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Survey of entomopathogenic nematodes and fungi in agricultural areas

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Abstract: In 2016–2018, in north-western Poland, field studies were carried out on the coexistence of various taxonomic groups, such as soil nematodes and fungi, including beneficial species that comprise the environment's natural resistance to pests in agrocenoses. The research aimed to find a connection between select biotic and abiotic factors in the chosen crops which could have practical applications in plant protection. Entomopathogenic nematodes *Steinernema feltiae* Filipiev, 1934 and entomopathogenic fungi *Cordyceps fumosorosea* and *Metarhizium anisopliae* (Metschn.) Sorokin were found to be present in all studied agrocenoses; however, they showed clear preferences for some types of crops or soil. The research shows that the effectiveness of the biological methods of plant protection depends on the selection of the right biopreparations, which strengthen the local populations of the beneficial organisms present in specific agriculture areas.

Keywords: crops; soil microorganisms; natural occurrence; plant protection

Microorganisms are an integral part of the soil environment, affecting the proper functioning of ecosystems, soil structure and productivity, soil-forming processes and finally the health of the plants themselves (Magdoff 2001; Nannipieri et al. 2003; Acosta-Martinez et al. 2007). The development of microorganisms in the soil depends on its physical and chemical properties, fertilisation, climatic conditions and agrotechnical factors, and especially on its abundance of organic matter, which is a source of energy and nutrients for microorganisms (Johansson et al. 1999). An important factor affecting the biodiversity, development and the number of microorganisms in the soil are anthropogenic transformations of the natural environment, including agroecosystems.

The regulation of local insect population densities, including many plant pests, by natural environmental resistance, including entomopathogenic nematodes and fungi, is one of the important links in trophic chains (Vega et al. 2009; Mudrončeková et al. 2013; Lacey et al. 2015; Johnson et al. 2016; Kergunteuil et al. 2016; Popowska-Nowak et al. 2016; Rasmann & Turlings 2016; Jabner & Ownley 2018; Benvenuti et al. 2019; Branine et al. 2019). Trophic chains form a network of food dependencies between organisms. Thanks to them, it is possible to circulate matter and the energy flow in ecosystems. Reducing the population of harmful insects by nematodes and fungi depends not only on the presence of the preferred host in the soil, but also on many environmental factors affecting the living organisms and their biological activity (Stuart et al. 2006).

The role of entomopathogenic fungi is much greater in the environment. Some species are able

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to stimulate plant growth and development as endophytes, induce systemic resistance and relieve the symptoms of abiotic stress (St. Leger 2008; Vega 2008; Bayat et al. 2009; Wyrebek et al. 2011; Sasan & Bidochka 2012; Liao et al. 2014; Barelli et al. 2016; Sánchez-Rodríguez et al. 2016; Raya-Diaz et al. 2017; Jabner & Ownley 2018; Branine et al. 2019; Nishi & Sato 2019). After introduction into the soil, entomopathogenic fungi coexist with each other (Nishi & Sato 2019), but during the colonisation of plant roots they can compete for a place in the rhizosphere (Wyrebek et al. 2011). In addition, as rhizospheric fungi, they play an important role in protecting plant roots against pests because they can infect insects (Keyser et al. 2014). They occur in the roots of wild and cultivated plants (Wyrebek et al. 2011; Nishi et al. 2013, 2017; Keyser et al. 2014; Behie et al. 2015; Nishi & Sato 2019). Despite numerous publications on the occurrence of entomopathogenic nematodes in Europe and around the world, little is known about the links between these organisms and their natural environment (Mráček et al. 2005; Hunt 2007). Therefore, the aim of the research was to assess the biological condition of the soil based on the presence of fungi and nematodes from the family Steinernematidae and Heterorhabditidae in agricultural crops.

MATERIAL AND METHODS

Soil samples, for analysis for the presence of soil microorganisms (fungi and entomopathogenic nematodes), were taken from a 100 m^2 research area in the spring and autumn in 2016–2017, and in the spring of 2018, taken from 36 representative sites in agricultural crops in north-western Poland. The plants were grown in rotation: potatoes – cereals (wheat, barley) – winter rape/beetroots – legumes (broad bean, lupine) – potatoes.

The soil was taken with Egner's cane from designated crops with a depth up to 30 cm: 100 individual samples, 1 200 cm^3 total. In the laboratory, each soil sample was thoroughly mixed, divided into 11 parts (subsamples) and then placed in 11 plastic containers with a volume of 100 cm^3. In order to maintain the proper humidity of the samples from which the nematodes were isolated (70–80%), the soil was successively moistened with distilled water (5–15 ml H_2O); in the trials from which the fungi were isolated, the soil moisture content was maintained at 30–35% (Kaya & Stock 1997).

The collected soil from the agriculture areas was classified into three soil types: Eutric/Epidystric Cambisols soil (78.4%; 28 sites), Eutric/Endocalcaric Cambisols soil (10.8%; 4 sites) and Albic Podzols (Ochric) soil (10.8%; 4 sites). The Cambisols soils of the studied fields were characterised by a high proportion of loamy sands, neutral pH, low organic carbon content (below 0.6%) in the humus layer and a low sulfur content (1.52 mg S-SO_4/100 g soil). The Albic Podzols (Ochric) soils with a thickness not usually exceeding 10 cm was characterised by the dominance of sands and a slightly acidic soil (pH = 5.5). No heavy metal impurities (Cd, Ni, Pb, Cu, Zn) were found in the tested soils (Siebielec et al. 2017).

All the examined agriculture areas were covered by integrated plant protection programmes IMP (Integrated Management Protection). Appropriate agrotechnics and plant fertilisation were used in all the crop types, adapted to the crop type and soil type. Chemical methods of protecting plants against pests, bacteria and fungi were used everywhere. Chemical treatments were carried out outside the bee flight hours, taking the low toxicity of pesticides to beneficial organisms into account. No biopreparations were used to protect the plants against pests, bacteria and fungi.

Total number of samples collected during the study was 185 (148 samples were collected in 2016–2017 and 37 samples – in spring 2018). A total of 2 035 subsamples were examined. Five samples came from every site, except place No. 6 (two samples were always taken there due to the different plant species grown on both sides of the road).

All the crop types were grown on the Eutric/Epidystric Cambisols (Eu/EcCM) soils and Eutric/Endocalcaric Cambisols (Eu/EdCM) soils, and only the potatoes, winter rape and broad bean were grown on the Albic Podzols (Ochric) (PZA) soils.

Isolation of the entomopathogenic nematodes from the soil: The presence of entomopathogenic nematodes in the soil samples was determined using a standard baiting technique, the trap insect *Galleria mellonella* Linnaeus. (Bedding & Akhurst 1975; Mráček 1980). The mortality of *G. mellonella* insects infested with nematodes was assessed 5 days after the experiment was initiated. The dead insects were placed on an inverted watch glass to obtain the larvae of the invasive nematodes for the taxonomic description (White 1927). The experiment continued until *G. mellonella* were no longer infected by the nematodes, until the 15^{th} day of the experiment. The isolation of the soil nematodes was carried out

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at 22 °C. The isolated nematodes were preserved in a 4% formalin solution and then identified based on the morphological and morphometric features of invasive larvae (J3) and the second generation (Hominick et al. 1997; Nguyen 2007).

Isolation of the fungi from the soil: The entomopathogenic fungi were isolated from the soil with the method of the trap insects *G. mellonella* (method adopted by Zimmermann 1986). Five L3-stage larvae were placed in containers with soil samples, and the closed samples were kept at room temperature (21–22 °C). A total of 25 caterpillars were placed in the soil samples from each crop. The control of the larvae mortality was performed on days 5, 8 and 12. The dead larvae, after being rinsed in distilled water, surface sterilised in sodium hypochlorite (1% solution) and re-rinsed in distilled water, were incubated in the absence of light. The fungi taxonomies were identified based on the morphological and morphometric characteristics of their structures according Humber (2012).

Isolated fungi from the trap insects were divided into two groups: Insect-pathogenic fungi [proven entomopathogenic interaction, according to the published literature (Meng et al. 2017; Gürlek et al. 2018)] and other fungi. The division into groups was adopted from Tkaczuk et al*.* (2012, 2014).

Statistical analysis. The statistical analyses were performed with STATISTICA 6.0 software (version 6.0) using statistical significance tests on the differences between the structural factors (the frequency of the nematodes present in the different ecosystems) and on the differences between the means, assuming a normal distribution of variables ($P < 0.05\%$).

Soil samples with entomopathogenic nematodes (Rhabditida: Steinernematidae, Heterorhabditidae) and fungi identified by the species, were analysed in terms of the dominance structure (percentage of nematode/fungal samples) and frequency (the frequency of the nematode/fungi findings in the tested crops) according to the following formulas (Krebs 2009).

ANOVAs were computed to test if the observation depended on one or several factors acting simultaneously. It would explain the probability that the distinguished factors may be the reason for the differences between the observed group means. Levene's test was computed to determine the homogeneity of variances. If the assumption was met, a post-hoc Tukey's t-test was applied to compare the groups for statistically significant differences (Webster 2007; Borcard et al. 2018; Kukla et al. 2019).

RESULTS

The research conducted in various types of crops showed a high diversity of species of soil microfauna (Table 1). Entomopathogenic nematodes were represented by two species: the predominant *Steinernema feltiae* Filipiev (67% of cases) and *Heterorhabditis megidis* Poinar, Jackson & Klein (33% of cases). The frequency of the nematodes in the researched sites, compared to the entomopathogenic coexisting fungi in the soil, was relatively low and did not exceed 30% (Table 1). The mortality of the trap insects was clearly higher in the case of *H. megidis*, however, it did not exceed 50% in relation to all the tested insects (Table 1).

It was found that the *S. feltiae* nematodes were not associated with one particular type of crop, but their significant share was recorded in the winter rape (80% of the cases) (Table 2). Comparable results (in about 20% of the cases) were obtained for this species in the cultivation of root crops and cereals*.* The *H. megidis* nematodes were only isolated from the soil samples taken from cereal crops (over 40% of the cases). The *S. feltiae* nematodes were the most responsive to the *G. mellonella* trap insects in the soils collected from the winter canola crops; however, the

Table 1. The occurrence of entomopathogenic nematodes and various taxa of fungi in the studied crops

Taxon	N	AM $x \pm$ SD	*F*	D
Fungi				
Beauveria bassiana	8	5.9 ± 3.5	21.6	8.2
Cordyceps farinosa	9	6.8 ± 3.8	24.3	9.5
Cordyceps fumosorosea	21	24.2 ± 6.2	56.8	33.7
Metarhizium anisopliae	17	12.9 ± 4.5	45.9	17.9
Aspergillus spp.	13	3.9 ± 1.5	35.2	5.4
Fusarium spp.	8	3.2 ± 1.7	21.6	4.5
Gliocladium spp.	4	1.8 ± 1.2	10.8	2.6
Mucor spp.	9	2.7 ± 1.3	24.3	3.8
Unsporulated mycelium	23	10.4 ± 2.7	62.2	14.4
Nematode				
Steinernema feltiae	10	30.8 ± 4.4	27.7	67
Heterorhabditis megidis	5	43.3 ± 3.5	13.8	33

N – the number of sites with nematodes/fungi; AM – average mortality (%) of the trap insects in the soil samples; *F* – frequency (%) of the fungi/nematodes at the researched sites; *D* – dominance (%) of fungi/nematodes at the sites; SD – standard deviation (sample); x – the mean

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Table 2. The occurrence of soil fungi and entomopathogenic nematodes in the various types of crops

Biological factors	Root plants (%)	Oil plants (%)	Cereals (%)	Legumes (%)
Insect-pathogenic fungi				
Beauveria bassiana	22.2	12.5	–	62.5
Cordyceps farinosa	22.2	25.0	16.7	25.0
Cordyceps fumosorosea	66.7	50.0	58.3	50.0
Metarhizium anisopliae	44.4	50.0	41.7	50.0
Others fungi				
Aspergillus spp.	44.4	37.5	41.7	12.5
Mucor spp.	22.2	25.0	16.7	37.5
Fusarium spp.	11.1	50.0	16.7	12.5
Gliocladium spp.	–	12.5	16.7	25.0
Unsporulated mycelium	55.6	37.5	66.7	75.0
Nematodes				
Steinernema feltiae	22.2	80.0	25.0	11.1
Heterorhabditis megidis	0.0	0.0	41.7	0.0

H. megidis nematodes were the most responsive in the samples collected from the cereals (Table 3).

Both isolated species of entomopathogenic nematodes preferred leached and acid brown soils; the nematodes from podzolic and podzolic soils were the least isolated. Overall, the effectiveness of *G. mellonella* test for the insect infestation by the nematodes in the different soil types was higher for *S. feltiae* and ranged from 9.7% (leached brown soils) to 23.6% (brown soils) (Table 4).

The presence of entomopathogenic fungi was found in the majority of the research sites (97.3%). In most of the samples (70.3%), there was only one species of fungus from each group. *Cordyceps fumosorosea* (Wize) Kepler, B. Shrestha & Spatafora (found at 21 sites and 56.8% of all the samples) was the species present at the largest number of sites and the most widespread. This taxon caused the highest mean mortality of the larvae in the studied soils (24.2%) and was the most dominant among the fungi (33.7%) (Table 1). It was the species most frequently found in the studied plant crops (Table 2 and 3), but this species preferred Eutric/Endocalcaric Cambisols and Eutric/Epidystric Cambisols soils (Table 4).

Levene's test showed a homogeneity of variance. The ANOVA test result showed that only the

Table 3. The average mortality (%) of the *Galleria mellonella* trap insects caused by the entomopathogenic nematodes* and the fungi in the soil from the various types of agricultural crops

Taxon	Root plants	Oil plants	Cereals	Legumes
	x ± SD			
Beauveria bassiana	4.4 ± 9.0	4.0 ± 11.3	0	18.3 ± 23.5
Cordyceps farinosa	11.6 ± 24.3	5.0 ± 9.8	5.3 ± 13.8	5.5 ± 10.2
Cordyceps fumosorosea	23.8 ± 22.3	19.5 ± 23.7	31.3 ± 29.8	19.0 ± 21.9
Metarhizium anisopliae	14.0 ± 18.2	13.8 ± 17.3	15.0 ± 23.3	7.5 ± 10.5
Aspergillus spp.	5.6 ± 7.3	3.3 ± 5.2	4.2 ± 5.7	2.3 ± 6.4
Mucor spp.	2.7 ± 6.1	2.5 ± 5.1	2.5 ± 5.9	3.5 ± 5.0
Fusarium spp.	1.8 ± 5.3	7.5 ± 8.7	3.2 ± 7.7	0.8 ± 2.1
Gliocladium spp.	0	1.3 ± 3.5	2.0 ± 4.7	4.3 ± 8.3
Unsporulated mycelium	10.4 ± 9.3	8.0 ± 12.8	9.5 ± 11.0	14.0 ± 10.2
*Steinernema feltiae**	9.2 ± 1.3	31.1 ± 6.5	11.6 ± 3.2	1.9 ± 1.0
*Heterorhabditis megidis**	0	0	18.1 ± 4.5	0

SD – the standard deviation (sample); x – the mean

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Table 4. The percentage of the trap insect *Galleria mellonella* infection by the entomopathogenic nematodes and fungi isolated from the various types of arable soils

Biological factors	Soil subtype*		
	PZA	Eu/EcCM	Eu/EdCM
	x ± SD		
Insect-pathogenic fungi			
Beauveria bassiana	0	5.0 ± 2.3	7.3 ± 3.9
Cordyceps farinosa	13.0 ± 4.3	0	7.6 ± 4.1
Cordyceps fumosorosea	7.0 ± 3.5	38.0 ± 8.0	25.7 ± 6.2
Metarhizium anisopliae	12.0 ± 4.0	24.0 ± 6.1	11.6 ± 4.4
Others fungi			
Aspergillus spp.	4.0 ± 1.8	5.0 ± 2.3	4.4 ± 1.4
Mucor spp.	11.0 ± 2.1	0	2.4 ± 1.1
Fusarium spp.	3.0 ± 1.5	3.0 ± 1.3	3.7 ± 1.8
Gliocladium spp.	0	0	2.6 ± 1.4
Unsporulated mycelium	15.0 ± 2.8	12.0 ± 2.3	10.4 ± 2.8
Nematodes			
Steinernema feltiae	18.1 ± 6.5	23.6 ± 4.9	9.7 ± 2.7
Heterorhabditis megidis	13.9 ± 5.0	0	6.0 ± 2.9

*soil subtype (after IUSS Working Group WBR 2015): PZA – albic podzols (ochric); Eu/EcCM – eutric/endocalcaric cambisols; Eu/EdCM – eutric/epidystric cambisols; SD – the standard deviation (sample); x – the mean

changes were statistically significant for the type of soil and *Mucor* spp. and between the type of crop and *S. feltiae.* After applying Tukey's post hoc test, it showed that particularly statistically significant differences occur between the type of crops in the system and the legumes – root crops – cereals, cereals – root crops – legumes, cereals – oil plants – root crops. Analysing data on two grouping agents, i.e., the soil type and crop type, only *S. feltiae* showed statistically significant differences, in particular, between the PZA oil plants – legumes – root crops, and the Eu/EdCM cereals – oil plants – legumes, the Eu/EdCM legumes – root crops – cereals, the Eu/EdCM cereals – root crops – legumes, the Eu/EdCM cereals – oil crops – root crops, the Eu/EdCM root crops – cereals – oil plants, the PZA oil plants – legumes – root crops.

DISCUSSION

Biological plant protection methods involving the use of living organisms to fight plant pests, as well as increasing the natural resistance potential of the environment, are now becoming an important element of integrated plant protection methods (Lacey & Shapiro-Ilan 2008, Wu et al. 2014; Půža 2015; Půža et al. 2016; Cruz-Martínez et al. 2017; Labaude & Griffin 2018; Abd-Elgawad 2019; Jagodič et al. 2019). In relation to nematodes and entomopathogenic fungi, an important aspect of their use in practice is to supplement and feed the natural resources by introducing biopreparations produced on an industrial scale, as well as activities aimed at using local populations of organisms naturally occurring in the environment by providing them with adequate living conditions (Sevim et al. 2012; Shapiro-Ilan et al. 2012; Gürlek et al. 2018). A condition for increasing the effectiveness of the biopreparations with living organisms is the detailed recognition of their adaptation to the environment and the determination of the connections with various host insect groups (Kruitbos et al. 2010; Sevim et al. 2012; Shapiro-ilan et al. 2014; Helmberger et al. 2017). In order to effectively use these organisms in the biological fight against plant pests, it is necessary to take the ecological conditions of a given agro- or ecosystem into account, so that their introduction will bring the desired effect.

The conducted research shows that entomopathogenic nematodes, although present in various types of agrocenoses, show specific habitat and food preferences. For example, *S. feltiae* was found more often in winter rape crops, and *H. megidis* was found

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more often in cereals (Table 2). This is undoubtedly related to the insects associated with the specific agrocenoses (Hominick et al. 1995; Mráček et al. 1999; Sturhan 1999; Mráček & Bečvář 2000; Mráček et al. 2005; Helmberger et al. 2017; Shapiro-Ilan et. al. 2017). For example, the high species diversity and density of entomopathogenic nematodes were noted in orchards, where an increased occurrence of butterflies from the family of Tortricidae and Hymenoptera of the Tenthredinidae family occurred, especially fruit plants (Dzięgielewska 2012; Ulu et al. 2015). Numerous studies show that *S. feltiae* nematodes are characterised by a wide ecological flexibility (Abate et al. 2017). This is supported by the fact that, in the conducted tests, it was found in all types of agrocenoses and in the different soil types (De Nardo & Grewal 2003; Cuthbertson et al. 2007; Morton & Garcia-del-Pino 2008; Leite et al. 2018).

Entomopathogenic nematodes have been used for many years in biological methods of plant protection against pests. High sensitivity in field cultivations for grubs from the beetle family (Ehlers et al. 1996; Koppenhöfer & Fuzy 2004, 2008), weevil larvae from the Curculionidae family (Ehlers 1996), as well as maize beetle larvae (Kuhlmann & Burgt 1998) show nematodes from the Heterorhabditidae family: *Heterorhabditis bacteriophora* Poinar and *H. megidis*. In the United States, species from the family Steinernematidae, *S. feltiae* and *S. glaseri* Steiner, are used to control Colorado potato beetles and wireworms in potato cultivation (Toba et al. 1983; Berry et al. 1997; Rostislav et al. 2017). In Europe, biopreparations based on *H. megidis* and *S. feltiae* are recommended for biological warfare against wireworms of the *Agriotes lineatus* Linnaeus. (Kuhar et al. 2003; Ansari et al. 2009). The data in the literature show that the effectiveness of the soil pest control by nematodes depends on the nematode species and its food preferences (Ansari et al. 2009). For example, 65% mortality was observed using *H. bacteriophora*, and 50% when applied to *S. carpocapsae* Weiser soil.

Entomopathogenic fungi also show preferences for various habitats and vegetation (Meyling & Eilenberg 2006; Jarmuł-Pietraszczyk et al. 2011; Clifton et al. 2015; Popowska-Nowak et al. 2016; Ramos et al. 2017), but they have the ability to adapt to environmental conditions (habitat type and climate; Sevim et al. 2012). *Metarhizium anisopliae* (Metschn.) Sorokīn is more common in habitats with cultivated plants, while *Beauveria bassiana* (Bals.-Criv.) Vuill. prefers natural sites and soil from orchards (Miętkiewski et al. 1991; Bidochka et al. 1998; Klingen et al. 2002; Sun et al. 2008; Jarmuł-Pietraszczyk et al. 2011; Medo & Cagáň 2011; Kepler et al. 2015; Steinwender et al. 2015). The research in north-western Poland has led to similar conclusions: the occurrence of *B. bassiana* has rarely been observed in comparison with other entomopathogenic taxa. The species found most often in this studies, *C. fumosorosea*, is reported in the literature as often being found in cereal crops only in Poland (Miętkiewski et al. 1991), while in other parts of the world, it was most frequently recorded in permanent plant sites – with hedges and orchards (Meyling & Eilenberg 2006; Sun et al. 2008). However, in Poland, it was also often found in forest soils (Popowska-Nowak et al. 2016). Sun et al. (2008) state that *C. fumosorosea* is adapted to more natural positions with less human interference, while *Cordyceps farinosa* (Holmsk.) Kepler, B. Shrestha & Spatafora prefers natural positions without the influence of agricultural activity. In our research in north-western Poland, *C. farinosa* was noted less frequently than *C. fumosorosea*. According to previous studies, the occurrence of entomopathogenic fungi is more dependent upon the soil than the plants, but some fungal populations may be associated with vegetation groups (Fisher et al. 2011; Wyrebek et al. 2011; Behie et al. 2015; Steinwender et al. 2015; Nishi & Sato 2019).

Populations of entomopathogenic fungi are influenced by the cultivated plant species and the method of cultivation, including the use of crop rotation (Tkaczuk et al. 2012; Kolczarek & Jankowski 2014; Clifton et al. 2015; Trizelia et al. 2015; Ramos et al. 2017). These factors determine the diversity of the fungi and the mortality of the trap insects (Sun et al. 2008). Particularly favourable conditions for fungi prevail in organic crops (Mäder et al. 2002; Klingen et al. 2002; Uzman et al. 2019). Larger riches of entomopathogenic fungi and their low frequency of occurrence are observed in field crops, which are associated with the agrotechnical activities carried out on the fields causing large changes in all the habitat components (Sun et al. 2008). Chandler et al. (1997) also observes the negative impact of treatments carried out on farms, e.g., ploughing, on the occurrence and functionality of entomopathogenic fungi. The presence of more organic matter in the soil promotes the greater diversity of entomopathogenic fungi (Thiele-Bruhn et al. 2012; Nelly et al. 2019),

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however, Bruck and Lewis (2002) show that the presence of plant debris limits the spread of *B. bassiana* spores.

The activity of nematodes and entomopathogenic fungi in the environment is limited by many biotic and abiotic components. Important soil conditions – humidity, temperature and pH – are important abiotic factors affecting their biological activity (Quesada-Moraga et al. 2007; Bouamama et al. 2010; Dzięgielewska & Erlichowski 2011; Wyrebek et al. 2011; Popowska-Nowak et al. 2016). Studies have shown that their occurrence depends on the soil type. In poor Albic Podzols soils collected from under cereal crops and Luvisols soils from potato crops with a low humus and acid content, a coexistence was found with the Elateridae beetle larvae and two species of entomopathogenic nematodes from the Steinernematidae family: *S. feltiae* and *S. carpocapsae* (Dzięgielewska & Erlichowski 2011). Other research has shown that the native strain *Steinernema carpocapsae* can have the potential to control the larvae of Elateridae, for example *A. obscurus* (Morton & Garcia-del-Pino 2017).

Entomopathogenic nematodes were more often isolated from the Eutric/Endocalcaric Cambisols soils than the Albic Podzols (Ochric) soils in the study. However, in the faunistic research on entomopathogenic nematodes in various agrochemicals and biocenoses, the highest nematode percentage was obtained from sandy loam soils (Dzięgielewska & Skwiercz 2018).

Tkaczuk & Renella (2003) and Lacey et al. (2015) also show the dependence of entomopathogenic fungi on the soil type and structure: sandy soils favour more frequent occurrences of *C. fumosorosea* and *M. anisopliae* (Tkaczuk 2008 given by Tkaczuk et al. 2014), hence, they are known to be dominant in Poland. Clay soils are inhabited the most often by three taxons: *B. bassiana*, *C. fumosorosea* and *M. anisopliae*. Observations regarding the occurrence of the last two species in Poland are consistent with our results: in the studied soils, *C. fumosorosea* and *M. anisopliae* were the dominant taxa, while in all the studied crop types, the highest mortality of the trap insect larvae was caused by the first of these taxa. Although Eutric/Epidystric Cambisol soils were the dominant group in our studies, *B. bassiana* was the least frequent.

Entomopathogenic fungi show seasonal variability (Sun et al. 2008; Jarmuł-Pietraszczyk et al. 2011; Kolczarek & Jankowski 2014; Popowska-Nowak et al. 2016) and a variable occurrence depending on the year (Meyling & Eilenberg 2006), while the main factors determining the effectiveness of the host infection, the abundance of the sporulation and the ability to survive winter periods are the temperature (Kessler et al. 2003; Sun et al. 2008; Wyrebek et al. 2011) and soil moisture (Popowska-Nowak et al. 2016). Bruck and Lewis (2002) demonstrate a significant role of rainfall in the propagation of entomopathogenic fungal spores. These factors constitute a limitation on the possibility of using, and the effectiveness of the biopreparations introduced into the environment, and may cause changes in the effectiveness for a particular year.

Among the biotic factors, there are various groups that co-exist with each other in the environment and interact with each other. Meyling and Eilenberg (2006) often observed the coexistence of several species of fungi inhabiting trap insects. Kreft & Skrzypek (2002) confirm that entomopathogenic nematodes in competitive conditions can change their biological activity. The coexistence of fungi competing for a host with nematodes may significantly affect the synergistic or antagonistic behaviour of both competitors (Barbercheck & Kaya 1990; Ansari et al. 2005, 2006; Anbesse et al. 2008; El-Borai et al. 2011). In addition, favourable habitat conditions, e.g., low soil moisture, may reduce the viability of nematodes in favour of entomopathogenic fungi (Tkaczuk et al. 2014). However, sometimes taxa belonging to one group compete during plant colonisation, e.g., the introduced fungi strains – native taxa; as a result, non-native strains sometimes win (Wyrebek et al. 2011; Liao et al. 2014). Popowska-Nowak et al. (2016) found that a large number of fungal spores in the soil are not always associated with a large number of infected insects. According to the authors, the low activity of the fungus is a manifestation of the strategy described by Chandler (2009) as "sit and wait".

Understanding and defining the role of local populations of entomopathogenic nematodes and fungi adapted to specific environmental conditions is of key importance for reducing harmful insects in a given area, and may contribute to their wider use in plant protection. Comprehensive pest control, based on minimising the use of pesticides, is necessary to keep the environment in the best possible condition, with a biological balance. In summary, the recognition of local populations of beneficial, naturally occurring organisms in agrocenoses should be the basis for selecting a biopreparation to control

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specific plant pests, either occurring temporarily or seasonally in the soil. For the purpose of increased effectiveness, it is advisable to use biopreparations for plant pests that contain propagated beneficial organisms (e.g., nematodes and insecticidal fungi) whose natural occurrence has been confirmed by research. The confirmed coexistence of entomopathogenic nematodes and fungi in the soil suggests that the introduction of both components into the environment may increase the effectiveness of pest control in the case of seasonal changes in the habitat conditions.

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