www.czasopisma.pan.pl PAN POLSKA AKADEMIA NAUK www.journals.pan.pl

JOURNAL OF PLANT PROTECTION RESEARCH Vol. 50, No. 4 (2010)

DOI: 10.2478/v10045-010-0090-5

MORTALITY OF THE NUT-LEAF WEEVIL *STROPHOSOMA MELANOGRAMMUM* (FORSTER) AND DAMAGE RATE OF NEEDLES AFTER TREATMENT WITH ENTOMOPATHOGENIC FUNGI

*Anna Augustyniuk-Kram**

Centre for Ecological Research, Polish Academy of Science
Dziekanów Leśny, Konopnickiej 1, 05-092 Łomianki, Poland
Present address:
Cardinal Stefan Wyszyński University in Warsaw, Institute of Ecology and Bioethics, The Faculty of Christian Philosophy,
Wóycickiego 1/3, 01-938 Warsaw, Poland

Received: July 24, 2010
Accepted: November 14, 2010

Abstract: The susceptibility of the nut-leaf weevil, *Strophosoma melanogrammum* (Forster) (Coleoptera; Curculionidae) to the selected strains of *Beauveria bassiana* (Bals.-Criv.) Vuillemin, *Isaria farinosa* (Holm) Brown & Smith, *I. fumosorosea* (Wize) Brown & Smith, *Metarhizium anisopliae* (Metschnikoff) Sorokin and *M. flavoviride* Gams & Rozsypal, was tested under laboratory conditions. Two ways of insect exposure to fungus were studied; direct inoculation of weevils and indirect by contact with inoculated *A. procera* twigs that served as food for *S. melanogrammum*. All of the tested isolates were pathogenic to *S. melanogrammum*. Direct inoculation of adult weevils in fungal spore suspensions resulted in the greatest mortality compared to indirect inoculation where *A. procera* twigs were immersed in spore suspensions. Direct inoculation with *B. bassiana* and *Metarhizium* spp. isolates caused over 80% mortality after 28 days at 20°C, while LT_{50} values ranged from 15.0 to 21.6 days. Indirect inoculation with the most effective isolate *M. anisopliae* (275) resulted in 72% mortality after 28 days with a LT_{50} value of 22.1 days. Both direct and indirect spore application failed to reduce needle damage compared with the control. However, significant differences between particular isolates have appeared.

Key words: *Strophosoma melanogrammum*, nut-leaf weevil, entomopathogenic fungi, pathogenicity, virulence, damage, noble fir, *Abies procera*

INTRODUCTION

The nut-leaf weevil *Strophosoma melanogrammum* (Forster) is a serious pest of coniferous trees, mainly in *Abies* plantations for greenery production. Damage is primarily due to adult weevils feeding on needles (Kolk and Starzyk 1996; Eilenberg *et al*. 2000; Frølander and Harding 2000; Nielsen *et al*. 2006). Curculionid adults and larvae are commonly attacked under natural conditions by the entomopathogenic fungi *Beauveria bassiana* (Bals.-Criv.) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin (Keller and Zimmermann 1989). Vestergaard *et al*. (2000) reported natural occurrence of *B. bassiana*, *Lecanicillium lecanii* (Zimmermann) Viégas (formerly *Verticillium lecanii*) (Gams and Zare 2001), *Isaria farinosa* (Holm) Brown & Smith and *I. fumosorosea* (Wize) Brown & Smith (formerly *Paecilomyces farinosus* and *P. fumosoroseus*) (Gams *et al*. 2005) on weevils of *Strophosoma* spp., while *Metarhizium* spp. has not been found so far. Poprawski *et al*. (1985), however, found that eggs and larvae of the other curculionid weevils – *Sitona lineatus* L. and *Otiorhynchus sulcatus* L. – were susceptible to *B. bassiana, M. anisopliae*, *M. flavoviride* Gams & Rozsypal, *I. farinosa* and *I. fumosorosea*.

Entomopathogenic fungi are used for the control of many pests and can restrict damage caused by pest insects (Keller *et al*. 1997; Burges 1998; Butt 1999). Compared to chemical insecticides, entomopathogens are often slow acting and days or even weeks can elapse before an infected insect dies. During this time, hosts can feed and thereby add to crop or forest damage before their death. However, fungal infection may affect feeding behaviour and food consumption resulting in decreased food intake (Moore *et al*. 1992; Tefera and Pringle 2003). The repellent effect of Hyphomycete fungi (Noma and Strickler; 2000), and the fungi's production of secondary metabolites (Roberts 1981; Brousseau *et al*. 1996) can also reduce feeding. Antifeedant effects have been ascribed to destruxins from *Metarhizium* spp. (Amiri *et al*. 1999) and to efrapeptins from *Tolypocladium* spp. (Bandani and Butt 1999).

The leaf surface is the feeding site for many insects and often the site where fungal infection can take place (Hajek 1997). In the present study, the virulence of isolates of the entomopathogenic fungi *B. bassiana*, *I. farinosa*, *I. fumosorosea*, *M. anisopliae* and *M. flavoviride* to adult *S. melanogrammum* and subsequent damage to noble fir needles by the the weevils was determined. Two ways of

*Corresponding address:
aaugustyniuk-kram@cbe-pan.pl

insects exposure to fungus were studied; direct inoculation of weevils and indirect by contact with inoculated *A. procera* twigs that served as food for *S. melanogrammum*.

MATERIALS AND METHODS

Origin of insects, fungi and conidia preparation

Adult weevils of *S. melanogrammum* were collected from noble fir *A. procera*, (Bidstrup, Denmark), and kept at 6–8°C in a plastic box (30x22x12 cm) containing a 2 cm layer of standard sphagnum (Pindstrup Mosebrug, DK) and twigs of *A. procera*.

The thirteen isolates of entomopathogenic fungi used in this study were obtained from different host species and different geographical locations (Table 1).

The isolates were plated onto fresh Sabouraud dextrose agar (SDA) (Oxoid LTD., Basingstoke, UK) and incubated for two weeks at 23±1°C. After sporulation, all isolates were stored at 5°C until needed. Conidia and hyphae were harvested from the SDA plates by flooding with sterile 0.05% aqueous Triton®-X-100 (wetting agent) and agitated with a glass rod. Conidia were separated by centrifugation for 3 min at 4 500 rpm. The pellet was washed twice with sterile 0.05% Triton®-X-100, and the concentration of the inoculum was adjusted to 10^7 conidia/ml. The viability of the conidia was assessed on SDA plates after incubation for 24 h at 23°C, by examining the germination of 300 conidia per suspension (Goettel and Inglis 1997).

Virulence of tested isolates to *S. melanogrammum*

Direct inoculation bioassay

Adult weevils were immersed for 10 s in 10 ml of conidial suspension (10^7 conidia/ml). The conidial suspension and weevils were poured onto filter paper which was in a Buchner funnel (8 cm diam.), and excess suspension was removed by vacuum. Insects immersed in 0.05% Triton®-X-100 served as the controls. A twig (containing 10–40 needles) and one treated weevil were placed in a cup (30 ml) containing 5 ml of 3% water agar to maintain high humidity. The cup was sealed with polyethylene cling film and closed with a plastic lid containing a hole. Weevils were incubated at 20°C and had a 16L : 8D photoperiod. After 2 weeks cups and twigs were renewed. Three replicates of 10 weevils were used per treatment. Mortality, and the presence of mycelia on the bodies of dead insects were recorded every second day for 28 days.

Indirect inoculation bioassay

The following isolates were used: *B. bassiana* (Chot), *I. farinosa* (MS 2/3-10/00), *I. fumosorosea* (S-1/3-06/00), *M. anisopliae* (YF 1/1-05/00) and *M. anisopliae* (275) (Table 1). Twigs of *A. procera* (containing 10–40 needles) were immersed for 10 s in 20 ml of conidial suspension (10^7 conidia/ml) or in a similar volume of 0.05% Triton®-X-100 (the control). Insects were transferred to the inoculated twigs in medicine cups and maintained and observed as previously described.

Damage rate determination

In both bioassays, at the same time the mortality control was done, the initial number of needles on each twig was counted. The number of damaged needles was determined every second day.

Statistical analysis

Mortality data were subjected to a probit program (Mathematica®, Version 4, Wolfram Research, Inc.), which incorporated natural control mortality, LT_{50} and confidence values were calculated using complimentary log-log, logit or probit transformation of proportion of insects killed (Throne *et al*. 1995). Differences between LT_{50} values of *S. melanogrammum* for the two methods (twig inoculation and weevil inoculation) were assessed by comparison of slopes and intercepts of each isolate (Throne *et al*. 1995). The significance of the slopes and intercepts of the time-mortality data was tested at the 0.05 level (Robertson and Preisler 1992).

Analysis of the effect of the fungi on damaged needles in each treatment was conducted using analysis of variance (ANOVA, SAS) and the Duncan comparison test (PROC GLM, SAS Institute 1990). The square root arcsin transformation was used on all percentage values subjected to ANOVA. The weevil mortality level and needle damage 28 days after exposure to fungi were compared between particular isolates.

Table 1. Origin and host of fungal isolates tested against weevils of *S. melanogrammum*

Fungal species	Code	Host	Country of origin
B. bassiana	Chot	*Melolontha melolontha,* (Coleoptera; Scarabaeidae)	Poland
B. bassiana	MS1/1-05/00	*Leptinotarsa decemlineata,* (Coleoptera; Chrysomelidae)	Poland
B. bassiana	00-88	*Otiorhynchus singularis,* (Coleoptera; Curculionidae)	Denmark
M. anisopliae	3-1/2-08/00	soil (bait method-*Galleria mellonella*) (Lepidoptera; Pyralidae)	Poland
M. anisopliae	YF1/1-05/00	soil (bait method-*Galleria mellonella*) (Lepidoptera; Pyralidae)	Poland
M. anisopliae	275 ATCC No 90448	Cydia pomonella, (Lepidoptera; Tortricidae)	Austria
M. flavoviride	KVL 01-8	soil (bait method-*Galleria* spp.) (Lepidoptera; Pyralidae)	Denmark
I. farinosa	MS2/3-10/00	soil (bait method-*Galleria mellonella*), (Lepidoptera; Pyralidae)	Poland
I. farinosa	3-2/2-10/00	soil (bait method-*Galleria mellonella*) (Lepidoptera; Pyralidae)	Poland
I. farinosa	KVL 00-124	*Strophosoma melanogrammum,* (Coleoptera; Curculionidae)	Denmark
I. fumosorosea	5-1/1-08/00	soil (bait method-*Galleria mellonella*) (Lepidoptera; Pyralidae)	Poland
I. fumosorosea	S-1/3-06/00	*Otiorhynchus sulcatus,* (Coleoptera; Curculionidae)	Poland
I. fumosorosea	KVL 01-17	soil (bait method-*Galleria mellonella*) (Lepidoptera; Pyralidae)	Denmark

RESULTS

Weevils infected with entomopathogenic fungi began to die 2–11 days after inoculation. Isolates of *Metarhizium* spp. and *B. bassiana* were the most virulent. After direct spore application was used, *B. bassiana* (Chot) and *M. anisopliae* (3-1/2-08/00) were found to be highly virulent – with mortalities of 93 and 91%, respectively. However, indirect application of *B. bassiana* (Chot) resulted in only a 30% mortality (Tables 2, 3). *M. anisopliae* 275 also caused high mortality of *S. melanogrammum* – 87.0% after direct inoculation, and 71.6% after indirect spore application and after 28 days (Tables 2, 3). Direct and indirect spore application of *I. fumosorosea* (S-1/3-06/00) resulted in mortalities of about 50%. *I. farinosa* (MS 2/3-10/00) gave mortalities of 53% after indirect application and only 33% after direct application (Tables 2, 3).

LT_{50} values of *S. melanogrammum* for the most virulent isolates in both experiments ranged between 15.0 and 22.1 days, and two isolates of *Metarhizium* spp. (KVL 01-8 and 3-1/2-08/00) and *B. bassiana* (00-88 and MS1/1-05/00) were highly virulent (Table 2). LT_{50} values were only significantly different for the isolates *B. bassiana* (Chot) and *I. farinosa* (MS 2/3-10/00) when direct and indirect inoculation were compared (Table 4).

Most of the isolates, applied directly to the weevils, induced very high mortality of *S. melanogrammum*, but failed to reduce damage compared with the control. Similarly, when twigs were inoculated with conidial suspension there was no significant difference in damage needles between treatment and control. However, significant differences between particular isolates have appeared. In the case of direct inoculation, weevil damage to *A. procera* needles was significantly higher with *M. anisopliae* (YF1/1-05/00) and *I. farinosa* (MS2/3-10/00) compared with two isolates of *B. bassiana* (Chot and 00-88) and one isolate of *I. fumosoreseaus* (5-1/1-08/00). When twigs were inoculated with the conidial suspension, damage was higher (over 30%) for *M. anisopliae* 275 compared with other species of entomopathogenic fungi, *i.e. B. bassiana* (Chot), *I. farinosa* (MS2/3-10/00) and *I. fumosorosea* (5-1/1-08/00) (Table 5).

Table 2. Mortality and LT_{50} values of *S. melanogrammum* when weevils were inoculated with different entomopathogenic fungi

Fungal species	Code	Mortality [%] [a] 28 days post-inoculation	LT_{50} [days] [b]	Transformation model
Control		16.4 (3.5–36.1)		
M. anisopliae	275	87.0 (83.6–90.0)	18.1 (14.9–21.2)	Logit
M. anisopliae	3-1/2-08/00	90.7 (80.0–97.6)	19.1 (15.2–23.2)	Probit
M. anisopliae	YF1/1-05/00	81.6 (71.4–90.0)	17.4 (13.2–21.7)	Probit
M. flavoviride	KVL 01-8	87.0 (83.6–90.0)	15.0 (11.8–18.3)	Probit
B. bassiana	Chot	93.3 (84.9–98.5)	20.4 (16.6–24.6)	Probit
B. bassiana	00-88	83.6 (80.0–87.0)	19.5 (15.5–23.8)	Probit
B. bassiana	MS1/1-05/00	80.7 (74.5–86.2)	21.6 (18.0–24.4)	CLL
I. fumosorosea	5-1/1-08/00	68.4 (54.7–80.6)	25.3 (18.2–34.5)	Logit
I. fumosorosea	KVL 01-17	60.3 (50.1–70.0)	29.0 (23.8–37.5)	Probit
I. fumosorosea	S-1/3-06/00	53.4 (41.1–65.4)	27.0 (19.4–38.4)	Probit
I. farinosa	KVL 00-124	62.0 (44.7–77.8)	25.9 (19.1–35.7)	Probit
I. farinosa	3-2/2-10/00	43.2 (34.4–52.1)	33.1 (23.7–53.0)	Probit
I. farinosa	MS2/3-10/00	33.3 (30.0–36.6)	56.6 (undefined)	Logit

[a] mean ±SE; [b] values in brackets give 95% confidence limits; CLL = complimentary log-log

Table 3. Mortality and LT_{50} values of *S. melanogrammum* when twigs were inoculated with different entomopathogenic fungi

Fungal species	Code	Mortality [%] [a] 28 days post-inoculation	LT_{50} [days] [b]	Transformation model
Control		19.3 (13.8–25.2)		
M. anisopliae	275	71.6 (58.9–82.8)	22.1 (17.7–26.7)	Logit
I. farinosa	MS2/3-10/00	53.5 (44.5–62.4)	27.0 (23.3–31.8)	Logit
I. fumosorosea	S-1/3-06/00	50.0 (44.2–55.8)	33.7 (undefined)	Probit
M. anisopliae	YF1/1-05/00	43.2 (36.5–50.0)	34.5 (26.7–52.3)	Probit
B. bassiana	Chot	29.6 (24.0–35.6)	43.0 (undefined)	Probit

[a] mean ±SE; [b] values in brackets give 95% confidence limits

Table 4. Comparison of LT_{50} values for each isolate used in the two methods direct and indirect inoculation of *S. melanogrammum*

Isolate	Code	Test for the slopes	Test for the intercepts
B. bassiana	Chot	3.006*	0.937 n.s.
I. farinosa	MS 2/3-10/00	2.926*	1.607 n.s.
I. fumosorosea	S-1/3-06/00	0.471 n.s.	0.085 n.s.
M. anisopliae	YF1/1-05/00	1.542 n.s.	0.655 n.s.
M. anisopliae	275	1.319 n.s.	0.487 n.s.

* slopes are different at the 0.05 level; n.s. – slopes or intercepts are not different at the 0.05 level

Table 5. Damaged needles caused by weevils after direct and indirect spore application (mean ±SE; 28 days after inoculation)

Fungal species	Code	Direct inoculation	Indirect inoculation
		damage [%]	damage [%]
Control		35.6±22.0 abc*	61.2±15.1 ab
B. bassiana	Chot	32.8±9.5 ab	42.4±21.6 a
I. farinosa	MS2/3-10/00	60.7±15.6 c	44.7±15.4 a
I. fumosorosea	S-1/3-06/00	54.9±21.9 bc	46.6±21.3 a
M. anisopliae	YF1/1-05/00	64.8±21.2 c	54.1±10.6 ab
M. anisopliae	275	40.0±14.8 abc	78.4±15.5 b
B. bassiana	MS1/1-05/00	27.9±16.5 ab	not compared
B. bassiana	00-88	20.9±14.2 a	not compared
I. farinosa	3-2/2-10/00	35.6±11.2 abc	not compared
I. farinosa	KVL 00-124	42.2±19.8 abc	not compared
I. fumosorosea	5-1/1-08/00	22.4±21.1 ab	not compared
I. fumosorosea	KVL 01-17	36.1±16.8 abc	not compared
M. anisopliae	3-1/2-08/00	47.5±25.3 abc	not compared
M. flavoviride	KVL 01-8	48.5±17.9 abc	not compared

* means with the same letter are not significantly different

DISCUSSION

In the present study *S. melanogrammum* weevils appeared susceptible to all the entomopathogenic fungi tested, with *M. anisopliae, M. flavoviride* and *B. bassiana* showing the highest virulence. The present study also demonstrated the high virulence of *M. anisopliae* 275, irrespective of spore application method. The susceptibility of *S. melanogrammum* and *S. capitatum* to *Metarhizium* spp., *Isaria* spp. and *Beauveria* spp. was also studied by Vestergaard *et al*. (2001), who found most of the *Metarhizium* isolates (among them isolate 275 used in this study) were highly virulent, while *Beauveria* spp. was moderately or weakly virulent. Nielsen *et al*. (2006) also confirmed high efficiency of *Metarhizium* strains against *Strophosoma* weevils under laboratory and field conditions (after soil application).

The results of other studies (Latch 1976; Poprawski *et al*. 1985; Moorhouse *et al*. 1993, Steenberg and Humber 1999) indicate that most virulent strains are isolated from the original host or from a closely related species. Our experiments have demonstrated that isolates from a different insect species and different geographical origins were pathogenic to the nut-leaf weevil. Only two isolates out of thirteen were from insects closely related to *S. melanogrammum*. It is interesting that isolates of *Metarhizium* spp. are very virulent against *S. melanogrammum*, but not found naturally occurring on those weevils. This could be explained by the fact that *Metarhizium* spp. are isolated more frequently from disturbed, agricultural soils than from undisturbed forest soils (Vänninen 1995; Qesada-Moraga *et al*. 2007).

Direct and indirect inoculation of insects did not change the damage on needles in comparison with the control but there were significant differences between isolates. In spite of the fact that there were not significant differences compared to the control, decreased food consumption was observed for some isolates, even when the isolate did not cause a high mortality. Mohamed (1982) suggested that development of a fungal infection could affect hormonal control of feeding and result in reduced food intake. It could also be that the fungi act as antifeedants. For example Amiri *et al*. (1999) and Bandani and Butt (1999) found that specific secondary metabolites secreted by entomopathogenic fungi, such as destruxins and efrapeptins, act as antifeedants. As an explanation for the increased weevil food consumption, Roberts and Humber (1981) suggested that entomopathogenic fungi could derive nutrients from the insect's hemolymph and thus necessitate increased food intake by the insect.

The presented results are similar to data given by Ignoffo *et al*. (1983) and contrary to Moore *et al*. (1992). The first author determined that presence of *B. bassiana* (Boverol) on leaflets did not inhibit feeding by larvae of *Leptinotarsa decemlineata* (Say) in spite of high mortality (73.7–98.7%). The second author obtained reduction in the total amount of food eaten per locust after direct in-

oculation with *M. flavoviride*. However, the effect on the food consumption may be related to the properties of the isolate rather than to the species of the fungus.

In conclusion, isolates of *M. anisopliae* and *B. bassiana* showed considerable potential as microbial control agents for *S. melanogrammum* adults. For some isolates spore applications of entomopathogenic fungi on plant surface can reduce food consumption and damage, even when *S. melanogrammum* are not killed.

ACKNOWLEDGEMENTS

I wish to thank C. Wolsted and R. Eliasen for assisting with bioassays. I also wish to thank J. Eilenberg, A. Gillespie and C. Nielsen for their comments on the manuscript.

REFERENCES

Amiri B., Ibrahim L. Butt T.M. 1999. Antifeedant properties of destruxins and their use with the entomogenous fungus *Metarhizium anisopliae* for improved control of crucifer pests. Biocontrol Sci. Technol. 9 (4): 487–498.

Bandani A.R., Butt T.M. 1999. Insecticidal, antifeedant, growth inhibitory activities ofefrapeptins, metabolities of the entomogenous fungus *Tolypocladium*. Biocontrol Sci. Technol. 9 (4): 499–506.

Brousseau C., Charpentier G. Belloncik S. 1996. Susceptibility of spruce budworm, *Choristoneura fumiferana* Clemens, to destruxins, cyclodepsipeptidic mycotoxins of *Metarhizium anisopliae*. J. Invertebr. Pathol. 68 (2): 180–182.

Burges H.D. 1998. Formulation of mycoinsecticides. p. 131–185. In: "Formulation of Microbial Biopesticides" (H.D. Burges, ed.). Kluwer Academic, Dordrecht, 399 pp.

Butt T.M., Harris J.G., Powell K.A. 1999. Microbial Biopesticides. p. 23–44. In: "Methods in Biotechnology" (F.R. Hall, J.J. Menn, eds.). Humana Press, Totowa, 630 pp.

Eilenberg J., Vestergaard S. Harding S., Nielsen C. 2000. Potential for microbial control of *Scarabaeidae* and *Curculionidae* in Christmas tree and greenery production. p. 22–23. In: "Improvements in Christmas Tree and Greenery Quality" (C.J. Christensen, ed.). Ministry of Environment, Danish Forest and Landscape Research Institute – Reports No. 7, 124 pp.

Frølander A., Harding S. 2000. *Strophosoma* spp. in *Abies* Christmas tree and greenery production – factors influencing weevils abundance and damage levels. p. 24–25. In: "Improvements in Christmas Tree and Greenery Quality" (C.J. Christensen, ed.). Ministry of Environment, Danish Forest and Landscape Research Institute – Reports No. 7, 124 pp.

Gams W., Hodge K.T., Samson R.A., Korf R.P., Seifert K.A. 2005. Proposal to conserve the name *Isaria* (anamorphic fungi) with a conserved type. Taxon 54 (2): 537.

Gams W., Zare R.A. 2001. A revision of *Verticillium* sect. Prostrata. III. Generic classification. Nova Hedwigia 72 (3–4): 329–337.

Goettel M.S., Inglis G.D. 1997. Fungi: Hyphomycetes. p. 221–224. In: "Manual of Techniques in Insect Pathology" (L.A. Lacey, ed.). Academic Press, New York, 409 pp.

Hajek A.E. 1997. Ecology of terrestrial fungal entomopathogens. p.193–247. In "Advances in Microbial Ecology" Vol. 15 (J.G. Jones, ed.). Plenum Press New York and London, 374 pp.

Ignoffo C.M., Garcia C., Kroha M. 1983. A leaf surface treatment bioassay for determining the activity of conidia of *Beauveria bassiana* against *Leptinotarsa decemlineata*. J. Invertebr. Pathol. 41 (3): 385–386.

Keller S., Schweizer C., Keller E., Brenner H. 1997. Control of white grubs (*Melolontha melolontha* L.) by treating adults with the fungus *Beauveria brongniartii*. Biocontrol Sci. Technol. 7 (1): 105–116.

Keller S., Zimmermann G. 1989. Mycopathogens of soil insects. p. 263–264. In: "Insect – fungus interactions" (N. Wilging, N.M. Collins, P.M. Hammond, J.F. Webber, eds.). Academic Press, London, 344 pp.

Kolk A., Starzyk J.R. 1996. Atlas szkodliwych owadów leśnych. Multico, Warszawa, 706 pp.

Latch G.C.M. 1976. Studies on the susceptibility of *Oryctes rhinoceros* to some entomogenous fungi. Entomophaga 21 (1): 31–38.

Mohamed A.K.A. 1982. Pathogenicity of *Nomuraea rileyi* and its effect of food consumption and utilization by *Heliothis virescens* larvae. J. Georgia Entomol. Soc.17: 369–376.

Moore D., Reed M., Le Patourel G., Abraham Y.G., Prior C. 1992. Reduction of feeding by the desert locust, *Schistocerca gregaria*, after infection with *Metarhizium flavoviride*. J. Insect Pathol. 60 (3): 304–307.

Moorhouse E.R., Gillespie A.T., Charnley A.K. 1993. Selection of virulent and persistent *Metarhizium anisopliae* isolates to control black vine weevil (*Otiorhynchus sulcatus*) larvae on glasshouse begonia. J. Invertebr. Pathol. 62 (1): 47–52.

Nielsen C., Vestergaard S., Harding S., Wolsted C., Eilenberg J. 2006. Biological control of *Strophosoma* spp. (Coleoptera: Curculionidae) in greenery (*Abies procera*) plantations using Hyphomycetes. Biocontrol Sci. Technol. 16 (6): 583–598.

Noma T., Strickler K. 2000. Effect of *Beauveria bassiana* on *Lygus hesperus* (Hemiptera:Miridae) feeding and oviposition. Environ. Entomol. 29 (2): 394–402.

Poprawski T.J., Marchal M., Robert P.H. 1985. Comparative susceptibility of *Otiorhynchus sulcatus* and *Sitonia lineatus* (Coleoptera: Curculionidae) early stages to five entomopathogenic Hyphomycetes. Environ. Entomol. 14 (3): 247–253.

Quesada-Moraga E., Navas-Cortes J.A., Maranhao E.A.A., Ortiz-Urquiza A., Santiago-Alvarez C. 2007. Factors affecting the occurrence and distribution of entomopathogenic fungi in natural and cultivated soils. Mycol. Res. 111 (8): 947–966.

Roberts D.W. 1981. Toxins of entomopathogenic fungi. p. 441–464. In: "Microbial Control of Pests and Plant Disease" (H.D. Burges, ed.). Academic Press, New York, 949 pp.

Roberts D.W., Humber R.A. 1981. Entomogenous fungi. p. 201–237. In: "Biology of Conidial Fungi" (I.T. Cole, B. Kendrick, eds.). Academic Press, New York, 486 pp.

Robertson J.L., Preisler H.K. 1992. Pesticide Bioassays with Arthropods. CRC Press, London. 199 pp.

SAS Institute Inc. 1990. SAS/STAT User's guide, version 6, 4th ed. Vol. 1–2. Cary, North Carolina.

Steenberg T., Humber R.A. 1999. Entomopathogenic potential of *Verticillium* and *Acremonium* species (Deuteromycotina: Hyphomycetes). J. Inverterb. Pathol. 73 (3): 309–314.

Tefera T., Pringle K.L. 2003. Food consumption by *Chilo partellus* (Lepidoptera: Pyralidae) larvae infected with *Beauveria*

bassiana and *Metarhizium anisopliae* and effects of feeding natural versus artificial diets on mortality and mycosis. J. Invertebr. Pathol. 84 (3): 220–225.

Throne J.E., Weaver D.K., Chew V., Baker J.E. 1995. Probit analysis of correlated data: multiple observations over time at one concentration. J. Econ. Entomol. 88 (5): 1510–1512.

Vänninen I. 1995. Distribution and occurrence of four entomopathogenic fungi in Finland: effect of geographical location, habitat type and soil type. Mycol. Res. 100 (1): 93–101.

Vestergaard S., Eilenberg J., Harding S. 2000. Potential for microbial control of scarabs and weevils in Danish forestry. IOBC/WPRS Bull. 23 (8): 35–37.

Vestergaard S., Eilenberg J., Nielsen C. Harding S. 2001. Microbial control of scarabs and weevils in Christmas trees and greenery. In: 34th Annual Meeting, Society for Invertebrate Pathology, Noordwijkerhout, The Netherlands. 25–30 August 2001, p. 81.

POLISH SUMMARY

WPŁYW GRZYBÓW OWADOBÓJCZYCH NA ŚMIERTELNOŚĆ I ŻEROWANIE ZMIENNIKA LESZCZYNOWCA (*STROPHOSOMA MELANOGRAMMUM* FORST.)

W warunkach laboratoryjnych testowano wybrane szczepy grzybów owadobójczych *Beauveria bassiana* (Bals.-Criv.) Vuillemin, *Isaria farinosa* (Holm) Brown & Smith, *I. fumosorosea* (Wize) Brown & Smith, *Metarhizium anisopliae* (Metschnikoff) Sorokin i *M. flavoviride* (Gams & Rozsypal) przeciwko chrząszczom zmiennika leszczynowca *Strophosoma melanogrammum* Forster. Porównywano dwa sposoby aplikacji badanych grzybów i ich wpływ na śmiertelność i żerowanie (uszkodzenia igieł) *S. melanogrammum*. Pierwszy polegał na bezpośredniej infekcji chrząszczy wodną zawiesiną zarodników, drugi – pośredni – poprzez kontakt chrząszczy z zainfekowanymi gałązkami jodły (*Abies procera*), które stanowiły ich pokarm. Wszystkie testowane szczepy okazały się patogeniczne dla chrząszczy zmiennika. Wyższą śmiertelność obserwowano w wyniku bezpośredniej infekcji chrząszczy zawiesiną zarodników *B. bassiana* i *Metarhizium* spp. Po 28 dniach eksperymentu ta aplikacja spowodowała ponad 80% śmiertelność chrząszczy, a wartość LT_{50} wahała się od 15.0 do 21.6 dni. Pośrednia infekcja jednym z bardziej patogenicznych szczepów *M. anisopliae* (275) dała śmiertelność 72% i LT_{50} 22.1 dni. Oba sposoby porażenia chrząszczy zmiennika grzybami owadobójczymi nie dały, w porównaniu z wariantem kontrolnym, oczekiwanej redukcji żerowania, chociaż zaobserwowano różnice pomiędzy niektórymi szczepami.