

The occurrence of entomopathogenic fungi in soils from mid-field woodlots and adjacent small-scale arable fields

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The aim of this study was to compare the species composition and the intensity of entomopathogenic fungi occurrence in the soil from mid-field woodlots and adjacent small farmlands. The study material consisted of soil samples taken from a mid-field woodlot and an adjacent small-scale arable field in three different localities in the vicinity of Siedlce. Entomopathogenic fungi were isolated from soil using two methods: the insect bait method and the selective medium. The comparative study showed that the soil from mid-field woodlots was characterized by a richer species composition of entomopathogenic fungi than of adjacent arable fields. A total of six fungal species representing the anamorphs of Hypocreales (Ascomycota) were isolated from the soil collected from mid-field woodlots: *B. bassiana*, *B. brongniartii*, *M. anisopliae*, *M. flavoviride*, *I. farinosa* and *I. fumosorosea*. The presence of only three species was reported in the farmland soil: *B. bassiana*, *M. anisopliae* and *I. fumosorosea*. This fact confirms the important role of semi-natural habitats as a source of biodiversity of entomopathogenic fungi in agricultural landscape. It was found that entomopathogenic fungi together formed more colony-forming units in the soil from arable fields than that of neighbouring mid-field woodlots. *B. bassiana* was the species of fungus which infected more bait insect larvae and formed significantly more colony-forming units (CFU) in the soil from mid-field woodlots than that of farmland in the localities studied, whereas the trend was the opposite in the case of *I. fumosorosea* and *M. anisopliae*. Given the presence of entomopathogenic fungi in the farmland soil in the three test places together, it was found that *I. fumosorosea* was dominant in the soil from the two arable fields, where this fungus infected more *G. mellonella* larvae and formed significantly more CFUs than the other species of fungi. *M. anisopliae* was the second most frequently isolated farmland species.

Key words: insect-pathogenic fungi, Hypocreales, (Ascomycota) anamorphs, soil, agricultural landscape

INTRODUCTION

Fungi are the largest group of microorganisms colonizing the soil environment after bacteria. Among them are entomopathogenic species which play an important role as one of the natural factors limiting populations of typical soil insect pests that overwinter or pupate in the soil environment (Ignoffo et al. 1978; Ferron 1981; Miętkiewski et al. 1994; Bajan et al. 1995). It is estimated that about 90% of arthropods harmful to plants spend at least part of their life cycle in soil (Gaugler 1988). Specimens which have died in soil as a result of mycoses spread diseases in the soil environment. The occurrence, development and pathogenicity of entomopathogenic fungi in soil are conditioned by a number of biotic and abiotic factors in the environment, as well as the agricultural and non-agricultural human activity.

Arthropods play a very important role among the biotic factors, as being potential hosts for these fungi, they determine their occurrence and survival in soil. The commonly accepted belief is that farmers affecting the physical and chemical properties of the environment, transforming the structure of vegetation both in fields and landscapes, contribute to the depletion of biological resources (Ryszkowski 1985; Karg 1989; Ryszkowski, Karg 1991). Issues related to the protection of biodiversity in rural areas are among the most important priorities set by the European Union in matters relating to environmental protection. Agri-environment schemes are an essential tool for the protection of valuable natural habitats associated with agricultural landscape. In this landscape, farmland are surrounded by semi-natural habitats such as mid-field woodlots and field margins which are specific refugia for flora and fauna not present in arable fields (Marshall, Moonen 2002; Meyling 2005). These habitats are the ideal place for many wintering arthropod species whose rich communities in turn determine a large variety and ease of propagation of pathogens, including entomopathogenic fungi. Therefore, unfarmed parts of natural vegetation clusters marginal among arable fields, water tanks and trees introduced in strips or on small areas of land become very important for the preservation of the biotic assemblages. While the botanical, avifaunistic and entomological characteristics of such structural elements of agricultural landscapes are relatively well developed, data relating to entomopathogens that are contained therein are quite sporadic (Bałazy, Cysewski 2003; Bałazy 2007). It is known, however, that the species composition of entomopathogenic fungi in semi-natural habitat soils, i.e. excluded from agrotechnical pressure, is different from that observed in soils intensively used for agriculture (Steenberg 1995; Bidochka et al. 1998; Klingen et al. 2002; Meiling, Eilenberg 2006; Tkaczuk 2008).

The aim of this study was to compare the species composition and the intensity of entomopathogenic fungi occurrence in the soil from mid-field woodlots and adjacent small farmlands.

MATERIAL AND METHODS

Sampling and characterization of soil samples. The study material consisted of soil samples taken at the beginning of December 2006 in three localities in the vicinity of Siedlce: Purzec, Borki Siedleckie and Pruszyń. In each place soil samples were collected from two environments: a mid-field woodlot and an adjacent arable field. Winter crops were grown in the fields, and their areas in different places were respectively 1.0, 1.2 and 2.0 ha. Mid-field woodlots were characterized by the following species composition of trees and shrubs:

1 - Purzec – trees on the area of about 500 m² with birch and pine dominance

2 - Borki Siedleckie – trees on the area of about 800 m² with birch and pine dominance

3 - Pruszyń – trees on the area of about 1200 m² with the dominance of poplar and oak.

Samples were taken at random, separately for each environment from 10-15 points on the test area. Samples from arable fields were taken at a distance of not less than 50 meters from the edge of trees. The material was collected using Egner's stick to a depth of 15 cm. A mixed sample was prepared from the collected material and stored in plastic bags at 0-4°C. Immediately before starting the experiment in the laboratory, the soil was sieved and dried up to a suitable moisture content, optimal for fungal growth and limiting the development of nematodes.

Soil samples from all points were analysed to assess their granulometric composition (soil type), pH and organic carbon content.

Isolation of fungi. Entomopathogenic fungi were isolated from soil using two methods: the insect bait method proposed by Zimmermann (1986) and the selective medium method developed by Strasser et al. (1996) and commonly used for the isolation of entomopathogenic fungi from soil (Keller et al. 2003). The Greater Wax Moth (*Galleria mellonella* L.) was used as a bait insect. Ten plastic boxes with a capacity of about 200 ml were filled with soil from each environment. Ten *Galleria mellonella* larvae were put in each box, a total of 100 larvae in the soil from each environment. The boxes of soil were placed in an incubator at 20-22°C. The first mortality control was conducted 7 days after the start of the experiment and then at 3-day intervals until the death of all larvae. Dead larvae were washed in distilled water and then surface-sterilized for 30 seconds in 1% sodium hypochlorite solution. After rinsing twice in distilled water, the larvae were put on Petri dishes with moistened filter paper. The dishes with the larvae were kept at 20-22°C in the dark. Fungi growing on insects were transferred on standard media and then examined macroscopically. Identification of isolated fungal species was done by using standard methods described by Goettel and Inglis (1997). Fungal nomenclature follows Sung et al. (2007) and Index Fungorum (www.indexfungorum.org).

Additionally, the concentration of colony-forming units (CFU) of entomopathogenic fungi in soil environments tested was determined applying a selective medium developed by Strasser et al. (1996).

For this purpose, 2 g of soil were weighed out from each mixed sample originating from a given point, and then 18 ml of distilled water were added with the addition of 0.05 Triton X-100, and the components were vigorously shaken for about 35

seconds. Then 0.1 ml of the soil solution was poured out and spread using a glass spatula on a selective medium in four Petri dishes which were the replicates. The dishes were placed in incubators at 22° C and after 8-10 days colonies of individual fungal species were counted. The results were expressed as the number of colony-forming units (CFU) of entomopathogenic fungi in 1 g of the soil. The selective medium used had the following composition: 10 g of peptone, 20 g of glucose and 18 g of agar were added to 1 litre of water. After sterilization and cooling, the following components were added to the medium: 0.6 g of streptomycin sulfate, 0.05 g of chlortetracycline, 0.05 g of cycloheximide and 0.1 g of dodine.

The results were analysed statistically by performing a 2-factorial analysis of variance. A detailed comparison of the environments was made using the Tukey's test at significance level $\alpha = 0.05$.

RESULTS

The comparative study of the composition and intensity of entomopathogenic fungi in the soil from mid-field woodlots and adjacent small-scale farmlands, conducted in 2006 in three localities in the vicinity of Siedlce showed that the soil from mid-field woodlots was characterized by a richer species composition of these fungi. A total of six fungal species representing the anamorphs of Hypocreales (Ascomycota) were isolated from the soil collected from mid-field woodlots using *G. mellonella* larvae: *Beauveria bassiana* (Bals.-Criv.) Vuill., *B. brongniartii* (Sacc.) Petch, *Metarhizium anisopliae* (Metschn.) Sorokin, *M. flavoviride* W. Gams & Rozsypal, *Isaria farinosa* (Holmsk.) Fr. and *I. fumosorosea* Wize. The presence of only three species was recorded in the farmland soil: *B. bassiana*, *M. anisopliae* and *I. fumosorosea* (Tabs 1 and 2). In addition, apart from typical insect-pathogenic fungi, some accompanying fungal species with unproved entomopathogenic abilities were isolated from investigated soils by means of *Galleria* bait method. The occurrence of fungi from the following genera was observed within this group: *Aspergillus* P. Micheli, *Fusarium* Link, *Gliocladium* Corda and *Mucor* P. Micheli ex L.

The use of selective media allowed the isolation of only three species of entomopathogenic fungi from soils of both investigated habitats, such as *B. bassiana*, *I. fumosorosea* and *M. anisopliae* (Tab. 3).

Four species of entomopathogenic fungi were isolated by means of bait insects from the soil samples taken from a mid-field woodlot in Purzec: *B. bassiana*, *M. anisopliae*, *M. flavoviride* and *I. fumosorosea*. The fungus *B. bassiana* strongly dominated in this soil, and it infected 52.7% of *G. mellonella* larvae and formed 4.5×10^3 colony-forming units (CFU) in 1 gram of soil. *M. anisopliae* and *I. fumosorosea* were of a mild intensity, infecting 6.3% and 1.1% of larvae and forming 1.7 and 1.5×10^3 CFU g⁻¹. Using the insect bait method only two species of entomopathogenic fungi were isolated from the adjacent farmland soil: *M. anisopliae* and *I. fumosorosea* that infected 96% and 1.0% of larvae (Tab. 1). The fungus *M. anisopliae* also developed the most numerous colony-forming units in the farmland soil in Purzec (15.0×10^3 CFU g⁻¹), and *B. bassiana* 1.2×10^3 CFU g⁻¹ (Tab. 3).

Table 1
Mortality of *Galleria mellonella* larvae (%) caused by entomopathogenic fungi in soils from mid-field woodlots and adjacent small-scale arable fields

Factor of mortality		Locality					
		Purzec		Borki Siedleckie		Pruszyn	
		A*	B	A	B	A	B
Entomopathogenic fungi	<i>B. bassiana</i>	52.7	0	23.0	8.1	65.0	0
	<i>B. brongniartii</i>	0	0	0b	0	3.0	0
	<i>M. anisopliae</i>	6.3	96.0	71.0	1.0	1.0	3.0
	<i>M. flavoviride</i>	1.1	0	0	0	0	0
	<i>I. farinosa</i>	0b	0	0	0	1.0	0
	<i>I. fumosorosea</i>	1.1	1.0	1.0	79.8	2.0	86.0
Total		61.2	97.0	95.0	88.9	72.0	89.0
Fungi of unproved entomopathogenic abilities	<i>Mucor</i> sp.	5.2	1.0	0	5.0	5.0	0
	<i>Gliocladium</i> sp.	3.1	0	0	2.0	1.0	2.0
	<i>Fusarium</i> sp.	2.1	1.0	1.0	0	2.0	0
	<i>Aspergillus</i> sp.	1.1	0	2.0	0	4.0	0
	Unsporulated mycelium	7.4	1.0	1.0	1.0	4.0	4.0
Total		18.9	3.0	4.0	8.0	16.0	6.0
Other causes	Nematodes	7.4	0	0	0	2.0	3.0
	Unidentified causes	9.5	0	1.0	3.0	10.0	2.0
Alive specimens		3.1	0	0	0	0	0

Abbreviations: * A – mid-field woodlot, B – arable field

It was found that entomopathogenic fungi invaded significantly more *G. mellonella* larvae and also formed more colony-forming units in the soil from the farmland than in the soil from the adjacent woodlot in this village – respectively 97.0 and 61.2%, and $16.2 \times 10^3 \text{ g}^{-1}$ and $7.7 \times 10^3 \text{ CFU g}^{-1}$.

The soil sampled from mid-field woodlots in Borki Siedleckie was dominated by *M. anisopliae* which formed $11.8 \times 10^3 \text{ CFU}$ in 1 gram of soil and infected 71% of larvae inserted into the soil. The fungus *B. bassiana* was also present on 23% of larvae ($1.8 \times 10^3 \text{ CFU g}^{-1}$), while *I. fumosorosea* infected only 1% of bait insects and formed $1.2 \times 10^3 \text{ CFU g}^{-1}$. The same fungal species were isolated from the adjacent farmland soil but their dominance was different. The highest mortality of *G. mellonella* larvae was caused by the fungus *I. fumosorosea* (79.8%), and other species such as *B. bassiana* and *M. anisopliae* invaded a small number of larvae, respectively, 8.1 and 1.0%. The fungus *I. fumosorosea* also formed the most numerous colony-forming units in the farmland soil in Borki Siedleckie ($9.7 \times 10^3 \text{ g}^{-1}$). It was found that entomopathogenic fungi infected more bait insect larvae and formed more colony-forming units in the woodlot soil than in the farmland soil in the village.

The soil collected from the mid-field woodlot in Pruszyn was found to have the richest entomopathogenic fungal species composition among all habitats studied. Up to five fungal species occurred in that soil: *B. bassiana*, *B. brongniartii*, *M. anisopliae*, *I. farinosa* and *I. fumosorosea*. The fungus *B. bassiana* was the most abundant among them and it infected 65.0% of bait insect larvae and formed $9.5 \times 10^3 \text{ CFU}$ in 1 gram of soil. Other species caused the mortality of individual larvae from 1.0 to 3.0%, and formed a small quantity of colony-forming units from 0.3 to $2.0 \times 10^3 \text{ CFU}$ in 1 gram of soil. Only two fungal species were isolated using bait insects in the adjacent farmland soil. The fungus *I. fumosorosea* definitely dominated there and it

Table 2
Mortality of *Galleria mellonella* larvae (%) caused by entomopathogenic fungi in soils from mid-field woodlots and adjacent small-scale arable fields (average from three localities)

Fungal species	Habitat	
	Arable field	mid-field woodlot
<i>B. bassiana</i>	2.7	47.1
<i>B. brongniartii</i>	0	1.0
<i>M. anisopliae</i>	33.3	26.1
<i>M. flavoviride</i>	0	0.4
<i>I. farinosa</i>	0	0.3
<i>I. fumosorosea</i>	55.5	1.4
Total	91.5	76.3

Table 3
Number of colony forming-units of entomopathogenic fungi in the soil (CFU x 10³ g⁻¹) from mid-field woodlots and adjacent small-scale arable fields

Fungal species	Locality					
	Purzec		Borki Siedleckie		Pruszczyn	
	A*	B	A	B	A	B
<i>B. bassiana</i>	4.5a**	1.2b	1.8b	0.5c	9.5a	1.3c
<i>M. anisopliae</i>	1.7c	15.0a	11.8a	1.7c	0.3c	8.0a
<i>I. fumosorosea</i>	1.5c	-	1.2c	9.7a	2.0c	13.2a
Total	7.7b	16.2a	14.8a	11.9ab	11.8b	22.5a

Abbreviations: * A – mid-field woodlot, B – arable field; ** values in lines followed by the same letters are not significantly different

Table 4
Characteristics of soil samples

Locality	Habitat	Soil pH (pH w mol × dm ³ KCL)	Soil type	Organic carbon content (%)
Borki Siedleckie	arable field	5.43	loamy sand	0.851
	mid-field woodlots	4.78	sandy loam	2.477
Purzec	arable field	4.72	weakly loamy sand	0.686
	mid-field woodlots	4.60	weakly loamy sand	1.114
Pruszczyn	arable field	4.49	weakly loamy sand	0.538
	mid-field woodlots	4.64	loamy sand	3.335

caused the death of 86.0% of larvae. Moreover, the presence of *M. anisopliae* was observed on 3.0% of *G. mellonella* larvae (Tab. 1). The fungus *I. fumosorosea* also formed by far the most numerous colony-forming units in the soil (13.2 x 10³ g⁻¹). *M. anisopliae* was also in a relatively high density (8.0 x 10³ g⁻¹), while *B. bassiana* formed the fewest colony-forming units (Tab.3).

Comparing the occurrence of entomopathogenic fungi in the soil from woodlots to their neighbouring farmlands in the three villages studied, we can conclude that *B. bassiana* infected most bait insect larvae in woodlot soil (a total of 47.1% of larvae) (Tab. 2). This fungus also formed the most numerous colony-forming units in two of the three mid-field woodlots studied. *M. anisopliae* was another species which infected most *G. mellonella* larvae in the soil under trees (26.1%). The fungus formed by far the most numerous colony-forming units in the woodlot soil in Borki Siedleckie.

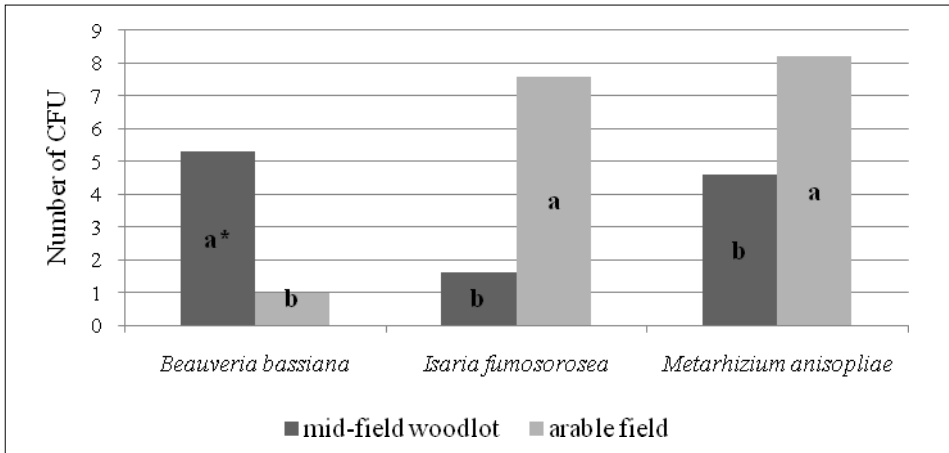


Fig. 1. Number of colony forming-units of entomopathogenic fungi in the soil (CFU $\times 10^3$ g⁻¹) from mid-field woodlots and adjacent small-scale arable fields (average from three localities)
* values followed by the same letters are not significantly different

Given the presence of entomopathogenic fungi in the farmland soil in the three test places together, it was found that *I. fumosorosea* was dominant in the soil from the two arable fields. In the farmland soil from Borki Siedleckie and Pruszyn, the fungus infected more *G. mellonella* larvae and formed significantly more CFUs than the other species of fungi. *M. anisopliae* was the second most frequently isolated farmland species. This fungus definitely dominated bait insect larvae in the farmland soil in Purzec, and it formed the most numerous colony-forming units in 1 gram of soil (Tabs 1 and 3).

Figure 1 illustrates the difference in density of colony-forming units of entomopathogenic fungi in soils from arable fields and adjacent woodlots. It was found that entomopathogenic fungi together formed more colony-forming units in the soil from arable fields than that of neighbouring mid-field woodlots. The fungus *B. bassiana* formed significantly more colony-forming units (CFU) in the soil from mid-field woodlots than that of farmland in the localities studied, whereas the trend was the opposite in the case of *I. fumosorosea* and *M. anisopliae* (Fig. 1).

DISCUSSION

Transformation of landscape relations, especially deforestation for agricultural production and the simplification of species composition and the age structure of forest stands lasting for at least two centuries are not conducive to the maintenance of species-rich groups of insects and their pathogens in anthropogenically transformed environments (Altieri 1999). For these reasons, unfarmed parts of natural vegetation clusters, marginal among arable fields, (field margins, roadsides, swamps), water tanks, as well as trees and shrubs introduced in strips on small areas of land become

very important for the preservation of the biotic assemblages (Bałazy, Cysewski 2003). These habitats are specific refugia for flora and fauna which are not present in farmland (Marshall, Monen 2002), and are the ideal place for many wintering species of arthropods.

It was found that in the area of semi-natural strips with a width of 3 to 6 m on the edges of fields, the number of living species of arthropods is more than two times higher than in their neighbouring farmland (Meek et al. 2002). According to Karg et al. (2003), the biomass of dipteran larvae in the soil from mid-field woodlots in Turew was 80-fold higher than in the soil from the adjacent field, and the total biomass of macrofauna was 90-fold higher. With regard to mesofauna, the biomass was 9-fold higher in the shelterbelt soil than in the soil from the adjacent field.

The species richness of arthropods, especially insects, in the area of mid-field woodlots undoubtedly affects the species diversity of their natural enemies, including entomopathogenic fungi. This was confirmed in our research. The species composition of entomopathogenic fungi was far richer in the soil from mid-field woodlots than in the soil from neighbouring farmlands. In the soil collected from woodlots, a total of six fungal species were isolated using *G. mellonella* larvae: *B. bassiana*, *B. brongniartii*, *M. anisopliae*, *M. flavoviride*, *I. farinosa* and *I. fumosorosea*. The presence of only three species was reported in the soil from arable fields: *B. bassiana*, *M. anisopliae* and *I. fumosorosea*. Chandler et al. (1997), Meyling and Eilenberg (2006) and Tkaczuk (2008) also draw attention to the richer species composition of entomopathogenic fungi in soils from shelterbelts surrounding arable fields, and emphasize the important role of natural habitats as specific refugia for the survival of these fungi in agroecosystems. Mayling (2005) showed, moreover, that shelterbelts around arable fields are also reservoirs for a variety of phylogenetic lines of the fungus *B. bassiana*. Studying strains of this species, obtained from a field of about 28 ha and an adjacent belt of trees with a total area of only 0.44 ha, he recorded a significantly higher genetic diversity of fungal isolates from under the trees than from the field where there was, in principle, only one genetic group of *B. bassiana*. According to the above-mentioned author, this fact confirms the important role of semi-natural habitats as a source of biodiversity of entomopathogenic fungi in agricultural landscape.

B. bassiana was the species of fungus which infected most bait insect larvae and formed the biggest number of CFUs in the soil from mid-field woodlots studied. This fungus is cited as co-dominant with *I. fumosorosea* in soils of semi-natural habitats in Poland (Tkaczuk 2008). According to Vanninen (1995) and Steenberg (1995), *B. bassiana* is characteristic of natural habitats (mid-field woodlots, shelterbelts) and is less common in agricultural soils. One of the concepts explains the dominance of *B. bassiana* in soils from mid-field woodlots which, as revealed by the study (Tab.4) are much richer in organic matter than farmland soils, is the ability of this fungus to grow in the saprophagous phase (Müller-Kögler, Zimmermann 1986; Storey et al. 1989). *B. bassiana* definitely dominates in soil and forest litter, i.e. the agricultural landscape environments that are probably the most similar to woodlots. It should also be noted that the survival of entomopathogenic fungi in soil largely depends on the stability of environmental conditions and the related continuous or at least frequent presence of potential insect hosts. In its

development cycle, the species *B. bassiana* seems to use a strategy that Ewald (1983, 1995) has called “sit and wait”, which means that the population growth and survival of the fungus in soil depend mainly on periodical infections of hosts, and factors limiting the population of hosts seem to substantially affect the survival of *B. bassiana* in the soil environment. Wojciechowska et al. (1977), Daoust & Pereira (1986) and Steenberg (1995) showed that the constant presence of arthropods in soil as potential hosts for *B. bassiana* has a significant impact on the survival of the species.

Given the presence of entomopathogenic fungi in the farmland soil in the three villages studied, it was found that *I. fumosorosea* was dominant in the soil from two arable fields, and *M. anisopliae* in one of the farmlands. As demonstrated by the analyses, all soils from the three fields studied were sandy loam (Tab.4). According to Tkaczuk (2008) the presence of entomopathogenic fungi in farmland soils in Poland was dependent on soil type. Sandy soils were dominated by *I. fumosorosea* and *M. anisopliae*, while *I. fumosorosea*, *M. anisopliae* and *B. bassiana* were of similar intensity in clay soils, whereas *B. bassiana* and then *M. anisopliae* were by far the most common in organic soils. The dominance of *I. fumosorosea* and *M. anisopliae* in farmland sandy soils is confirmed by earlier studies conducted by Tkaczuk & Miętkiewski (1996) and Kleespies et al. (1989). These authors also report a very low intensity of *B. bassiana* in these soils.

Examining the concentration of colony-forming units of entomopathogenic fungi in the soil from large-scale fields and neighbouring strips of trees in Austria and Poland, Tkaczuk (2008) showed that these fungi formed more colony-forming units in the soil under shelterbelts. In our study, in the case of soil from two of the three pairs of test environments (field-woodlots), there was a greater concentration of CFUs of entomopathogenic fungi in the farmland soil. The differences may be due to the fact that our study was conducted in small-scale fields (up to 2 ha) where farmers use minimal chemical protection, while research conducted by Tkaczuk (2008) related to fields with an area of more than 5 ha where extensive chemical treatments were applied (this is particularly true of research conducted in Austria), which could adversely affect the development of entomopathogenic fungi in the soil. According to Karg and Bałazy (2009), the simplification of the structure of crops, chemical control of pests and cyclically repeated tillage practices aimed at the intensification of crops directly or indirectly eliminate pathogens of arthropods, on a par with parasites and predators, as trophic groups with specific environmental and nutritional requirements. However, most of them are characterized by a high adaptability to adverse environmental conditions, and usually a high reproductive and migration potential, they can quickly colonize the environments from which they have been eliminated as a result of agricultural practices, as long as there are suitable refugia around the fields. Among such refugia can be undoubtedly small mid-field woodlots which are characteristic of the agricultural landscape in the Nizina Południowopodlaska lowland where this study was conducted. According to Marshall & Monen (2000) and Meek et al. (2002), especially the transition area (ecotone) between a cultivated field and a stable semi-natural environment is a habitat for species characteristic of both environments, and it makes it possible to enrich depleted entomofauna of farmland.

CONCLUSIONS

The occurrence, development and pathogenicity of entomopathogenic fungi in soil are conditioned by a number of biotic and abiotic factors in the environment, as well as the agricultural and non-agricultural human activity. In agricultural landscape, arable fields are surrounded by semi-natural habitats such as mid-field woodlots which are specific refugia for flora and fauna not present in arable fields. Our comparative study showed that the soil from mid-field woodlots was characterized by a richer species composition of entomopathogenic fungi than of adjacent arable fields. A total of six fungal species representing the anamorphs of Hypocreales (Ascomycota) were isolated from the soil collected from mid-field woodlots: *B. bassiana*, *B. brongniartii*, *M. anisopliae*, *M. flavoviride*, *I. farinosa* and *I. fumosorosea*. The presence of only three species was reported in the farmland soil: *B. bassiana*, *M. anisopliae* and *I. fumosorosea*. This fact confirms the important role of semi-natural habitats as a source of biodiversity of entomopathogenic fungi in agricultural landscape.

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Występowanie grzybów entomopatogenicznych w glebach z zadrzewień śródpolnych i sąsiadujących z nimi małoobszarowych pól uprawnych

Streszczenie

Celem podjętych badań było porównanie składu gatunkowego i nasilenia występowania grzybów entomopatogenicznych w glebach z zadrzewień śródpolnych i sąsiadujących z nimi pól uprawnych. Materiał do badań stanowiły próby gleby pobrane w trzech miejscowościach w okolicach Siedlce z dwóch środowisk: zadrzewienia śródpolnego i sąsiadującego z nimi małoobszarowego pola uprawnego. Grzyby owadobójcze izolowano z gleby stosując metodę owadów pułpkowych (z użyciem larw *Galleria mellonella*) oraz podłoże selektywne. Przeprowadzone badania porównawcze wykazały, że gleby spod zadrzewień śródpolnych charakteryzowały się bogatszym składem gatunkowym tych grzybów. Z gleby pobranej spod zadrzewień śródpolnych na larwy *G. mellonella* wyizolowano w sumie sześć gatunków grzybów, były to: *B. bassiana*, *B. brongniartii*, *M. anisopliae*, *M. flavoviride*, *I. farinosa* i *I. fumosorosea*. W glebie z pól odnotowano obecność tylko trzech gatunków: *B. bassiana*, *M. anisopliae* i *I. fumosorosea*. Fakt ten potwierdza istotną rolę środowisk seminaturalnych jako źródła bioróżnorodności grzybów owadobójczych w krajobrazie rolniczym. Stwierdzono że, grzyby entomopatogeniczne łącznie tworzyły więcej jednostek infekcyjnych (CFU) w glebie z pól uprawnych niż sąsiadujących z nimi zadrzewień śródpolnych. Stwierdzono, że grzyb *B. bassiana* zainfekował więcej larw owada pułpkowego i tworzył istotnie więcej jednostek infekcyjnych w glebach z zadrzewień śródpolnych niż pól w badanych miejscowościach, natomiast w przypadku *I. fumosorosea* i *M. anisopliae* odnotowano tendencję odwrotną. Biorąc pod uwagę występowanie grzybów entomopatogenicznych w glebie z pól uprawnych w trzech badanych miejscowościach łącznie, stwierdzono, że w glebie z dwóch pól uprawnych dominował *I. fumosorosea*. W glebach tych zainfekował on więcej larw *G. mellonella* i tworzył istotnie więcej jednostek CFU niż pozostałe gatunki grzybów. Drugim najczęściej izolowanym gatunkiem z pól uprawnych był *M. anisopliae*.