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NEMATODES IN THE SOIL AND ROOTS OF SPRING BARLEY GROWN IN CROP ROTATION AND LONG-TERM MONOCULTURE

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ABSTRACT

Background. Species structure of plant parasitic nematode populations from the rhizosphere of spring barley grown in an 18-year-old crop rotation and in a 48-year-old monoculture were analyzed and compared.

Material and methods. Plots were established in fields of spring barley grown in an 18-year-old crop rotation and in a 48-year-old monoculture. Four 1 m^2 plots were located in each corner of each field. Four soil samples from each 1 m^2 plot were taken by a pedestrian cane of a 3 cm section at a depth of 40 cm in the vicinity of the barley roots at start (BBCH 09) and a day after harvest (BBCH 99). Each soil sample weighed 1 kg and contained 50 g of fresh roots and spikes. The sample from each of the four 1 m^2 plots was a replicate (and at the same time, the combination 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 .

Results. Populations of dominating species such as *Bitylenchus dubius*, *Merlinius microdorus*, *Pratylenchus neglectus* and *Heterodera avenae* became higher in the monoculture than in the crop rotation. *H. avenae* eggs and larvae were infected by pathogenic fungi in 50% of samples from the monoculture (vs. 60% of the cysts from the crop rotation), and 18–35% of *Pratylenchus* specimens were colonized by bacteria, mainly by *Bacillus penetrans*.

Conclusion. The results illustrated nematological changes occurring in long-term cropping systems and provided additional information necessary to fight dangerous viral vectors for the examined cereal.

Key words: nematode, pathogenic fungi, vector of cereal virus

INTRODUCTION

In 2015 spring barley plantations, an important product in our agriculture, covered 608 thousand ha of Polish territories. Soil nematodes seem to be a serious pest of barley (Witkowski,1962; Corbett, 1972; van Bezoijen, 1979; Wilski, 1979; Wolny, 1989b; Brzeski, 1998; Winiszewska *et al.*, 2012). The earlier reports about soil nematode species near roots of barley in Poland were reported by: Domurat and Kozłowska (1970), Kornobis (1984), Głąba (1986), Wolny (1986; 1989a). The Bałcyny Station holds a 48-year-old monoculture of spring barley. For the first time, occurrence of nematode in monocultured

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spring barley was studied by Skwiercz and Wolny (1988). Throughout this time, populations of soil nematodes interacted with different agricultural pressures, as well as with many genera and species of bacteria and fungi (Hoestra, 1994; Sosnowska, 2004; Szczygieł and Zepp, 2004). Exposure of barley to the development of its soil pests, including parasitic nematodes on the roots, has increased as a result of the simplification of crop rotation and the intensification of cereal production. The study of densities of the nematode population should give an answer to the scale of the threat, which species develop under monoculture conditions, and the increasing population density may be seen as an indicator of the need to apply a nematicide.

MATERIAL AND METHODS

The research was carried out in 2015 at the Experimental Station of the University of Warmia and Mazury in Bałcyny. The soil was a medium loam. Plots were established in fields of spring barley grown in an 18-year-old crop rotation and in a 48-year-old monoculture. Four 1 m² plots were located in each corner of each field. The replicated 1 m² plots provided the opportunity to conduct a statistical analysis of the results. Samples were collected in periods as it is written 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 1 m^2 plot were taken by a pedestrian cane of a 3 cm section at a depth of 40 cm in the vicinity of the barley roots at start (shooting in the spike) and a day after harvest. Each soil sample weighed 1 kg and contained 50 g of fresh roots and spikes. The sample from each of the four 1 m^2 .

Isolation of nematodes was made in three steps. The first step, made by Baermann method: incubation of 20 g of fresh roots of barley on sieves in water during 5 days for large virus vectors (*Longidorus*, *Trichodorus* and *Xiphinema* specimens) and for endoparasitic specimens belonging to the genus *Pratylenchus*. The same method was used for isolating foliar nematodes from the spikes of barley. The second step used the centrifugation method (made by Szczygieł, 1971): this procedure helped to

extract isolates of other mobile nematodes from the soil. The third step depended on the extraction of cysts of nematodes using the simple bottle method. All cysts were collected, washed 4 times in distilled water, smashed and centrifuged. Dyeing sediment by 0.05% lactophenol separated parasitized and dyed eggs by intense blue color (Wronkowska, 1990). For fungal parasite analysis, the cysts were washed in water. Nematodes were obtained by isolation from incubation and centrifuging with water and killed in hot 6% formaldehyde. The number of eggs and larvae obtained from cysts were checked separately for the living and total number, part of them were parasited by fungal pathogens. After processing to glycerin by the Seinhorst rapid method (1959), permanent slides of nematodes were made. Species of nematodes were identified using keys of Brzeski (1998) and Andrassy (2007).

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 Statistica version 13 software.

Fungal isolation

A microscopic examination of nematodes' cysts obtained from the soil of barley revealed spores and mycelium of different fungus taxons. These were identified characteristics of both species' saprophytes and pathogens. A taxonomic affiliation to verify the fungi associated with *Heterodera avenae* was prepared on PDA (Potatoes Dextrose Agar) medium on which blurs the periphery filtrate from the cysts and other forms of nematodes. Sterile Petri dishes with sprouting fungi cultures were incubated for seven days at 24°C. After using the single spore technique transplanted fungi were assessed as a pure culture.

RESULTS AND DISCUSSION

Eleven species of parasitic plant nematodes were identified from the soil and roots in surveyed plots under spring barley growing in the crop rotation and in long-term monoculture. The population density was counted in spring (= Pi-initial population density) and in autumn, after harvest (= Pf-final population density) (Fig. 1–11).

Nematodes, vectors of plant viruses

The stubby root nematode *Trichodorus viruliferus* (Hooper, 1963) was obtained in the soil from the plots of both the crop rotation and both 18-year-old and 48-year-old monocultures. The maximal population density was 18 specimens/100 cm³ of soil under 18-year-old spring barley in crop rotation (11 specimens in monoculture) and 24 specimens after 48 years monoculture of barley (Fig. 1). Both densities were enough to translate virus between deceased and healthy roots. All *Trichodoridae* are known as a vector of tobacco rattle virus group during feeding (as ectoparasites) on the roots of plants (Decreamer and Robbins, 2007). The most important virus diseases in barley in major vectored by *Trichodoridae* are:

barley yellow dwarf virus BYDV-MAV and BYDV-PAV, cereal yellow dwarf virus RPV (CYDV-RPV), barley yellow mosaic virus (BaYMV), barley mild mosaic virus (BaMMV). These diseases are also vectored by *Polymyxa graminis* (Hoestra, 1994; Jeżewska, 1998; Miller *et al.*, 2002).

Polish investigations on nematodes as soil vectors of plant viruses in barley are still lacking. The occurrence of species of the genera *Longidoridae*, *Xiphinema* and species of *Trichodoridea* in Polish soils needs observation of its role in potential virus (especially latent form) diseases in barley and other cereals (Wolny, 1986; 1989a; Hoestra, 1994; Miller *et al.*, 2002).



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-year monoculture D – Pf1m final population density after 18-year monoculture E – Pi2r initial population density after 48-year crop rotation F – Pf2r final population after 48-year crop rotation G – Pi2m initial population density after 48-year monoculture H – Pf2m final population density after 48-year monoculture

Fig. 1. Population density of *Trichodorus viruliferus* (Hooper, 1963) from 100 cm³ of soil under spring barley cultivated in crop rotation and long-term (18-year-old or 48-year-old) monoculture

Migratory endoparasitic nematodes

There were two species of the genus *Pratylenchus*: *P. neglectus* (Rench, 1924) builds higher population density at the level of 147 specimens under 18-year monoculture and 210 specimens after 48-year monoculture (Fig. 2). Population densities noted in surveyed plots from Bałcyny experiments were lower than those observed by Wolny (1989b), which concluded that 278–393 specimens in 200 cm³ of soil of barley rhizosphere significantly decreased the yield of barley. *P. fallax* noted after 18- and 48-year experiments were equal to population densities of 92 specimens after 18 years and 180 specimens after 48 years of monoculture. That population density can

also take a part of decrease the yield of barley (Fig. 3). *P. fallax* were noted as a nematode associated with barley growth inhibition (Corbett, 1972; Winiszewska *et al.*, 2012). The tolerance limit of that *Pratylenchus* species for the top specific environments of monocultured barley on the plots are unknown; but feeding in the cortical tissues of the young roots leads to necrosis and death of part of roots due to phytotoxins arising from the hydrolysis of phenolic substances from enzymes extracted by nematodes inside plant cells. *Pratylenchus* specimens are harmful directly and indirectly in the way of tritrophic interaction with plant pathogenic fungi and bacteria (Skwiercz, 1987; Sosnowska, 2004).





Fig. 2. Population density of *Pratylenchus neglectus* (Rench 1924) from 100 cm³ of soil under spring barley cultivated in crop rotation and long-term (18-year-old or 48-year-old) monoculture



Fig. 3. Population density of *Pratylenchus fallax* (Seinhorst 1968) from 100 cm³ of soil under spring barley cultivated in crop rotation and long-term (18-year-old or 48-year-old) monoculture

Migratory ectoparasitic nematodes

Nematode species of that group were also recognized by Winiszewska et al. (2012) during a survey of nematodes that inhibited barley growth in the Wielkopolska region. Higher population density (370 specimens/100 cm³ of soil under 48-year monoculture) was obtained by Bitylenchus dubius (Fig. 4). (Butschli, 1873) which was associated with the plant growth inhibition of barley all around Poland (Brzeski, 1998; Winiszewska et al. 2012). Permanent species in soil under barley are also Paratylenchus projectus and Merlinius microdorus; which obtain the higher population densities, 210 and over 350 specimens respectively, under 48-year monoculture (Fig. 5 and 6). Barley is also a host for Scutylenchus tessellatus, with the maximal population density 130 specimens in soil under 48-year monoculture plots (Fig. 7). However, Paratylenchus nanus was not recognized during analysis after an 18-year experiment but was found in the population of 190 specimens in 48-year monoculture (Fig. 8). The same situation was described in the population of Rotylenchus robustus. Density of its population reached the highest level over 55 specimens/100 cm³ of soil under spring barley cultivated in 48-year-old monoculture. The difference of parasitic nematodes from Rotylenchus robustus' density between crop rotation and long-term monoculture seemed corned (Fig. 9). Ditylenchus dipsaci occurred in surveyed soils builds very low population density, probably specific environments of extremely long-term monoculture decreased the population (Fig. 10). No stem nematodes were observed in aerial parts of spring barley during survey in Bałcyny.



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Fig. 4. Population density of by *Bitylenchus dubius* (Butschli 1873) from 100 cm³ of soil under spring barley cultivated in crop rotation and long-term (18-year-old or 48-year-old) monoculture



Fig. 5. Population density of by *Paratylenchus projectus* from 100 cm³ of soil under spring barley cultivated in crop rotation and long-term (18-year-old or 48-year-old) monoculture



Fig. 6. Population density of by *Merlinuis microdorus* (Allen, 1955) from 100 cm³ of soil spring barley cultivated in crop rotation and long- term (18-year-old or 48-year-old) monoculture





Fig. 7. Population density of by *Scutylenchus tessellatus* (Goodey, 1952) from 100 cm³ of soil under spring barley cultivated in crop rotation and long-term (18-year-old or 48-year-old) monoculture



Fig. 8. Population density of *by Paratylenchus nanus* (Cobb, 1923) from 100 cm³ of soil under spring barley cultivated in crop rotation and long-term (18-year-old or 48-year-old) monoculture



Fig. 9. Population density of by *Rotylenchus robustus* from 100 cm³ of soil under spring barley cultivated in crop rotation and long-term (18-year-old or 48-year-old) monoculture





Fig. 10. Population density of by *Ditylenchus dipsaci* (Kuhn, 1857) from 100 cm³ of soil under spring barley cultivated in crop rotation and long-term (18-year-old or 48-year-old) monoculture

Sedentary nematodes

The biggest group of sedentary nematodes was formed by *Heterodera spp.* where the highest level was carried by species *Heterodera avenae*. Their cyst, eggs and larvae were observed during last 30 years of the Bałcyny experiment. Higher population density, 320 total eggs and larvae in 100 cm³ of soil, were observed in 48-year monoculture plots of

barley. After the examination of eggs and larvae, it was found that pathogenic fungi had killed 60% of specimens in the 48-year-old crop rotation plots and 49% after 48-years of monoculture (Fig. 11). There were found also unidentified species of *Heterodera spp.* and *Globodera spp.* which are also parasited by fungi (Fig. 12).



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-year monoculture
- $\mathsf{D}-\mathsf{Pf1m}$ final population density after 18-year monoculture
- $\mathsf{E}\,-\mathsf{Pi}2\mathsf{r}$ initial population density after 48-year crop rotation
- F Pf2r final population after 48-year crop rotation
- G Pi2m initial population density after 48-year monoculture
- H Pf2m final population density after 48-year monoculture a, b, c, d... statistical significance

a, b, c, u... – statistical significance

Fig. 11. Population density of by *Heterodera avenae* (Wollenweber, 1924) from 100 cm³ of soil under spring barley cultivated in crop rotation and long-term (18-year-old or 48-year-old) monoculture





Species of pathogenic fungi isolated from surveyed material are listed as follow:

- 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 Pyrenophora teres (Drechs. (1923)
- G Penicillium spp. (Link 1809)
- H Trichothecium roseum (Link 1809)
- I Rhizopus nigricans (Ehrenberg 1820)

a, b, c, d... - statistical significance



Some aspects of the natural control of nematodes genus *Pratylenchus* and cereal cyst nematode *Heterodera avenae* (Wollenweber 1924)

Specimens of Pratylenchus neglectus were occupied by Bacillus penetrans in 18-25% in soil under crop rotation and 20-35% in the samples from monocultured barley. There were no bacteria observed on the other nematode species occurred in surveyed soil samples. During the examination of the trains inside eggs of *H*. avenae, ten species of parasitic fungi were isolated: Paecilomyces lilacinus (Thom) inside eggs and larvae, Pochonia chlamydosporia (Goddard) Zare et Gams which attack only eggs of nematodes. Alternaria alternata (Keissl), Cladosporium spp. (Persoon), Fusarium roseum (Schwein), Penicillium spp. (Link), Trichothecium roseum (Link) and Rhizopus nigricans (Ehrenberg) which are also known as nematophagous fungus. The mechanism of plants' susceptibility with nematophagous fungi

living around the roots which way of infection was provided by numerous nematode populations was mostly discussed by Kerry (2000) and Kerry et al. (1984). A decreased number of Globodera rostochiensis which can be seen as evidence of interactions between fungi and nematodes was also observed by Wronkowska and Janowicz (1986). Multitrophic interactions of soil organisms works probably due to specific soil network on the base of fungus strain system. The soil stability system very often disturbed by parasites and pathogens correlates with the problem in the case of replant spring barley what was observed in this paper and works of many other researchers (Hoestra, 1994; Szczygieł and Zepp, 2004). This evidence was also confirmed by pathogenic fungus strain and bacteria of Pratylenchus specimens's eggs and larvae. The most dangerous for barley were Heterodera and Pratylenchus species, which by the way have each been limited by their Skwiercz, A.T., Adamiak, E., Stefanovska, T., Szelągowska, P., Zatoń, K., Sobolewska, M. (2018). Nematodes in the soil and roots of spring barley grown in crop rotation and long-term monoculture. Acta Sci. Pol. Agricultura, 17(3), 115–124

natural fungal and bacterial pathogens. Species of the genera *Bitylenchus* and *Merlinius* noted in the experiment are not known in the literature as significant barley parasites. The results of this research do not indicate the need to use of nematicides but put strong suggestion to keep seasonal monitoring of nematodes and microorganisms in crops.

CONCLUSIONS

- 1. Soil stability system involved pathogenic fungi and bacteria to decrease some populations of pathogens due to multitrophic interaction between bacteria, fungus and nematodes.
- 2. Spring barley cultivation in intensive production need knowledge of virus diseases vectored in root zone of soil by nematodes genus *Trichodoridae* and *Longidoridae*.
- 3. Many years' monoculture involved a specific reaction of the soil to decrease parasite nematode population density.

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NICIENIE W GLEBIE I KORZENIACH JĘCZMIENIA JAREGO UPRAWIANEGO W PŁODOZMIANIE I MONOKULTURZE DŁUGOOKRESOWEJ

Streszczenie

W pracy przedstawiono wyniki badań struktury gatunków pasożytniczych populacji nicieni roślinnych z ryzosfery jęczmienia jarego uprawianego w 18-letnim płodozmianie i 48-letniej monokulturze. W każdym rogu obu pól znajdowały się cztery poletka o powierzchni 1 m². Cztery próbki gleby z każdego poletka pobrano laską glebową o przekroju 3 cm na głębokości 40 cm w pobliżu korzeni jęczmienia na początku (BBCH 09) i dzień po zbiorach (BBCH 99). Każda próbka gleby ważyła 1 kg, z czego 50 g stanowiły świeże korzenie i kłosy. Każda próbka, z której wyizolowano nicienie, stanowi połączenie powtórzeń każdego wariantu doświadczenia (połączenie gleba + korzenie + łodygi). Populacje gatunków dominujących, takich jak *Bitylenchus dubius, Merlinius microdorus, Pratylenchus neglectus* i *Heterodera avenae*, są znacznie wyższe w monokulturze niż w płodozmianie. Jaja i larwy gatunku *H. avenae* zostały zainfekowane chorobotwórczymi grzybami w 60% próbek pochodzących z monokultury jęczmienia (w porównaniu z płodozmianem, którego infekcja dotyczyła 50% cyst). Z kolei bakterie, głównie *Bacillus penetrans*, skolonizowały nicienie z rodzaju *Pratylenchus*, co stanowiło przedział 18–35% pobranych próbek. Uzyskane wyniki obrazują zmiany nematologiczne zachodzące w długotrwałych systemach uprawy jęczmienia jarego. Dostarczają one dodatkowych informacji niezbędnych do podjęcia odpowiednich działań w celu zwalczania niebezpiecznych wektorów wirusowych dla badanego zboża.

Słowa kluczowe: grzyby patogenne, nicienie, wektory wirusów zbóż