Rench 1924) which had a higher population density of 340 specimens after 18 years of monoculture. After 48 years of monoculture the population was 389 (Fig. 2) from 315 specimens (Fig. 3) of *P. pseudopratensis* (Seinhorst 1968). The tolerance limits of that *Paratylenchus* species for highest specific environments of monocultured wheat on the plots are unknown. Phytotoxins arising from hydrolysis of phenolic substances from enzymes extracted by nematodes inside plant cells and in the cortical tissues of the young roots lead to necrosis and death of part of the roots. *Paratylenchus* specimens are harmful both directly and indirectly due to tritrophic interaction with plant pathogenic fungi and bacteria (Skwiercz 1987; Sosnowska 2004).

Migratory ectoparasiting nematodes

Higher population density, 502 specimens/100 cm^3 of soil (Fig. 4) of *Bitylenchus dubius* (Butschli 1873) is associated with plant inhibition of wheat all around Poland (Brzeski 1998; Winiszewska *et al.* 2012). *Merlinuis brevidens, Rotylenchus robustus* (Fig. 5) and *M. microdorus* (Fig. 6) are also permanent species in soil under wheat. These nematodes create higher population densities, 310 and 350 specimens, respectively, after 48 years of monoculture. Wheat is also a good host for *Scutylenchus tessellates* (Fig. 7), with maximal population density of 253 specimens in soil after 18 years of monoculture. *Paratylenchus nanus* (Fig. 8) and *P. fallax* (Fig. 9) have lower tolerance limits than several plant

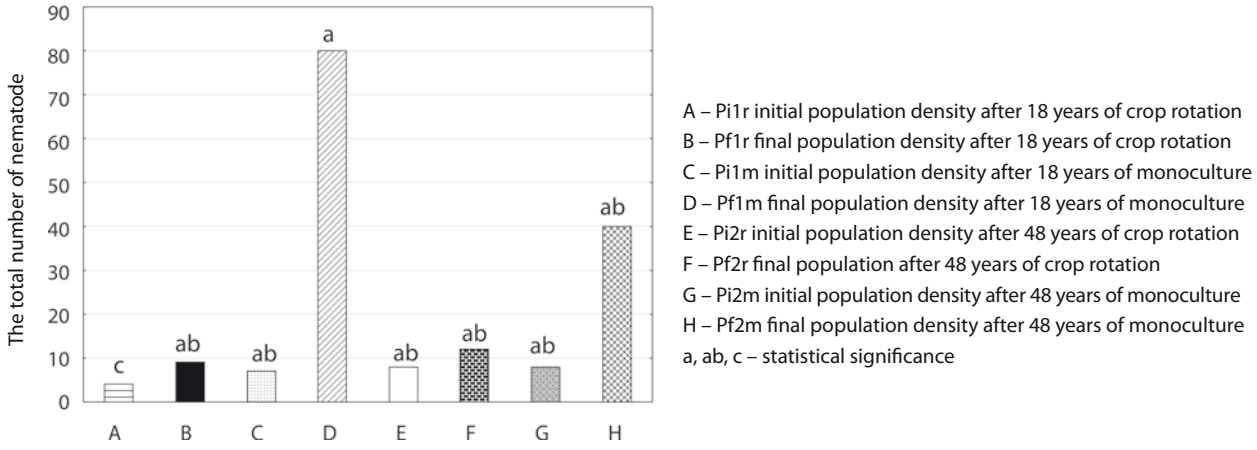


Fig. 1. Population density of *Trichodorus viruliferus* (Hooper, 1963) from 100 cm^3 of soil under winter wheat cultivated in crop rotation and long-term monoculture

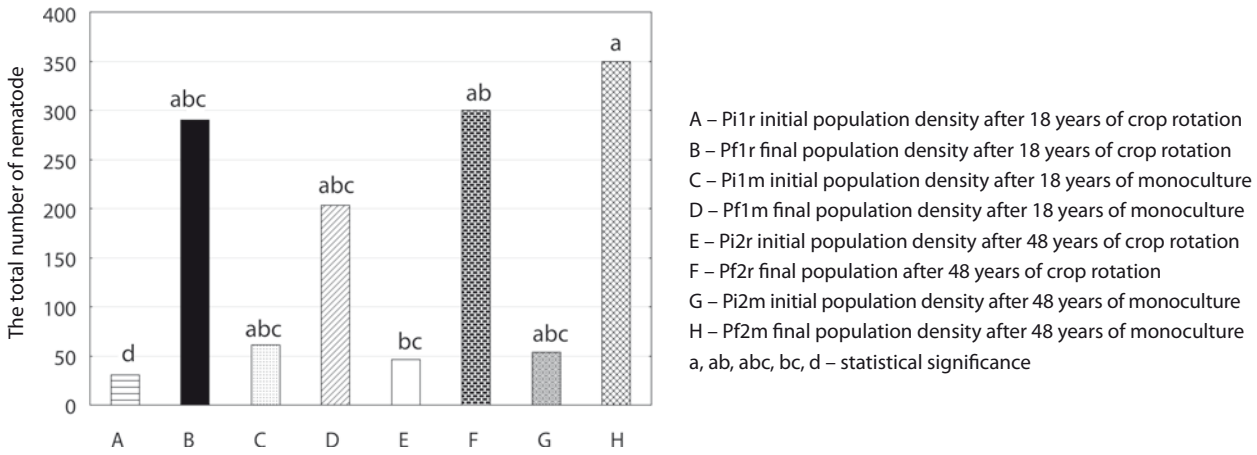
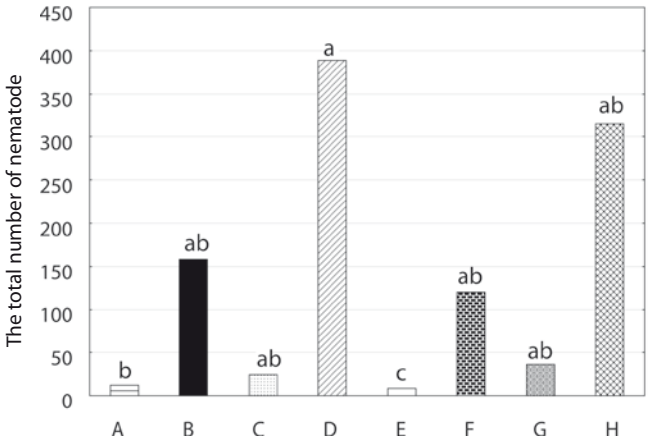
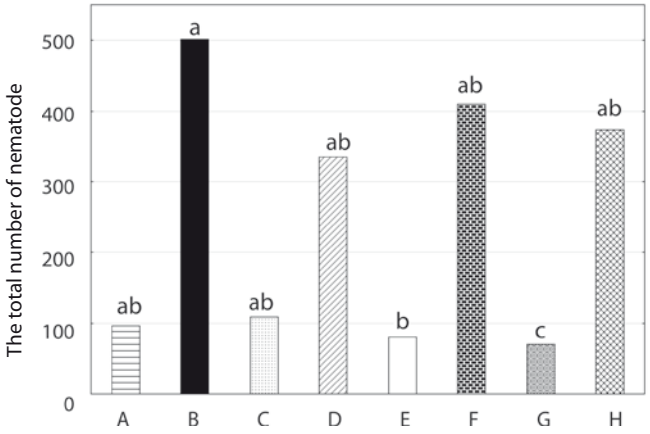


Fig. 2. Population density of *Pratylenchus neglectus* (Rench 1924) from 100 cm^3 of soil under winter wheat cultivated in crop rotation and long-term monoculture



A – Pi1r initial population density after 18 years of crop rotation
B – Pf1r final population density after 18 years of crop rotation
C – Pi1m initial population density after 18 years of monoculture
D – Pf1m final population density after 18 years of monoculture
E – Pi2r initial population density after 48 years of crop rotation
F – Pf2r final population after 48 years of crop rotation
G – Pi2m initial population density after 48 years of monoculture
H – Pf2m final population density after 48 years of monoculture
a, b, ab – statistical significance

Fig. 3. Population density of *Pratylenchus pseudopratensis* (Seinhorst, 1968) from 100 cm^3 of soil under winter wheat cultivated in crop rotation and long-term monoculture years of monoculture



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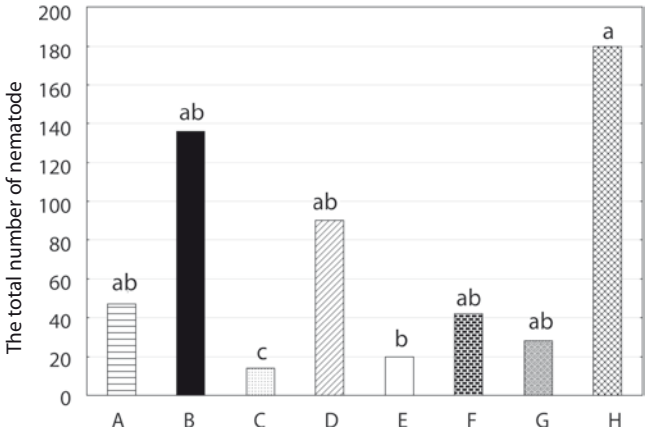
B – Pf1r final population density after 18 years of crop rotation
C – Pi1m initial population density after 18 years of monoculture
D – Pf1m final population density after 18 years of monoculture
E – Pi2r initial population density after 48 years of crop rotation
F – Pf2r final population after 48 years of crop rotation
G – Pi2m initial population density after 48 years of monoculture
H – Pf2m final population density after 48 years of monoculture
a, b, ab, c – statistical significance

Fig. 4. Population density of *Bitylenchus dubius* (Butschli, 1873) from 100 cm^3 of soil under winter wheat cultivated in crop rotation and long-term monoculture



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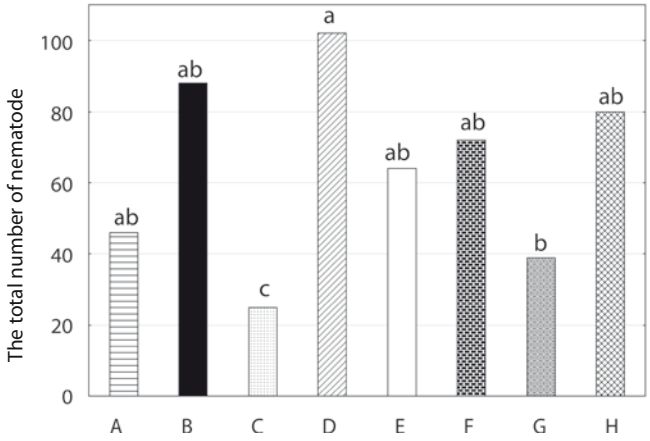
C – Pi1m initial population density after 18 years of monoculture
D – Pf1m final population density after 18 years of monoculture
E – Pi2r initial population density after 48 years of crop rotation
F – Pf2r final population after 48 years of crop rotation
G – Pi2m initial population density after 48 years of monoculture
H – Pf2m final population density after 48 years of monoculture
a, b, ab, c – statistical significance

Fig. 5. Population density of *Rotylenchus robustus* from 100 cm^3 of soil under winter wheat cultivated in crop rotation and long-term monoculture

species. *Ditylenchus dipsaci* (Fig. 10) was found to have very low population density, probably due to the specific environment of extremely long-term monoculture. No stem nematodes were observed in the aerial parts of wheat.

Sedentary nematodes

Heterodera avenae cysts, eggs and larvae were observed over the last 30 years of the Bałcyny experiment. Higher population densities, 480 specimens



A – Pi1r initial population density after 18 years of crop rotation
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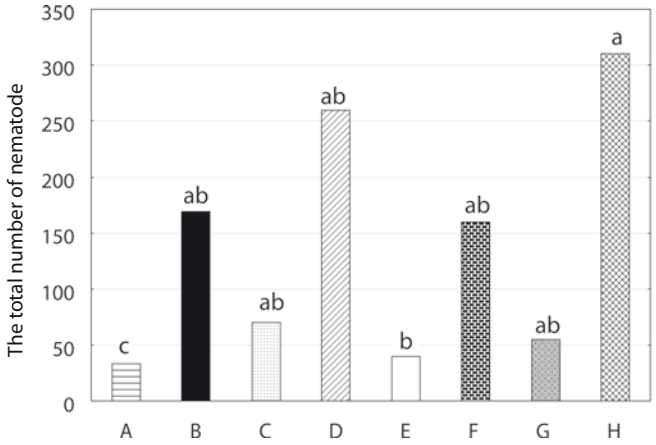
C – Pi1m initial population density after 18 years of monoculture
D – Pf1m final population density after 18 years of monoculture
E – Pi2r initial population density after 48 years of crop rotation
F – Pf2r final population after 48 years of crop rotation
G – Pi2m initial population density after 48 years of monoculture
H – Pf2m final population density after 48 years of monoculture
a, b, ab, c – statistical significance

Fig. 6. Population density of *Merlinuis microdorus* (Allen, 1955) from 100 cm^3 of soil under winter wheat cultivated in crop rotation and long-term monoculture



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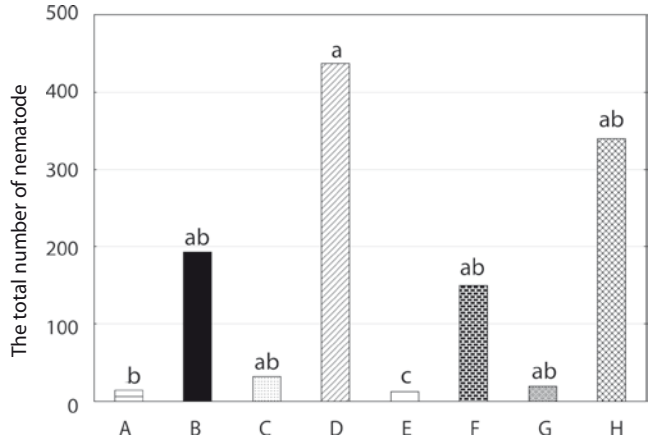
B – Pf1r final population density after 18 years of crop rotation
C – Pi1m initial population density after 18 years of monoculture
D – Pf1m final population density after 18 years of monoculture
E – Pi2r initial population density after 48 years of crop rotation
F – Pf2r final population after 48 years of crop rotation
G – Pi2m initial population density after 48 years of monoculture
H – Pf2m final population density after 48 years of monoculture
a, b, ab, c – statistical significance

Fig. 7. Population density of *Scutylenchus tessellates* (Goodey, 1952) from 100 cm^3 of soil under winter wheat cultivated in crop rotation and long-term monoculture



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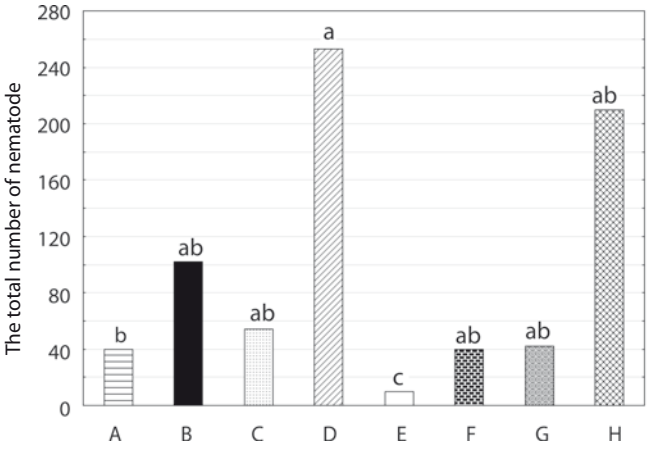
B – Pf1r final population density after 18 years of crop rotation
C – Pi1m initial population density after 18 years of monoculture
D – Pf1m final population density after 18 years of monoculture
E – Pi2r initial population density after 48 years of crop rotation
F – Pf2r final population after 48 years of crop rotation
G – Pi2m initial population density after 48 years of monoculture
H – Pf2m final population density after 48 years of monoculture
a, b, ab, c – statistical significance

Fig. 8. Population density of *Pratylenchus nanus* (Cobb, 1923) from 100 cm^3 of soil under winter wheat cultivated in crop rotation and long-term monoculture

per 100 cm^3 of soil were observed in plots of wheat after 48 years of monoculture (Fig. 11).

After examining the eggs and larvae it was concluded that pathogenic fungi specimens deceased by 65% after 18 years in plots with crop rotation, and 66% after 48 years of crop rotation. In plots from both lengths of monoculture pathogenic fungi infected 68% of *H. avenae* specimens. Figure 12 lists species of different fungi isolated from the surveyed material.



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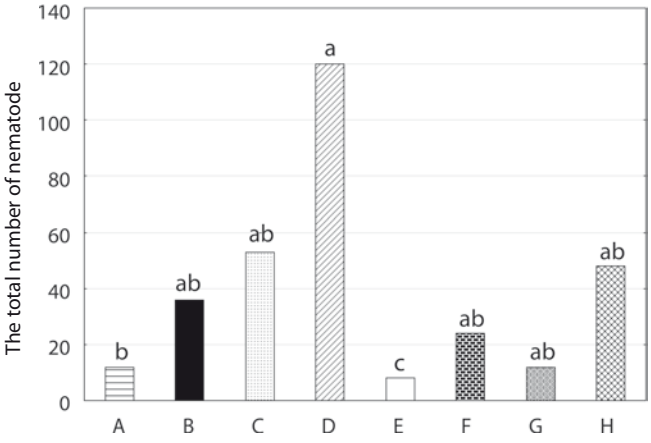
F – Pf2r final population after 48 years of crop rotation
G – Pi2m initial population density after 48 years of monoculture
H – Pf2m final population density after 48 years of monoculture
a, b, ab, c – statistical significance

Fig. 9. Population density of *Paratylenchus fallax* from 100 cm³ of soil under winter wheat cultivated in crop rotation and long-term monoculture



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B – Pf1r final population density after 18 years of crop rotation
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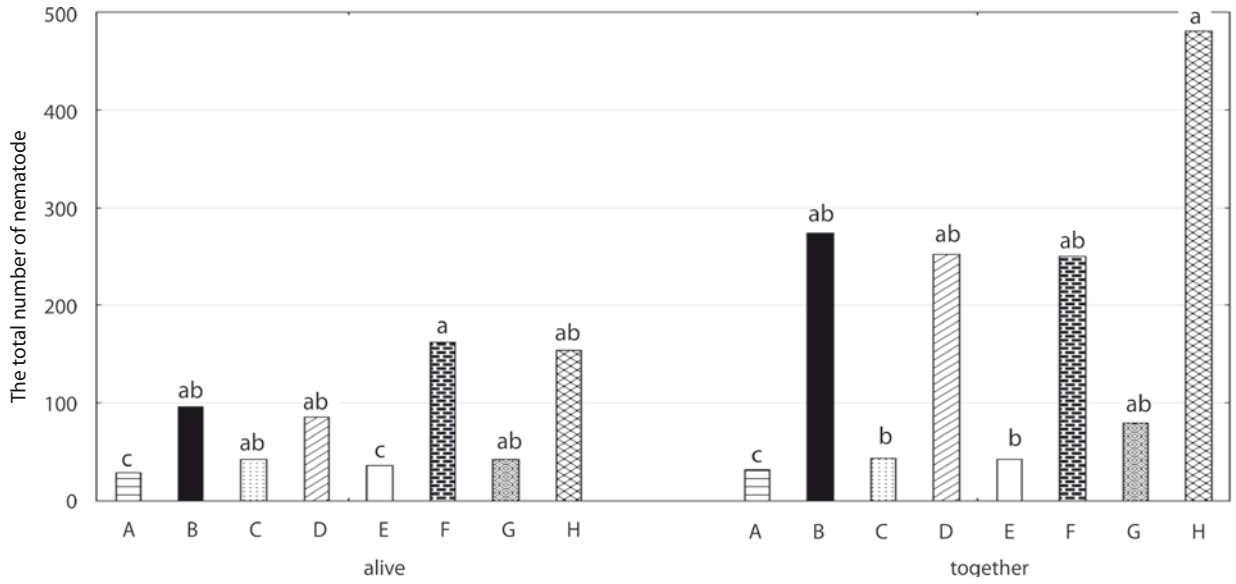
E – Pi2r initial population density after 48 years of crop rotation
F – Pf2r final population after 48 years of crop rotation
G – Pi2m initial population density after 48 years of monoculture
H – Pf2m final population density after 48 years of monoculture
a, b, ab, c – statistical significance

Fig. 10. Population density of *Ditylenchus dipsaci* (Kuhn, 1857) from 100 cm³ of soil under winter wheat cultivated in crop rotation and long-term monoculture

Some aspects of natural control of the nematode genus *Paratylenchus* and cereal cyst nematode *Heterodera schachtii* (Wollenweber, 1924)

Specimens of *Paratylechus neglectus* were occupied by *Bacillus penetrans* in 12–15% of the soil under crop rotation and in 15–20% of the samples from monocultured wheat. *Paratylenchus pseudopratensis* was infected relatively in 24 and 28% of all genus. Three species of parasitic fungi: *Paecilomyces lilacinus* (Thom) Samsom inside eggs and larvae, *Pochonia chlamydosporia* (Goddard Zare et Gams) which attack only nematode eggs and *Verticillium chlamydosporum* – nematophagous fungi, were isolated while examining the strains inside *H. avenae* eggs. The mechanism of plant susceptibility to the impact of nematode populations on nematophagous fungi living around the roots has been identified. The multitrophic interactions of soil organisms are probably due to a specific soil network on the base of the fungus strain system. It confirmed suggestions of many authors before: Ettema and Bongers (1993), Kerry (2000), Sosnowska (2004), Augustyniuk-Kraus (2012). Long-term monocultured wheat shows that the soil stability system still provides resistance to disturbance by parasites and pathogens. This is seen by the parasitism of eggs and larvae by pathogenic fungus strains and bacteria inside *Paratylenchus* specimens. As a result of this research, it was concluded that the simplification of shifting towards monoculture has not caused the development of parasitic nematode populations in a way that requires the use of nematicides. Species of the genus *Paratylenchus* and *Heterodea,* which are the most dangerous for wheat, have been limited by their natural fungal and bacterial pathogens. Species of the genera *Bitylenchus* and *Merlinius* noted in the experiment are not known in the literature as significant wheat parasites.



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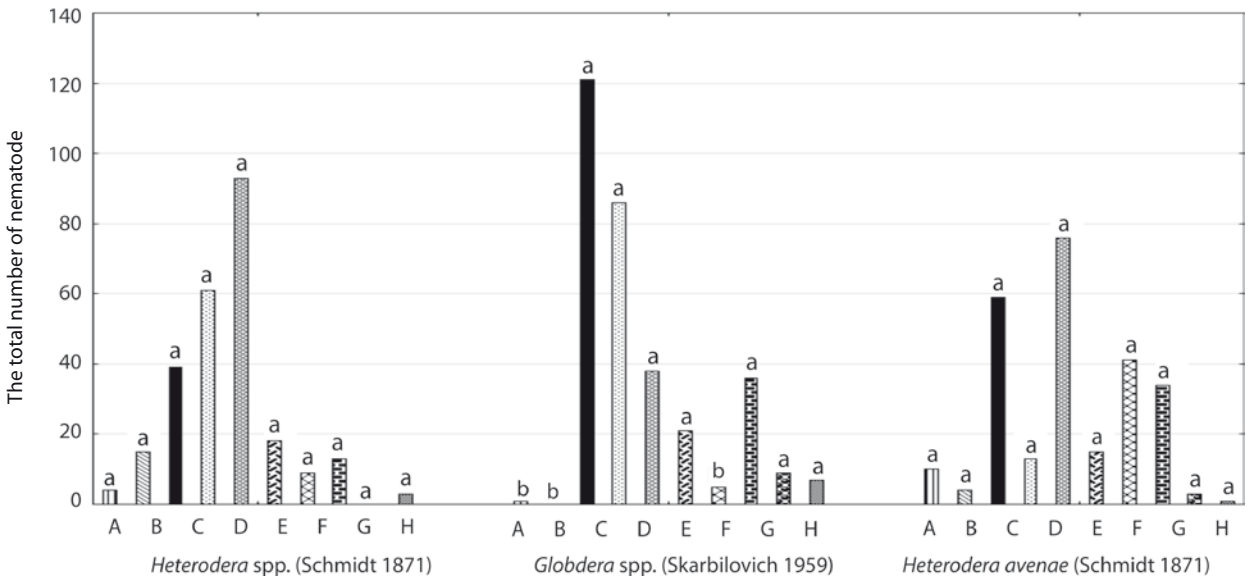
E – Pi2r initial population density after 48 years of crop rotation
F – Pf2r final population after 48 years of crop rotation
G – Pi2m initial population density after 48 years of monoculture
H – Pf2m final population density after 48 years of monoculture
a, ab, abc, bc, c, d – statistical significance

Fig. 11. Population density of *Heterodera avenae* (Wollenweber, 1924) from 100 cm^3 of soil under winter wheat cultivated in crop rotation and long-term monoculture



A – *Pochonia chlamydosporia* (Goddard 2001)
B – *Paecillomyces lilacinus* (Thom 1910)
C – *Alternaria alternata* (Keissl 1912)
D – *Cladosporium* spp. (Persoon 1816)
E – *Fusarium roseum* (Schwein 1936)
F – *Fusarium solani* (Schwein 1936)
G – *Penicillium* spp. (Link 1809)
H – *Trichothecium roseum* (Link 1809)
I – *Rhizopus nigricans* (Ehrenberg 1820)
J – *Rhizoctonia solani* (Kühn 1858)
a, b – statistical significance

Fig. 12. Fungal parasites of cereal cyst nematode collected from the long-term monocultured wheat in the plots of Experimental Station Bałcyny

Conclusions

Soil stability system involved pathogenic fungi and bacteria to decrease some populations of pathogens due multitrophic interaction between bacteria, fungi and nematodes.

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