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Entomopathogenic fungi isolated in the vicinity of Szczecin

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The spectrum and occurrence of entomopathogenic fungi in litter beech forest, meadow soil and arable soil were evaluated using an "insect bait method". Soil and litter samples taken in autumn and spring from each stand were baited with *G. mellonella* larvae at 22°C. Six species of entomopathogenic fungi infected these larvae. In the litter *Beauveria bassiana* was the dominant species. Besides *B. bassiana*, 23% of *G. mellonella* larvae were infected by *Paecilomyces farinosus* in autumn. In the forest soil the dominant entomopathogenic fungus was not found. In this stand the number of infected larvae was the lowest.

Metarhizium anisopliae was the dominant species in the meadow soil in both sampling periods. In the soil taken from a rye field *P. fumosoroseus* and *B. bassiana* infected *G. mellonella* larvae almost to the same degree.

It was established that *B. bassiana* and *M. anisopliae* infected more *G. mellonella* larvae in spring than in autumn. In the case of *P. farinosus* and *P. fumosoroseus* an opposite tendency was observed.

Key words: entomopathogenic fungi, *Beauveria bassiana, Metarhizium anisopliae, M. flavoviride, Paecilomyces farinosus, P. fumosoroseus.*

INTRODUCTION

By applying the "insect-bait method", the occurrence and species spectrum of entomopathogenic fungi was assessed in the soils of eastern Poland, particularly in the vicinity of Siedlce (Miętkiewski and Miętkiewska 1993; Miętkiewski and Kolczarek 1995; Miętkiewski, Tkaczuk and Zasada 1991/1992; Miętkiewski, Machowicz-Stefaniak and Górski 1996;

Tkaczuk and Miętkiewski 1996). The species composition of the fungi depends on the type of soil (Tkaczuk and Miętkiewski 1996), and soil utilization (Miętkiewski, Miętkiewska and Sapieha 1992; Miętkiewski and Kolczarek 1995; Miętkiewski, Machowicz-Stefaniak and Górski 1996; Miętkiewski, Tkaczuk and Badowska-Czubik 1992). In sandy arable soils such species as *Metarhizium anisopliae* (Metsch.) Sorok. and *Paecilomyces fumosoroseus* (Wize) Brown et Smith dominate (Miętkiewski and Miętkiewska 1993; Miętkiewski and Kolczarek 1995; Miętkiewski, Tkaczuk and Zasada 1991/1992; Miętkiewski, Tkaczuk and Badowska-Czubik 1992). In heavy soils the *Beauveria bassiana* (Bals.) Vuill. is the most frequent species (Tkaczuk and Miętkiewski 1996). In the meadow soil *M. anisopliae* dominates (Miętkiewski, Tkaczuk and Zasada 1991/1992). In the forest litter *B. bassiana* was the dominatet species and *M. anisopliae* and *P. farinosus* were also present (Miętkiewski et al. 1991b; Bajan et al. 1995). A comparative analysis of the litter and soil of pine and beech forests showed that there were differences in depending on the litter composition, processes during its decomposition, and the soil type.

No studies on entomopathogenic fungi in soils of north-western Poland and in the litter and soil of beech forests have been undertaken to date.

The present investigations were conducted in the vicinity of Szczecin where the occurrence and species spectrum of entmopathogenic fungi were examined in litter and soil of a beech-forest and in the adjacent meadow and arable land.

MATERIAL AND METHODS

In the autumn (Nov. 10.1996) and in spring (May 14.1997), samples of the litter and soil were taken from the beech forest and also soil samples from adjacent meadow and rye field were collected. The litter and soil were passed through 0.4 cm screens, to remove pebbles and other debris, and subsequently stored in 100 ml plastic containers. Ten larvae L_5 of *Galleria mellonella* L. were introduced, into each Petri dish with the soil or litter using 10 dishes for each sampling sites. The samples were kept at 22°C. After 5 days, and later every 3 days, up to the 25th day, the mortality of larvae was determinated. The dead larvae were first sterilized with 1% sodium hypochlorite and then washed three times in sterile distilled water. After wards the larvae were placed in wet chambers for the development of spores needed for the identification of the fungus. Larvae on the surface, of which a mycelium was visible, were not sterilized but only washed 3 times in sterile distilled water.

The results were evaluated statistically by the Chi square [χ^2] method.

RESULTS

Altogether 6 species of fungi were isolated from the litter and soil samples collected in the two sampling periods (Tab. 1). In the litter of beech-forest 4 species of entomopathogenic fungi were found. In both sampling periods *B. bassiana* dominated and infected most of the larvae (only a few were infected by *P. farinosus*) in spring. In autumn, besides *B. bassiana*, *G. mellonella* larvae were infected by *P. farinosus* and, in some cases, by *P. fumosoroseus* and *V. lecanii*.

The percentage of larvae infected by entomopathogenic fungi was several times lower in the soil taken from underneath the litter than in other areas. Of the 3 species found in this soil, no dominant species could be discerned. In autumn, most larvae were infected by *P. fumosoroseus* whereas in spring – by *M. anisopliae*, which was not found in the soil samples collected in autumn.

Three entomopathogenic fungi species were isolated from the meadow soil of which *M. anisopliae* was the dominant species. The number of *M. anisopliae* increased in spring. In autumn, besides of *M. anisopliae*, a large number of *G. mellonella* larvae were infected by *B. bassiana*, and a few by *P. fumosoroseus*.

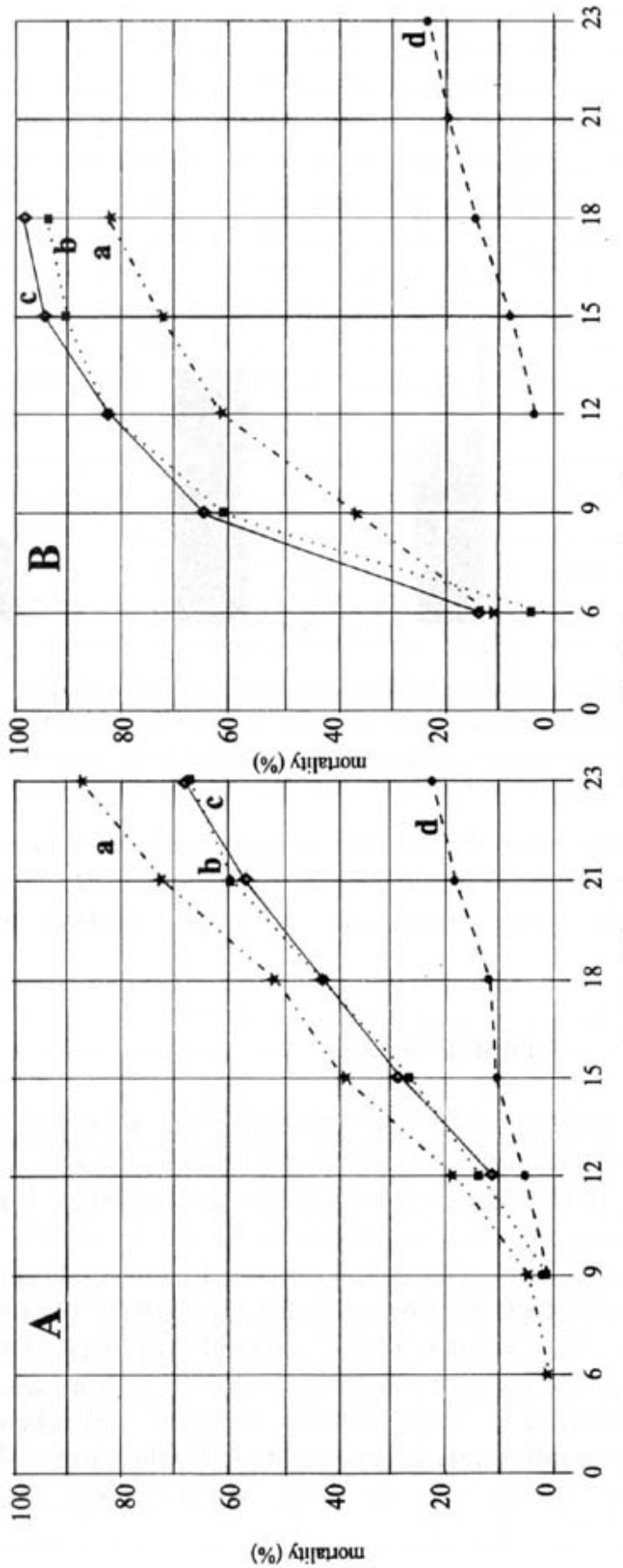
Four species of entomopathogenic fungi were isolated from the soil of the rye field. *B. bassiana* and *P. fumosoroseus* were found to occur abundantly. In autumn *P. fumosoroseus*, prevailed. In samples taken in spring, larvae attacked by this species were less numerous, whereas the number of those infected by *M. anisopliae* increased. It should be emphasized that, *M. flavoviride* was sporadically found in samples taken in spring and autumn, was absent in other sampling sites.

On the dead *G. mellonella* larvae, fungi of unproved entomopathogenic abilities were noted (Tab. 1). Among them, *Aspergillus parasiticus* Speare occurred frequently. Over 50% of the larvae put into soil taken from underneath the litter sampled in autumn were infected by *A. parasiticus* and sporadically from meadow soil. In spring this species was not noted in the samples.

On the whole, no significant differences in the larvae mortality could be found as far as the sampling time was concerned. A tendency towards higher rate of mortality of the larvae kept in litter and arable soil taken in spring was observed. The rate of mortality of larvae in the meadow soil was similar in both sampling periods, whereas in the soil from underneath the litter, more larvae died in autumn than in spring. A higher rate of mortality of larvae was observed in the case when the larvae had been kept in litter and soil sampled in autumn. The larvae began to die after being kept in the substrate for 6 days, with the exception of larvae from underneath the litter, when the first larvae died after 12 days. Larvae, which were kept in the soil taken from underneath

Table 1
Mortality (%) of *Galleria mellonella* larvae after 23 days of contact with litter and soil

Organisms found in insect cadavers		beech litter		forest soil		meadow soil		rye field	
		autumn	spring	autumn	spring	autumn	spring	autumn	spring
Entomopathogenic species	*Beauveria bassiana* (Bals.) Vuill.	36.0	96.4	3.3	5.5	26.4	0.9	4.8	56.3
	Metarhizium anisopliae (Metsch.) Sorokin				11.0	55.6	80.9	3.7	15.4
	Metarhizium flavoviride Gams et Rozsypal							1.8	1.8
	Paecilomyces farinosus (Holm) Brown et Smith	23.0	0.9	5.3	6.5				
	Paecilomyces fumosoroseus (Wize) Brown et Smith	6.0		13.8		5.0		56.3	19.9
	Verticillium lecanii (Zimm.) Viegas	3.0							
	Total	**68.0**	**97.3**	**22.4**	**23.0**	**87.0**	**81.8**	**66.6**	**93.4**
Fungi of unproved entomopathogenic abilites	*Aspergillus parasiticus* Speare			55.2		0.9			
	Cylindrocarpon sp.							0.9	
	Fusarium sp.				1.1	0.9			
	Mucor sp.				3.3	1.8	1.7	2.8	0.9
	Non-sporulating mycelium			2.2	8.8	2.8	0.9	5.7	2.8
	Total	–	–	**57.4**	**13.2**	**6.4**	**2.6**	**9.4**	**3.7**
Others	Nematodes	8.0			17.7		6.1	0.9	
	undefined causes	24.0	2.7	20.2	36.3	6.6	9.5	23.1	2.9
	Total	**32.0**	**2.7**	**20.2**	**54.0**	**6.6**	**15.6**	**24.0**	**2.9**



a — meadow soil b — rye field c — beech litter d — forest soil

Fig. 1. Mortality of *Galleria mellonella* larvae in beech litter and soil (autumn-A; spring-B)

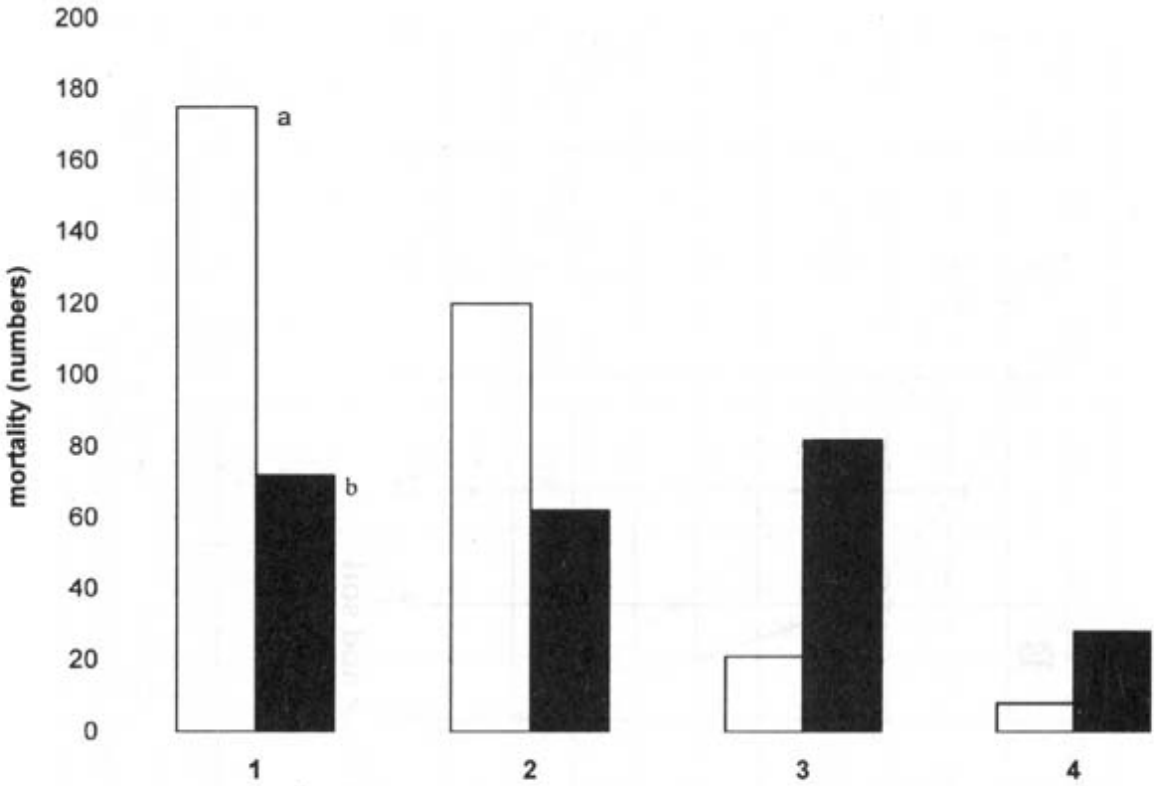


Fig. 2. Mortality rate of *Galleria mellonella* larvae in spring and autumn
1 — *Beauveria bassiana*, 2 — *Metarhizium anisopliae*, 3 — *Paecilomyces fumosoroseus*, 4 — *Paecilomyces farinosus*; a — spring, b — autumn

the litter, began to die earlier when the substrat samples were taken in autumn (Fig. 1). In spring more larvae were infected by *B. bassiana* and *M. anisopliae*. An opposite tendency was noted in the case of the *P. farinosus* and *P. fumosoroseus* (Fig. 2).

DISCUSSION

The present results confirm earlier data indicating the dominant role of *B. bassiana* in the litter of deciduous and coniferous forests (Miętkiewski and Tkaczuk 1997; Miętkiewski et al. 1991b; Bajan et al. 1995).

The lowest number of *G. mellonella* larvae infected by fungi was recorded in the soil taken from underneath the litter. Similar results were obtained by Miętkiewski and Tkaczuk (1997) in hornbeam forest. This was not the case for pine forests (Miętkiewski et al. 1991a; Bajan et al. 1995). The species composition of fungi isolated from the soil taken from underneath the litter was usually more diversified than in the litter (Miętkiewski et al. 1991b; Bajan et al. 1997; Miętkiewski and Tkaczuk 1997).

In the meadow soil *M. anisopliae* prevailed, which confirms the findings of Miętkiewski et al. (1991/1992), who compared the presence of entomopathogenic fungi in meadow and arable soils. Yip et al. (1992) point to the abundance occurrence of *M. anisopliae* in meadow soils of Tasmania. In arable soils the variety of species depends largely on the soil type, which was indicated by Tkaczuk and Miętkiewski (1996). *M. anisopliae*, *P. fumosoroseus* and *B. bassiana* were mostly found to occur in arable soils (Kleespies et al. 1989; Miętkiewski et al. 1991a; Miętkiewski and Kolczarek 1995; Miętkiewski and Miętkiewska 1993; Vänninen 1996; Vänninen et al. 1989). The arable soil of a 20-year-old barley monoculture in Rothamsted is the only one not to contain *P. fumosoroseus* (Miętkiewski et al. 1997).

Some attention should be paid to *M. flavoviride* isolated from the rye field, since it appears rather rarely in such habitats. In Poland it was noted only sporadically in the soils of mid-forest meadows of the Sudety region (Miętkiewski et al. 1994). This species was found sporadically also in the meadow soils of Tasmania (Yip et al. 1992).

The present study showed that a higher rate of mortality of larvae was noted in substrates samples collected in spring with the exception of the soil from underneath the litter. This is in consistent with our earlier observations, which showed that the rate of mortality of the larvae was higher in autumn (Miętkiewski et al. 1994, Miętkiewski et al. 1996).

REFERENCES

Bajan C., Kmitowa K., Mierzejewska E., Popowska-Nowak E., Miętkiewski R., Górski R., Miętkiewska Z., Głowacka B. 1995. Występowanie grzybów owadobójczych w ściółce i glebie borów sosnowych w gradiencie skażenia środowiska leśnego. Prace IBL, ser. B, 24: 87–97.

Kleespies R., Bathon H., Zimmermann G. 1989. Untersuchungen zum natürlichen Vorkommen von entomopathogennen Pilzen und Nematoden in verschidenen Boden in Umgebung von Darmstadt. Gesunde Pflanzen 41 (10): 350–355.

Miętkiewski R., Klukowski Z., Bałazy S. 1994. Entomopathogenic fungi isolated from soil of mid-forest meadows of Sudety mountains. Roczn. Nauk Roln. ser. E, 24 (1/2): 33–38.

Miętkiewski R., Kolczarek R. 1995. Grzyby izolowane z gleby pobranej z plantacji koniczyny i ziemniaków. Zesz. Nauk. WSRP Siedlce 39: 91–96.

Miętkiewski R., Machowicz-Stefaniak Z., Górski R. 1996. Occurrence of entomopathogenic fungi in soil of the hop plantations and adjacent arable fields. Rocz. Nauk Rol., ser. E, 25 (1/2): 49–53.

Miętkiewski R., Miętkiewska Z. 1993. Grzyby entomopatogenne w glebie. Acta Mycol. 28 (1): 77–82.

Miętkiewski R., Miętkiewska Z., Sapieha A. 1992. Występowanie grzybów owadobójczych w glebie pochodzącej z sadu. Zesz. Nauk. WSRP Siedlce 31: 209–220.

Miętkiewski R., Pell J. K., Clark S. J. 1997. Influence of pesticide use on the natural occurrence of entomopathogenic fungi in arable soils in the UK: Field and laboratory comparisions. Biocontrol Sci. Technol. 7: 565–575.

Miętkiewski R., Tkaczuk C. 1997. The spectrum and frequency of entomopathogenic fungi in litter, forest soil and arable soil. European Meeting Kopenhaga 1997.

Miętkiewski R., Tkaczuk C., Badowska-Czubik T. 1992. Entomogenous fungi isolated from strawberry plantation soil infested by *Otiorhynchus ovatus* L. Rocz. Nauk Rol. ser. E, 22 (1/2): 39–46.

Miętkiewski R., Tkaczuk C., Zasada L. 1991/1992. Występowanie grzybów entomopatogennych w glebie ornej i łąkowej. Acta Mycol. 27 (2): 197–203.

Miętkiewski R., Żurek M., Miętkiewska Z., Tkaczuk C. 1991a. Przydatność wybranych gatunków owadów do wychwytywania grzybów owadobójczych z gleby. Zesz. Nauk. WSRP Siedlce 29: 231–242.

Miętkiewski R., Żurek M., Tkaczuk C., Bałazy S. 1991b. Występowanie entomopatogennych grzybów w glebie ornej, leśnej oraz ściółce. Rocz. Nauk Rol. ser. E, 21 (1/2): 61–68.

Tkaczuk C., Miętkiewski R. 1996. Occurrence of entomopathogenic fungi in different kinds of soil. Rocz. Nauk Rol. ser. E, 25 (1/2): 44–48.

Yip H. Y., Rath A. C., Koen T. B. 1992. Characterization of *Metarhizium anisopliae* isolated from Tasmanian pasture soils and their pathogenicity to redheaded cockchafer (*Coleoptera*: *Scarabaeidae*: *Adoryphorus couloni*). Mycol. Res. 96 (2): 92–96.

Vänninen I., Husberg G. B., Hokkanen H. M. T. 1989. Occurrence of entomopathogenic fungi and entomoparasitic nematodes in cultivated soils in Finland. Acta Entomol. Fennica 53: 65–71.

Vänninen I. 1996. Distribution and occurrence of four entomopathogenic fungi in Finland: effect of geographical location, babitat and soil type. Mycol. Res. 100 (1): 93–101.

Grzyby entomopatogenne izolowane z gleb okolic Szczecina

Streszczenie

Stosując metodę owadów pułapkowych ustalono skład gatunkowy i nasilenie grzybów owadobójczych w ściółce lasu bukowego, glebie leśnej, glebie łąkowej oraz glebie ornej. Próbki gleby i ściółki pobrano jesienią i wiosną. Do ściółki i gleby wykładano larwy *Galleria mellonella* i przetrzymywano je w temperaturze 22°C. Larwy *G. mellonella* zainfekowane zostały przez 6 gatunków grzybów entomopatogennych. Grzyb *Beauveria bassiana* dominował w ściółce, a ponadto wystąpił *Paecilomyces farinosus* porażając w próbach pobranych jesienią 23 larw *G. mellonella*. W glebie pobranej spod ściółki stwierdzono najniższy procent larw zainfekowanych przez grzyby. Wyizolowano 3 gatunki grzybów lecz żaden z nich nie przeważał zdecydowanie. W glebie łąkowej, w próbach pobranych w obydwu terminach, dominował *Metarhizium anisopliae*. W glebie pobranej spod żyta w podobnym nasileniu wystąpiły: *B. bassiana* i *P. fumosoroseus*.

Ustalono, że w próbach pobranych wiosną w większym nasileniu wystąpiły *B. bassiana* i *M. anisopliae*, natomiast w próbach jesiennych w większym nasileniu wystąpiły *P. farinosus* i *P. fumosoroseus*.