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Pathogenicity assessment of entomopathogenic fungi infecting *Leptoglossus occidentalis* (Heteroptera: *Coreidae*)

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Leptoglossus occidentalis, an insect species native to North America, is a pest of conifer seed orchards in its natural distribution area. Recently, it was accidentally introduced into Europe where its populations have been expanding throughout the continent. In this study we evaluated the pathogenicity of *Beauveria bassiana*, *Isaria fumosorosea*, and *Metarhizium anisopliae* to this pest under laboratory and outdoor conditions. Pathogenicity varied depending upon isolates, exposure methods and location of bioassay. In the laboratory, LC_{50} values were highest for *I. fumosorosea* and lowest for *M. anisopliae*, and an indirect exposure was less effective than a direct one. In outdoor experiments the overall mortality was in all isolates significantly lower than mortality in the laboratory, and inter-specific variability in pathogenicity was not as prominent outdoors as in the laboratory. The results of this bioassay showed that *I. fumosorosea* has the potential as a microbial control agent of *L. occidentalis*.

Key words: Ascomycota, *Hypocreales*, seed bug, natural enemies, virulence.

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Leptoglossus occidentalis, bzdocha pochádzajúca zo Severnej Ameriky, je považovaná za významného škodcu semenných porastov v areáli svojho prirodzeného rozšírenia. Nedávno bola táto bzdocha náhodne zavlečená do Európy a jej populácia začala expandovať do ostatných častí kontinentu. V tejto práci sme hodnotili patogenitu entomopatogénnych húb *Beauveria bassiana*, *Isaria fumosorosea* a *Metarhizium anisopliae* k dospelým jedincom tejto bzdochy. Miera patogenity varírovala medzi jednotlivými izolátmi, metódami expozície a miestami pokusov. V laboratóriu, boli hodnoty LC_{50} najnižšie pre *I. fumosorosea*, a najvyššie pre *M. anisopliae*. Nepriama metóda expozície bola menej účinná než priama expozícia. Pri exteriérových testoch priamo na hostiteľských rastlinách bola celková mortalita imág pri všetkých izolátoch preukazne nižšia než v laboratóriu a medzidruhová variabilita patogenity nebola taká výrazná než pri laboratórnych pokusoch. Výsledky laboratórnych pokusov naznačujú, že huba *I. fumosorosea* má potenciál ako mikrobiálny bioagens pre reguláciu bzdochy *L. occidentalis*.

INTRODUCTION

The western conifer seed bug, *Leptoglossus occidentalis* Heidemann (Heteroptera: *Coreidae*), is an insect species native to North America, where it is considered a major pest of conifer seed orchards (McPherson et al. 1990). This seed bug, originally described from California in 1910, has been expanding eastward from the west coast of North America since the second half of the last century (McPherson et al. 1990, Wheeler 1992). Recently, the western conifer seed bug (WCSB) has also been recorded outside of its native continent, in Europe (Tescari 2001) and Japan (Ishikawa and Kikuhara 2008). In Europe, this species was first reported from northern Italy in 1999 (Tescari 2001) and this first European record was soon followed by finds in other countries throughout the continent (e.g. Bernardinelli and Zandigiacomo 2001, Gogala 2003, Moullet 2006, Aukema and Libeer 2007, Beránek 2007, Lis et al. 2008). In Slovakia, the occurrence of WCSB was studied in collections of conifers in the Mlyňany Arboretum SAS as well as in parks and public greenery of several towns and villages in south-western Slovakia during the summer of 2008 (Barta 2009). During this survey, the occurrence of indigenous entomopathogenic fungi in populations of this exotic species was also studied and two entomopathogens were identified from collected individuals. They were *Isaria fumosorosea* Wize and *Beauveria bassiana* (Balsamo) Vuillemin (Barta 2009). Although there is limited information on the activity of these entomopathogenic fungi in populations of this seed bug in the literature, these findings may be considered the first records of natural infection by these fungi in WCSB populations. However, WCSB susceptibility to an artificial inoculation with *B. bassiana* has already been demonstrated under laboratory conditions in Italy (Rumine and Barzanti 2008). Recent laboratory evaluation of WCSB susceptibility to Slovak isolates of the hypocrealean insect-pathogenic fungi (Barta 2010) has shown their potential of possible use in biocontrol of this invasive insect.

The hypocrealean entomopathogens are ubiquitous microorganisms attacking various arthropods by causing acute mycoses. They can spread fast among host populations horizontally via aerially produced conidia and infect their host by penetration of the cuticle with germ hyphae. After crossing the insect integument, they grow within the internal fluids, depleting nutrients from degraded proteins and fat bodies, and produce toxins which kill the host. After the host's death, the mycelium grows throughout the cadaver and protrudes outside completing the life cycle by abundant conidial formation (Hajek and St Leger 1994). Many strains of entomopathogenic fungi have been isolated and tested on different insect pests in a variety of cropping systems (e.g. Legaspi et al. 2000, Leland et al. 2005, Pu et al. 2005, Liu and Bauer 2008). To date, several fungal strains have been successfully licensed for commercial use against whiteflies, aphids, thrips and numerous other

pests (e.g. Shah and Pell 2003). However, development of microbial control agents against WCSB has drawn only marginal attention (Rumine and Barzanti 2008).

To evaluate the potential of fungal pathogens for WCSB control, we initiated research in Slovakia to screen for virulent isolates in the laboratory. Preliminary results show that indigenous isolates of *I. fumosorosea*, *B. bassiana* and *Metarhizium anisopliae* (Metschn.) Sorokin successfully infected WCSB adults whereby *I. fumosorosea* isolates were the most virulent (Barta 2010). It is generally accepted that susceptibility of insects to entomopathogens in the laboratory usually do not relate to that in field, therefore the main objective of this study was to compare laboratory and field pathogenicity of selected isolates against WCSB adults.

MATERIALS AND METHODS

Insects. Adults of *Leptoglossus occidentalis* (4060 individuals) used in the bioassays were collected by sweep netting in the Mlyňany Arboretum SAS (48° 19' 12'' N, 18° 22' 09'' E) in Slovakia. Collected individuals were placed in rearing cages (300 × 300 × 400 mm, covered with fine nylon fabric) in the laboratory at 20 ± 2 °C, 70 ± 10 % relative humidity (RH) and under a natural photoperiod. Insects were kept in the cage until their utilisation (usually no longer than 48 h) and provided with fresh food (unripe Douglas-fir cones).

Fungal isolates. After preliminary pathogenicity tests of *B. bassiana*, *I. fumosorosea* and *M. anisopliae* isolates (Barta 2010), the isolates AMSAS-03 (*B. bassiana*), AMSAS-06 (*I. fumosorosea*) and SUA-d26A (*M. anisopliae*) were selected and used in the present study. Three additional isolates obtained from USDA-ARS (Collection of Entomopathogenic Fungal Cultures, Ithaca, New York) were also tested. The geographic origin and hosts of all the isolates are given in Tab. 1. The fungi were cultivated on Sabouraud-dextrose agar (SDA) (Biomark™ Laboratories, India) in Petri dishes and incubated at 25 ± 2 °C with a 16/8 (L/D) photoperiod. Conidia of 15-day-old cultures were harvested and suspended in 100 ml of sterile distilled water with 0.05 % (v/v) Tween 80 (Sigma–Aldrich, India). The conidial suspensions were filtered through several layers of cheesecloth to remove mycelial mats. Conidial concentrations were adjusted to 1×10^8 conidia/ml (stock suspensions). Conidia in the suspensions were quantified by direct counting with an optical microscope using an improved Neubauer chamber. Viability of conidia was assessed before preparing final suspensions in germinating tests. Aliquots of 500 µl of stock suspensions were pipetted on an SDA plate and incubated at 20 °C. After 24 h the rate of conidial germination was determined by counting 100 conidia in four different view fields (400 spores per plate, magnification = 500×). The conidia were categorised into two groups: viable conidia identified by production of germ tubes, and non-germinating conidia. Only conidia

with a germ tube longer than its width were considered germinated. Only fungal cultures in which more than 90 % of the conidia germinated were used in the bioassay.

Virulence bioassay. For all test isolates, five aqueous suspensions were prepared from the stock in logarithmic series from 1×10^4 to 1×10^8 conidia/ml in Tween 80 (0.05 %, v/v). The concentrations were determined based on pre-tests, in which a concentration that would kill about 10 % and another that would kill about 90 % of treated insects was identified. The other concentrations used were spread between these extremes. For each concentration, a group of 20 WCSB adults were directly immersed in the conidial suspension for 10 s. Another 40 adults were immersed in 0.05 % Tween 80 (v/v) as controls. The treated and control insects were then incubated in groups of 20 in transparent polypropylene boxes (500 ml) for a period of 10 days at 23 ± 2 °C, saturated RH and under a natural photoperiod. The test insects were observed at 24-h intervals to record daily mortality, and unripe Douglas-fir cones were changed at 2-day intervals. All dead individuals were surface sterilised in a sodium hypochlorite solution (1 %, w/v) for 30 s, rinsed twice in sterile distilled water and incubated individually in Petri dishes containing water agar (2 %, w/v) for 7 days to stimulate fungal growth and confirm infection by the test fungi. Mortality caused by the fungi was confirmed by microscopic examination. The bioassay was repeated three times at intervals of 1 week for all isolates.

Laboratory bioassay – direct exposure to inoculum. In this bioassay two conidial concentrations of each test isolate corresponding to LC_{50} and LC_{90} were prepared in aqueous suspensions as mentioned above. The two concentrations were designed individually for each isolate based on the results of the virulence bioassay. A group of 20 WCSB adults were then directly immersed in the conidial suspensions for 10 s and another 20 adults were immersed in 0.05 % Tween 80 (v/v) as controls. The treated insects were maintained and mortality was evaluated as described above. The bioassay was repeated three times.

Laboratory bioassay – indirect exposure to inoculum. Two suspensions (conidial concentrations corresponding to LC_{50} and LC_{90}) prepared for each isolate were used for surface contamination of Douglas-fir cones with conidia by immersing them directly in the suspensions. Twenty unripe cones were individually dipped in 500 ml of conidial suspension for 30 s and then left on a laboratory desk in Petri dishes lined with filter paper to allow drying for 60 min. The treated cones were then placed on wet cotton in transparent polypropylene boxes (500 ml). A single cone together with one WCSB adult was placed into each box and closed with a ventilated lid. The boxes were held at 23 ± 2 °C, saturated RH and under a natural photoperiod. Two days after the treatment the experimental insects were transferred into new boxes with fresh untreated cones. The WCSB adults were monitored at 24-h intervals to record mortality for as long as 10 days,

and fresh food was changed every other day. Dead individuals were surface sterilised and incubated for infection confirmation as above. In the control variant, 20 cones were immersed in 0.05 % Tween 80 (v/v). The treatment was replicated three times on separate dates (at about 1-week intervals).

Outdoor bioassay. This experiment was initiated by installing insect rearing sleeves (20 × 50 cm) made of fine nylon netting on twigs of Douglas-fir trees in June. Only twigs with a single unripe developing cone were selected for sleeve setting. The Douglas-fir trees were about 15 years old and were grown in the Mlyňany Arboretum SAS. For the treatment, the indirect exposure method was used and inoculations of Douglas-fir cones were performed in situ by their immersing in the two conidial suspensions (LC_{50} and LC_{90}) prepared as described above. The sleeves were rolled up and cones developing on twigs were individually dipped into 500 ml of conidial suspension for 30 s. One WCSB adult was introduced into each sleeve 60 min. after the treatment and both ends of the test sleeves were tied to prevent the adults from escaping. The treatments were carried out between 8:00 and 9:00 a.m. Two days after the treatment the insects were transferred into new sleeves with untreated cones. They were monitored daily and any mortality was recorded for 10 days. All dead individuals were checked for fungal infection as in the previous experiments. For each conidial concentration 20 cones were treated and altogether 20 adults were exposed to contaminated cones. A further 20 cones treated with 0.05 % Tween 80 (v/v) acted as a control variant. This bioassay was repeated three times at intervals of 1 week for all isolates.

Statistical analysis. Cumulative percentage mortality data from the virulence bioassay were corrected for natural (control) mortality using Abbott's formula (Abbott 1925) and analysed with the Probit analysis (Finney 1971) in Minitab 14® (© 2004 Minitab Inc.) to estimate LC_{50} and LC_{90} for each isolate. Analysis of variance (ANOVA) ($\alpha = 0.05$) was used to determine the significant differences between the treatments and Tukey's HSD multiple comparison ($\alpha = 0.05$) followed if significant differences were detected. Treatment mortalities from outdoor trials, direct and indirect exposure bioassays in the laboratory, after correction for control mortality by Schneider-Orelli's formula (Finney 1971), were transformed using the arcsine square root function and subjected to ANOVA ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Six isolates of three entomopathogenic fungi (*B. bassiana*, *I. fumosorosea* and *M. anisopliae*) were screened for their virulence against adults of *Leptoglossus occidentalis* in the laboratory and in the field (in situ). The test insects were exposed to conidial suspensions using two different methods, a direct and an indirect exposure. The basic measure of virulence generated in the virulence bioassay

were lethal concentrations LC_{50} and LC_{90} expressed as conidia/ml of test suspensions and based on mortality recorded on the 10^{th} day after the inoculation. Our results indicate that WCSB adults are sensitive to isolates of all the three hypocrealean fungi irrespective of their geographical and host origin. In general, the percentage mortality of experimental insects increased with conidium concentration in the suspensions, which allowed for estimating the median lethal concentration. Fig. 1 shows the percentage mortality caused by the fungal isolates at different rates of conidial concentration. Lethal concentration values (LC_{50} and LC_{90}) for adult WCSBs are presented in Tab. 2. The results show certain level of inter-specific variability in virulence and significant differences were detected among the isolates ($F_{5.06} = 49.39$, $P < 0.01$ for LC_{50} and $F_{5.06} = 21.56$, $P < 0.01$ for LC_{90}). The LC_{50} values, as estimated by probit analysis, ranged from 0.86 to 47.13×10^5 conidia/ml and LC_{90} values varied from 0.11 to 33.87×10^7 conidia/ml. The level of virulence was highest for *I. fumosorosea* isolates and lowest for those of *M. anisopliae*. The mean conidial viability of the test isolates was 90.05–94.00 % (Tab. 2) during the virulence bioassays with no significant differences among the test isolates ($F_{2.77} = 1.18$, $P > 0.05$). Mortality of insects in the control groups ranged from 0 to 10 % ($\overline{x} = 4.44 \pm 0.89$ %, n = 18). This level of control mortality can be considered low confirming that the corrected mortalities obtained in the treatments were due to the pathogenicity of the entomopathogens rather than to other factors.

The three fungal species tested in the present study are considered facultative insect pathogens (Bidochka et al. 2002, Cory and Ericsson 2010) and we successfully demonstrated their pathogenicity to the coreid bug, *L. occidentalis*. A previous study presented by Italian authors (Rumine and Barzanti 2008) also successfully manifested *B. bassiana* virulence to WCSB adults under laboratory conditions. In addition, several other coreid bug species were tested for their susceptibility to entomopathogenic fungi. For instance, in Nicaragua, pathogenicity of *B. bassiana* and *M. anisopliae* to adults of *Leptoglossus zonatus* Dallas was assessed, and application of these isolates was effective both in the laboratory and in field conditions (Grimm and Guharay 1998). Similarly, *B. bassiana* or *M. anisopliae* successfully infected adults of other coreid bugs, *Riptortus linearis* (Fabricius) with $LC_{50} = 1.1 \times 10^6$ conidia/ml (Hu et al. 1996), *Paradasynus rostratus* (Distant) (Mohan et al. 2001) and *Clavigralla tomentosicollis* Stål with LC_{50} ranging from 9.8×10^4 to 1.8×10^5 (Ekesi 1999). Preliminary laboratory tests (Barta 2010) and the virulence bioassay in the current study confirmed that the hypocrealean fungi have a potential as biological control agents of *L. occidentalis* and could be considered for use in forest pest management. The use of entomopathogenic fungi in pest control is not a new idea and the three fungal species tested in this bioassay are often studied for their use in pest management. They are the most commonly applied fungal species in insect pest control and form the basis of a number of commercially available bio-pesticides (Shah and Pell 2003).

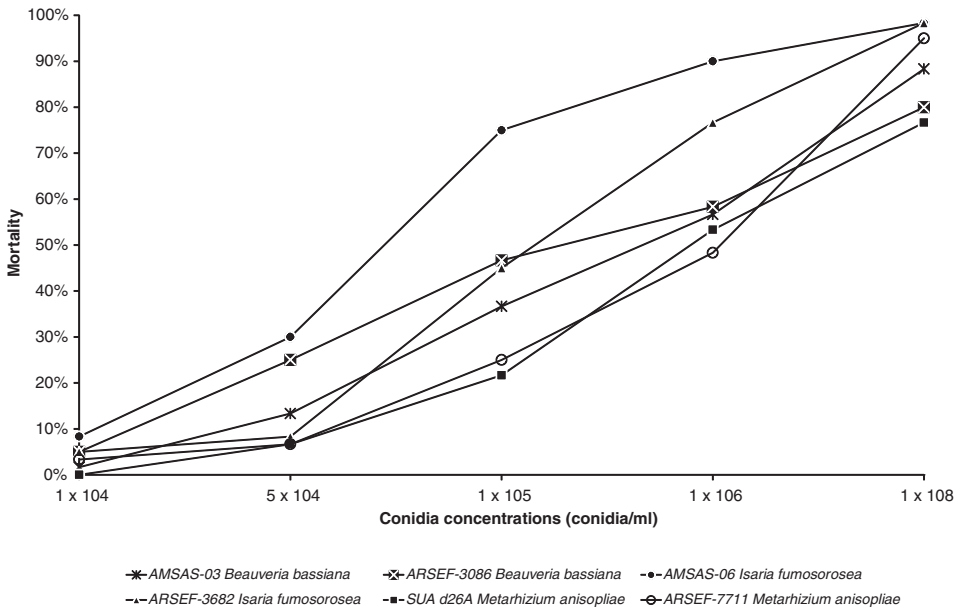

Fig. 1. Mean percentage mortality of *L. occidentalis* adults at different levels of conidia concentration 10 days after exposure to the six test isolates of entomopathogenic fungi in the virulence bioassay.

Tab 1. Fungal isolates assayed against adults of *Leptoglossus occidentalis*.

Beauveria bassiana	
Isolate Host Geographic origin	AMSAS-03 *L. occidentalis* Heidemann (Heteroptera: *Coreidae*) Slovakia (48° 19' 12.66" N, 18° 22' 08.51" E), 2009
Isolate Host Geographic origin	ARSEF-3086 *Leptoglossus fulvicornis* Westwood (Heteroptera: *Coreidae*) Homestead, Florida, USA
Isaria fumosorosea	
Isolate Host Geographic origin	AMSAS-06 *L. occidentalis* Heidemann (Heteroptera: *Coreidae*) Slovakia (48° 19' 12.66" N, 18° 22' 08.51" E), 2009
Isolate Host Geographic origin	ARSEF-3682 Homoptera: *Aphididae* Apopka, Florida, USA
Metarhizium anisopliae	
Isolate Host Geographic origin	SUA-d26A *G. mellonella* (L.) (Lepidoptera: *Pyralidae*) as bait from soil Slovakia (48° 17' 29.55" N, 18° 07' 20.80" E), 2008
Isolate Host Geographic origin	ARSEF-7711 unknown Austria

Tab. 2. Probit analysis results for test fungal isolates against *L. occidentalis* adults evaluated 10 days after exposure to conidial suspensions in the laboratory.

	Beauveria bassiana		*Isaria fumosorosea*		*Metarhizium anisopliae*	
Isolates	AMSAS-03	ARSEF-3086	AMSAS-06	ARSEF-3682	SUA-d26A	ARSEF-7711
LC_{50} [a]	10.35×10^5 ab [c]	10.18×10^5 ab	0.86×10^5 a	3.25×10^5 a	47.13×10^5 c	16.04×10^5 b
95% fiducial *CI* [a]	$5.85–19.98 \times 10^5$	$4.92–23.65 \times 10^5$	$0.59–1.25 \times 10^5$	$2.16–5.23 \times 10^5$	$23.38–108.13 \times 10^5$	$9.21–30.30 \times 10^5$
LC_{90} [a]	6.33×10^7 a	28.61×10^7 b	0.11×10^7 a	0.39×10^7 a	33.87×10^7 b	3.34×10^7 a
95% fiducial *CI* [a]	$2.31–26.92 \times 10^7$	$6.812–26.85 \times 10^7$	$0.06–0.27 \times 10^5$	$0.19–1.13 \times 10^7$	$10.50–185.61 \times 10^7$	$1.43–11.30 \times 10^7$
Slope ± SE	0.31 ± 0.03	0.23 ± 0.03	0.50 ± 0.06	0.51 ± 0.06	0.30 ± 0.03	0.42 ± 0.05
χ^2 [b]	3.60	2.99	3.36	2.47	3.61	2.03
P	0.000	0.000	0.000	0.000	0.000	0.000
Conidial viability (%)	92.50 ± 1.19	90.25 ± 2.01	91.00 ± 0.70	90.05 ± 1.55	94.00 ± 0.91	93.50 ± 1.94

[a] Values of lethal concentrations and 95 % fiducial confidence intervals are expressed in conidia per millilitre.
[b] Pearson chi-square goodness-of-fit test on the probit model ($\alpha = 0.05$, $df = 3$).
[c] Values followed by the same letter in the row are not significantly different (95 % Tukey's HSD test).

It is generally admitted that susceptibility of insects to entomopathogens demonstrated in a laboratory usually do not relate to infection rates obtained in fields. These discrepancies are often observed in bioassays and are attributed to differences between physiological and ecological susceptibility of hosts. In this study we carried out a bioassay to verify WCSB susceptibility to the entomopathogens in the laboratory as well as in the host's natural environment. In Tab. 3 we present mortality data of WCSB adults exposed to two different concentrations of conidial suspensions of all the tested fungal isolates in laboratory and outdoor bioassays. Mortality observed in the bioassays varied greatly depending on isolates, exposure methods and place of the assay. The exposure of adults to conidial suspensions resulted in 5–46 % mortality for LC_{50} concentrations and 20–86 % mortality for LC_{90} concentrations. Significant differences ($P < 0.05$) were observed in the mortality between the exposure methods and between laboratory and outdoor assays.

The results indicate that the indirect exposure method is less effective than the direct one. In other words, WCSBs treated directly with fungi were more susceptible to mycoses than were untreated individuals walking and feeding on conidia-treated cones. Adults' mortality in the indirect exposure bioassay decreased by 24–90 % in comparison with the direct exposure method and the mortality decrease varied depending on isolates and conidial concentrations. The differences in mortality are probably due to the fact that adults in the direct exposure collect higher quantities of conidia during the immersion than adults contacting the treated cones. The host's behaviour may encourage or discourage an infection

Tab. 3. Mortality data (mean mortality ± SE (%)) of *Leptoglossus occidentalis* adults exposed to two different concentrations of entomopathogens in laboratory and outdoor bioassays.

	Laboratory bioassays						Outdoor bioassay		
Bioassay	**Direct exposure**			**Indirect exposure**			**Indirect exposure**		
mortality ± SE (%)	**mortality**			**mortality**			**mortality**		
	observed	**corrected**[b]		**observed**	**corrected**		**observed**	**corrected**	
Beauveria bassiana									
AMSAS-03									
LC_{50}[a]	45.00 ± 5.77	43.86 ± 6.79	a[c]	15.00 ± 2.89	15.00 ± 2.89	b	16.67 ± 3.33	10.38 ± 4.81	b
LC_{90}	76.67 ± 3.33	76.32 ± 3.17	a	65.00 ± 2.89	65.00 ± 2.89	a	35.00 ± 2.89	29.83 ± 6.11	b
control	1.67 ± 1.67	–		0.00	–		6.67 ± 4.41	–	
ARSEF-3086									
LC_{50}	46.67 ± 7.26	46.67 ± 7.26	a	25.00 ± 2.89	21.09 ± 0.64	b	13.33 ± 1.67	5.46 ± 0.10	c
LC_{90}	80.00 ± 5.00	80.00 ± 5.00	a	66.67 ± 1.67	64.94 ± 1.01	a	48.33 ± 4.41	43.47 ± 5.59	b
control	0.00	–		5.00 ±2.89	–		8.33 ± 1.67	–	
Isaria fumosorosea									
AMSAS-06									
LC_{50}	48.33 ± 4.41	46.11 ± 6.55	a	31.67 ± 4.41	29.30 ± 4.57	a	13.33 ± 1.67	8.77 ± 1.75	b
LC_{90}	86.67 ± 1.67	86.30 ± 1.30	a	78.33 ± 1.67	77.54 ± 1.95	b	55.00 ± 2.89	52.63 ± 3.04	c
control	3.33 ± 3.33	–		3.33 ±1.67	–		5.00 ± 0.00	–	
ARSEF-3682									
LC_{50}	48.33 ± 6.01	45.61 ± 6.33	a	33.33 ± 1.67	30.93 ± 2.14	a	21.67 ± 1.67	12.55 ± 4.10	b
LC_{90}	81.67 ± 6.01	80.70 ± 6.33	a	70.00 ± 5.77	68.89 ± 5.88	ab	58.33 ± 6.01	53.92 ± 5.46	b
control	5.00 ± 0.00	–		3.33 ± 3.33	–		10.00 ± 5.00	–	
Metarhizium anisopliae									
SUA-d26A									
LC_{50}	41.67 ± 4.41	38.57 ± 4.43	a	15.00 ± 2.89	15.00 ± 2.89	b	15.00 ± 2.89	5.57 ± 0.18	c
LC_{90}	80.00 ± 5.77	79.08 ± 5.66	a	56.67 ± 4.41	56.67 ± 4.41	a	30.00 ± 5.77	21.65 ± 8.94	b
control	5.00 ± 2.89	–		0.00	–		10.00 ± 2.89	–	
ARSEF-7711									
LC_{50}	40.00 ± 2.89	37.98 ± 2.13	a	14.00 ± 3.06	9.16 ± 5.49	b	16.67 ± 1.67	7.41 ± 1.85	b
LC_{90}	78.33 ± 4.41	77.72 ± 4.15	a	58.33 ± 6.01	55.96 ± 6.94	b	28.33 ± 3.33	20.37 ± 3.70	c
control	3.33 ± 3.33	–		5.00 ± 2.89	–		10.00 ± 0.00	–	

[a] Conidial concentration levels at which 50 % (LC_{50}) or 90 % (LC_{90}) of tested WCSB adults died in the virulence bioassays.
[b] Mortality data corrected for control mortality according to Schneider-Orelli's formula (Finney 1971).
[c] Values followed by the same letter in the row are not significantly different (95 % Tukey's HSD test).

process and movement of WCSBs on treated cones during the course of their feeding was the only means of contact with infection propagules. Moreover, as mentioned above, the quantity of conidia which get in contact with a host is directly proportional to mortality. The differences in mortality may also be attributed to limited survival of conidia on a cone surface. This was mainly the case in the outdoor experiment, where the overall mortality decreased by 21–81 % in comparison with the laboratory test (indirect exposure in both assays). Although

conidial longevity on the cone surface was not measured during the bioassays, it is well known that viability of conidia may be limited by a range of environmental factors, including reduced aerial humidity, solar radiation (particularly the UV-B component), temperature, etc. (e.g. Ferron 1977, Zimmermann 1982, Fernandes et al. 2007). Conidial germination may also be inhibited by the presence of phytochemicals found on a plant surface (Fernandez et al. 2001). Longevity of conidia on plant surfaces may vary from several hours to a few days. For instance, *B. bassiana* conidia survived for 3 days on maize leaves (Mulock and Chandler 2000).

Based on data presented in this study, adults of *L. occidentalis* were more susceptible to the isolates of *I. fumosorosea* than to the isolates of the other two fungal species, irrespective of exposure method. However, the inter-specific variability in virulence was not as considerable in the outdoor bioassays as in the laboratory. Generally, in the natural field habitat, overall mortality was significantly lower for all isolates than mortality in the laboratory.

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