Available online at www.sciencedirect.com

ScienceDirect

Journal of Stored Products Research 43 (2007) 535–539

Journal of STORED PRODUCTS RESEARCH

www.elsevier.com/locate/jspr

Desiccation increases the efficacy of *Beauveria bassiana* for stored-grain pest insect control

Jeffrey C. Lord*

Grain Marketing and Production Research Center, USDA-ARS, 1515 College Avenue, Manhattan, KS 66502, USA

Accepted 1 March 2007

Abstract

The effect of desiccation stress on the efficacy of *Beauveria bassiana* for controlling stored-product insects was investigated in laboratory bioassays. The mortality of *B. bassiana*-treated *Plodia interpunctella* larvae was greater at a vapor pressure deficit (VPD) of 2.42 or 1.87 kPa than at 1.06 kPa. Moisture also had significant effects on the mortalities of adult rice weevils, *Sitophilus oryzae* and maize weevils, *Sitophilus zeamais*. Mortality of *S. zeamais* was higher at 2.42 and 1.87 kPa than at 1.06 kPa, while mortality of *S. oryzae* was higher at 1.87 kPa than at either 2.42 or 1.06 kPa. Higher control mortality at the higher two VPDs indicated that S. *zeamais* was less desiccation tolerant than *S. oryzae*. The mortalities of *B. bassiana*-treated adult *Cryptolestes ferrugineus*, larval *Lasioderma serricorne* and larval *Oryzaephilus surinamensis* were not significantly affected by VPD. These results demonstrate that dry stored-grain conditions are favorable for *B. bassiana* efficacy.

Keywords: *Beauveria bassiana*; *Plodia interpunctella*; *Sitophilus*; *Lasioderma serricorne*; Desiccation; Humidity; Grain beetle

1. Introduction

It is widely believed that entomopathogenic fungi are not only most efficacious in high moisture conditions, but that they are not at all effective with low moisture. However, many studies have demonstrated that this is often not the case. Ambient humidity has been found to have little impact on *Beauveria bassiana* (Balsamo) Vuillemin efficacy in some insect systems (Moore, 1973; Ferron, 1977). Ramoska (1984) reported that *B. bassiana* conidia were infective against the chinch bug, *Blissus leucopterus* (Say), at all relative humidities (r.h.) tested, ranging from 30% to 100%. Marcandier and Khachatourians (1987) also reported no significant effects of r.h. on *B. bassiana*-related mortality of grasshoppers at r.h. ranging from 12% to 100%. The preceding reports differ from those of Huafeng et al. (1998) and Luz and Fargues (1999) who reported dependence on high humidity on the germination as well as infection of *B. bassiana*. Similarly, a significant increase in the mortality of coffee berry borer, *Hypothenemus hampei* (Ferrari), due to *B. bassiana* was reported with an increase in r.h. from 50% to 90% (Haraprasad et al., 2001). Luz et al. (1998) screened isolates of *B. bassiana* for the control of *Triatoma infestans* (Klug) and reported varying effects of 50% and 100% r.h. on the efficacy of different isolates. In these studies, the differences in the effects of r.h. on *B. bassiana* efficacy may have occurred because of differences in the microclimate on insect cuticles or possibly due to differences in the cuticular chemical composition of different hosts.

Previous reports show that mortality of *B. bassiana*-treated *Tribolium castaneum* (Herbst) was not significantly different at 56% or 75% r.h., but the trend was for higher mortality at the lower r.h. (Akbar et al., 2004). The mortality of *Rhyzopertha dominica* (F.) treated with *B. bassiana* was enhanced by reduced moisture (Lord, 2005). Considering that stored-product environments are often dry and that controlled atmosphere pest management can include further drying, bioassays were conducted with some of the major insect pests of stored grain and grain products to determine whether reduction in moisture would correlate with improvement of *B. bassiana* efficacy as determined by insect mortality.

*Tel.: +1 785 776 2705; fax: +1 785 537 5584.
E-mail address: jeffrey.lord@gmprc.ksu.edu.

0022-474X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jspr.2007.03.002

2. Materials and methods

2.1. Insects and fungus

All of the experiments were conducted at the USDA Grain Marketing and Production Research Center, Manhattan, KS with insects from laboratory colonies that have been maintained for several years without wild input. *Plodia interpunctella* (Hübner), the Indian meal moth, and *Sitophilus zeamais* Motschulsky, the maize weevil, colonies originated in Georgia, USA. *Lasioderma serricorne* (F.), the cigarette beetle, *Oryzaephilus surinamensis* (L.), the sawtoothed grain beetle, *Cryptolestes ferrugineus* (Stephens), the rusty grain beetle, and *Sitophilus oryzae* (L.), the rice weevil, were from eastern Kansas, USA. *Plodia interpunctella* larvae were in the third instar and were reared on a diet of dried milk, brewer's yeast, wheat germ, bran, and flour without preservatives. *Oryzaephilus surinamensis* larvae were used 2 weeks after overnight oviposition on a diet of rolled oats and brewer's yeast, and *L. serricorne* larvae were used 3 weeks after oviposition on wheat flour. *Cryptolestes ferrugineus* adults were assayed at 1–2 weeks after emergence and were reared on rolled oats, wheat germ and brewer's yeast. *Sitophilus oryzae* and *S. zeamais* adults were of mixed ages and had been cultured on hard red winter wheat and corn kernels, respectively.

Commercially produced conidia of *B. bassiana* strain GHA (Laverlam, Butte, MT) were used in all experiments. The *B. bassiana* technical powder contained 9.4×10^{10} conidia/g. The viability of the conidia was at least 90% throughout the study as confirmed by germination rates on Sabouraud dextrose agar after incubation for 18 h at 26 °C.

2.2. Assays

Crimped hard red winter wheat adjusted to target moistures was used as diet for all insects. For *P. interpunctella* and *L. serricorne*, the wheat was supplemented with 1% brewer's yeast. Incubation was over saturated salt solutions that provided the selected humidities (Table 1) (Greenspan, 1977; Winston and Bates, 1960). The humidities were selected to extend to the upper limits of vapor pressure deficit (VPD) tolerance for the insects based on preliminary tests. The humidities were confirmed with BK Precision 625 (BK Precision, Yorba Linda, CA) and Vaisala HmI41 (Vaisala Oyj, Helsinki) hygrometers. The final moisture content was measured with a Dickey-John GAC 2000 or, when out of the meter's range, by weight loss after heating to 120 °C until weight stabilized. The wheat was crimped to facilitate insect feeding. The assay vessels were 118 ml (4 oz) wide-mouth glass jars with filter paper inserted into the lid rims.

Table 1
Grain and ambient moistures achieved with saturated salt solutions at 30 °C

% r.h.	% grain moisture	VPD (kPa)	Salt
75	13.2	1.06	NaCl
56	11.0	1.87	NaBr
43	10.0	2.42	K_2CO_3
32	8.7	2.97	$MgCl_2$
22	7.9	3.31	KAc
16	6.3	3.56	$CaBr_2$
12	5.1	3.78	LiCl

The doses were selected from results of preliminary assays to be near the median lethal concentrations in order to allow detection of moisture effect on efficacy. Multiple *B. bassiana* doses were used for *P. interpunctella* only. They were 0, 100, 200, 400, and 800 mg of conidia/kg of wheat with yeast. There were 30 *P. interpunctella* larvae per replicate in jars with 45 g of diet. Mortality was scored after 10 d of incubation. There were five temporal replicates.

Single-dose assays were used for all of the beetles. *Sitophilus oryzae* and *S. zeamais* adults were exposed to 400 mg of *B. bassiana*/kg of grain at four moistures, viz. six replicates at 1.06, 1.87, and 2.42 kPa and four replicates of 3.31 kPa. *Lasioderma serricorne* was exposed to 5 mg/kg at 1.06, 2.42, and 2.97 kPa with four replicates each. *Cryptolestes ferrugineus* adults were exposed to 30 mg/kg at 1.06, 3.31, 3.56 and 3.78 kPa with four replicates each. *Oryzaephilus surinamensis* larvae were exposed to 10 mg/kg at 1.06, 3.31, 3.56 and 3.78 kPa with four replicates each. Incubation was at 30 ° under subdued constant light for all assays. All beetle replicates had 20 insects and 20 g of diet. Replicates for all assays were temporal to avoid the problems of pseudoreplication that occur when replicates are set up and incubated jointly.

2.3. Statistical analysis

ANOVAs and *t*-tests were carried out with SigmaStat (Systat Software Inc., Richmond, CA), and differences in means were detected by Fisher's LSD test. The *t*-tests were used to detect mortality differences between weevil species treated with *B. bassiana* at each VPD and between treated and control *L. serricorne* at each VPD. Mortalities of *B. bassiana*-treated larvae were adjusted by Abbott's formula (Abbott 1925). All data passed the Kolmogorov–Smirnov test for normality (Zar, 1999) and were not transformed.

3. Results

Plodia interpunctella mortality was affected significantly by *B. bassiana* dose ($F = 12.0$; df = 3, 45; $P < 0.01$) and by moisture ($F = 4.3$; df = 2, 45; $P = 0.02$) (Fig. 1). There was no significant dose–moisture interaction ($F = 0.4$; df = 6, 45; $P = 0.87$). Over all doses collectively, the mortality was significantly greater at 2.42 kPa ($P = 0.009$) and 1.87 kPa ($P = 0.024$) than at 1.06 kPa. Mean control mortalities were 12.0% at 1.06 kPa, 17.2% at 1.87 kPa, and 16.0% at 2.42 kPa. The mean control mortalities were not significantly different ($F = 1.08$; df = 2, 12; $P = 0.37$).

Moisture had significant effects on the mortalities of *B. bassiana*-treated *S. oryzae* ($F = 6.30$; df = 2, 15; $P < 0.01$) and *S. zeamais* ($F = 4.03$; df = 2, 15; $P = 0.04$) (Table 2). *Sitophilus zeamais* mortality was significant higher at 2.42 and 1.87 kPa than at 1.06 kPa, while *S. oryzae* mortality was significant higher at 1.87 kPa than at either 2.42 or 1.06 kPa. Mortality was significantly greater for *B. bassiana*-treated *S. zeamais* than for *B. bassiana*-treated *S. oryzae* only at 2.42 kPa ($t = 2.76$, df = 10, $P = 0.02$). In the weevil assays at 3.31 kPa, the *S. zeamais* control mortality of 88.8% was significantly greater than the 40% for *S. oryzae* ($t = 5.05$, df = 6, $P = 0.002$). The unadjusted mean mortalities of *B. bassiana*-treated beetles at 3.31 kPa were 91.3% for *S. oryzae* and 98.8% for *S. zeamais*. The 3.31 kPa data were not included in the analysis or Table 2 because of the high control mortalities.

There was no significant difference among the mean mortalities of *L. serricorne* larvae treated with *B. bassiana* under three moisture regimes ($F = 0.10$; df = 2, 9; $P = 0.91$). Nor was there a significant difference among the mean mortalities of larvae that were not exposed to fungus ($F = 2.10$; df = 2, 9; $P = 0.18$). The mortality was significantly greater for *B. bassiana*-treated larvae than for control larvae at each moisture (2.97 kPa, $t = 2.86$, df = 6, $P = 0.03$; 2.42 kPa, $t = 2.60$, df = 6, $P = 0.04$; 1.07 kPa, $t = 5.28$, df = 6, $P = 0.002$) (Table 3). In a preliminary test, 3.31 kPa was fatal for all *L. serricorne* larvae.

Neither *C. ferrugineus* nor *O. surinamensis* showed a moisture effect in its mortality response to *B. bassiana* in spite of high control mortality at the higher VPDs (Table 4). The adjusted mortality of *B. bassiana*-treated adult *C. ferrugineus* was not significantly affected by moisture ($F = 0.7$; df = 3, 12; $P = 0.57$). The mortality of *B. bassiana*-treated *O. surinamensis* larvae was also not significantly affected by moisture ($F = 0.3$; df = 3, 11; $P = 0.82$).

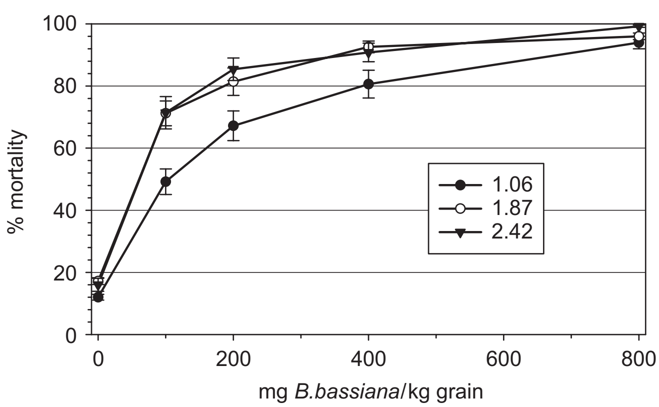

Fig. 1. *Plodia interpunctella* larval mortality (±SE) after exposure to *B. bassiana* at three vapor pressure deficits (kPa).

4. Discussion

According to the literature review of Howe (1965), *O. surinamensis* and *C. ferrugineus* are classified as tolerant of low r.h., *P. interpunctella* and *L. serricorne* need moderate r.h., and *S. oryzae* needs high r.h. The findings of this study confirm Howe's groupings with the exception of the *S. oryzae* results, wherein there were 5% control mortality and no significant increase in susceptibility to *B. bassiana* at 2.42 kPa as compared with 1.06 kPa.

The minimum r.h. for *P. interpunctella* according to Howe (1965) is 40%, or a VPD of 2.55 kPa at 30 °C. The significantly greater mortality of *B. bassiana*-associated mortality with VPDs of 1.87 and 2.42 than for 1.06 kPa suggests that there is a gradual increase in stress as moisture decreases under even moderate and commonly occurring conditions. VPDs of 1.87 and 2.42 correspond to moisture contents of 11% and 10%, respectively, for hard red winter wheat. The moisture content of hard red winter wheat at harvest in the 2006 USA crop ranged from 8.9% to 14.7% (US Wheat Associates, 2006). Consequently, moisture-stress *B. bassiana* efficacy enhancement for *P. interpunctella* could be operative without a special effort to alter moisture conditions.

Howe (1965) classified *S. oryzae* as needing high r.h., and several authors have indicated that *S. zeamais* does not tolerate dryness (Okelana and Osuji, 1985; Hwang et al., 1983). The control mortalities in this study at 2.42 and 3.31 kPa indicated that *S. oryzae* is more desiccation

Table 3
Percentage mortality ±SD of *Lasioderma serricorne* larvae exposed to 5 mg of *Beauveria bassiana*/kg of wheat at three vapor pressure deficits.

	2.97 kPa	2.42 kPa	1.06 kPa
Beauveria bassiana	53.8±13.2Aa	53.8±17.5Aa	50.0±9.1Aa
Control	33.8±4.8Ba	26.3±11.8Ba	22.5±5.0Ba

Means within rows followed by the same lower case letter are not significantly different by Fisher's LSD. Means within columns followed by the same upper case letter are not significantly different by Student's *t*-test ($\alpha = 0.05$).

Table 2
Percentage mortality ±SD of adult *Sitophilus oryzae* and *S. zeamais* exposed to 400 mg of *Beauveria bassiana*/kg of wheat at three vapor pressure deficits (control mortality in parentheses)

	2.42 kPa	1.87 kPa	1.06 kPa
Sitophilus oryzae	31.7±12.7Bb (5.0)	43.2±13.6Aa (4.2)	22.9±10.9Ab (1.7)
Sitophilus zeamais	67.0±28.7Aa (23.3)	61.6±24.3Aa (5.8)	25.1±9.2Ab (3.3)

Means within rows followed by the same lower case letter are not significantly different by Fisher's LSD. Means within columns followed by the same upper case letter are not significantly different by Student's *t*-test ($\alpha = 0.05$).

Table 4
Percentage mortality ±SD of adult *Cryptolestes ferrugineus* exposed to 30 mg of *Beauveria bassiana*/kg of wheat and larval *Oryzaephilus surinamensis* exposed to 10 mg of *B. bassiana*/kg of wheat at four vapor pressure deficits (control mortality in parentheses).

	3.78 kPa	3.56 kPa	3.31 kPa	1.06 kPa
C. ferrugineus	64.2±27.8a (66.3)	45.0±27.9a (25.0)	50.0±21.2a (0)	41.3±16.5a (0)
O. surinamensis	85.2±8.5a (25.0)	79.8±11.1a (31.3)	82.4±10.3a (1.3)	85.0±4.1a (0)

Means within rows followed by the same letter are not significantly different.

tolerant than the maize weevil. The mortality of *S. oryzae* with fungus at 2.42 kPa was an anomaly in the trend of increased mortality of fungus-treated beetles at higher VPD. Perhaps this is due to a biological effect, e.g. hydration speed effects, or it may be due to natural variation. In any case, desiccation-related improvement of *B. bassiana* efficacy for *S. oryzae* and *S. zeamais* adults, like *P. interpunctella* larvae, would be operative under normal grain storage conditions.

The lack of effect of the tested VPDs on the mortality of *B. bassiana*-treated *L. serricorne* may reflect a lack of desiccation-related decrease in fungus tolerance or it may reflect a narrow range between permissive and lethal moisture levels. In a preliminary test, 3.31 kPa was found to be lethal to most *L. serricorne* larvae. Accordingly, if there is a moisture range at which desiccation would improve efficacy, it would be too narrow for exploitation. There are, however, no published reports of *L. serricorne* treatment with *B. bassiana*. The significant fungus effect on mortality with the very low dose of 5 mg/kg indicates that it would be a good target for *B. bassiana* use as a mycoinsecticide regardless of efficacy-enhancing environmental conditions.

Some studies of *B. bassiana* efficacy for stored-product insects have been conducted with only high moisture and have consequently led to some questionable conclusions. For example, Searle and Doberski (1984) used r.h. of 70–100% for *O. surinamensis* adults and found no infections with less than 90% r.h.. Such high moistures do not normally occur in *O. surinamensis* habitat and would themselves be stressful to the beetles. Certainly they would encourage fungal growth, even on the grain diet itself. More fruitful investigations would concentrate on operational conditions, including those created by other pest management strategies.

The negative results in this study for *C. ferrugineus* adults and *O. surinamensis* larvae do not necessarily mean that desiccation stress does not favor fungal infection. It may be that there is only a narrow window of VPD where it occurs, unlike *P. interpunctella* and *S. zeamais*, or any desiccation enhancement of *B. bassiana* efficacy for these species may have been masked by the high stress mortality. Measurement of stress indicators, such as heat shock proteins would help to explain these effects.

The reference and highest moisture used in this study, a VPD of 1.06 kPa, was chosen because it is favorable for most stored-grain insects (Hagstrum, 1987; Storey et al., 1983) but is higher than that of most stored wheat. The lower end of the range of stored grain moisture, as indicated by the 2006 hard red winter wheat crop, would cause desiccation stress in all of the insects in this study except perhaps adult *C. ferrugineus* and larval *O. surinamensis*. Sound stored-product management should include maintenance of a dry environment to discourage insects and noxious fungi as a matter of course, thereby creating conditions favorable to the use of *B. bassiana*.

The results of this study demonstrate that the relatively dry conditions of stored-product environments are not an impediment to the use of *B. bassiana* for management of insect pests. In fact, such conditions are advantageous. Hong et al. (2005) reported variability of *B. bassiana* conidial survival over saturated $MgCl_2$ and suggested that it and atmospheres over it and other salts may have deleterious effects on the conidia. If that is the case, then the results presented here give a conservative estimate of the benefit of low moisture to efficacy. The longevity of *B. bassiana* conidia is best when dry (Lord, 2005; Wraight et al., 2001) Drying and aeration are common practices for conditioning and pest control in stored grains (Reed and Arthur, 2000). This work provides a basis for integrating *B. bassiana* with this approach.

Acknowledgments

I am grateful to Sheri Anderson for meticulous technical assistance. I thank Robert Everich and Clifford Bradley for helpful comments on an earlier version of this article. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture

References

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology 18, 265–267.

Akbar, W., Lord, J.C., Nechols, J.R., Howard, R.W., 2004. Diatomaceous earth increases the efficacy of *Beauveria bassiana* against *Tribolium castaneum* larvae and increases conidia attachment. Journal of Economic Entomology 97, 273–280.

Ferron, P., 1977. Influence of relative humidity on the development of fungal infection caused by *Beauveria bassiana* (Fungi Imperfecti, Moniliales) in imagines of *Acanthoscelides obtectus* (Col.: Bruchidae). Entomophaga 22, 393–396.

Greenspan, L., 1977. Humidity fixed points of binary saturated aqueous solutions. Journal of Research of the National Bureau of Standards, A. Physics and Chemistry 81, 89–96.

Hagstrum, D.W., 1987. Seasonal variation of stored wheat environment and insect populations. Environmental Entomology 16, 77–83.

Haraprasad, N., Niranjana, S.R., Prakash, H.S., Shetty, H.S., Wahab, S., 2001. *Beauveria bassiana*—a potential mycopesticide for the efficient control of coffee berry borer, *Hypothenemus hampei* (Ferrari) in India. Biocontrol Science and Technology 11, 251–260.

Hong, T.D., Edgington, S., Ellis, R.H., de Muro, M.A., Moore, D., 2005. Saturated salt solutions for humidity control and the survival of dry powder and oil formulations of *Beauveria bassiana* conidia. Journal of Invertebrate Pathology 89, 136–143.

Howe, R.W., 1965. A summary of estimates of optimal and minimal conditions for population increase of some stored products insects. Journal of Stored Products Research 1, 177–184.

Huafeng, L., Meizhen, F., Zengzhi, L., Cui, H., 1998. Pathogenic effect of *Beauveria bassiana* infected on *Dendrolimus* under different temperature and humidity. Chinese Journal of Applied Ecology 9, 195–200.

Hwang, J.S., Hsieh, F.K., Kung, K.S., 1983. Influences of temperature and relative humidity on the development of *Sitophilus zeamais* Motschulsky. Plant Protection Bulletin 25, 41–52.

Lord, J.C., 2005. Low humidity, moderate temperature, and desiccant dust favor efficacy of *Beauveria bassian*a (Hyphomycetes: Moniliales) for the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bruchidae). Biological Control 34, 180–186.

Luz, C., Fargues, J., 1999. Dependence of the entomopathogenic fungus, *Beauveria bassiana* on high humidity for infection of *Rhodnius prolixus*. Mycopathologia 146, 33–41.

Luz, C., Tigano, M.S., Silva, I.G., Cordeiro, C.M., Alajanabi, S.M., 1998. Selection of *Beauveria bassiana* and *Metarhizium anisopliae* isolates to control *Triatoma infestans*. Memórias do Instituto Oswaldo Cruz 93, 839–846.

Marcandier, S., Khachatourians, G.G., 1987. Susceptibility of migratory grasshopper, *Melanoplus sanguinipes* (Fab.) (Orthoptera: Acrididae), to *Beauveria bassiana* (Bals.) Vuillemin (Hyphomycetes): influence of relative humidity. Canadian Entomologist 119, 901–907.

Moore, G.E., 1973. Pathogenicity of three entomogenous fungi to southern pine beetle at various temperatures and humidities. Environmental Entomology 2, 54–57.

Okelana, F.A., Osuji, F.N.C., 1985. Influence of relative humidity at 30 °C on the oviposition, development and mortality of *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) in maize kernels. Journal of Stored Products Research 21, 13–19.

Ramoska, W.A., 1984. The influence of relative humidity on *Beauveria bassiana* infectivity and replication in the chinch bug, *Blissus leucopterus*. Journal of Invertebrate Pathology 43, 389–394.

Reed, C., Arthur, F.H., 2000. Aeration. In: Subramanyam, Bh., Hagstrum, D.W. (Eds.), Alternatives to Pesticides in Stored-Product IPM. Kluwer Academic Publishers, New York, pp. 193–227.

Searle, T., Doberski, J., 1984. An investigation of the entomogenous fungus *Beauveria bassiana* (Bals.) Vuill. as a potential biological control agent for *Oryzaephilus surinamensis* (L.). Journal of Stored Products Research 20, 17–23.

Storey, C.L., Sauer, D.B., Walker, D., 1983. Insect populations in wheat, corn, and oats stored on the farm. Journal of Economic Entomology 76, 1323–1330.

US Wheat Quality Associates, 2006. Crop Quality Reports. 〈http://www.uswheat.org/cropQualityReports〉.

Winston, P.W., Bates, D.H., 1960. Saturated solutions for the control of humidity in biological research. Ecology 41, 232–237.

Wraight, S.P., Jackson, M.A., de Kock, S.L., 2001. Production, stabilization and formulation of fungal biocontrol agents. In: Butt, T.M., Jackson, C., Magan, N. (Eds.), Fungi as Biocontrol Agents. CAB International, Wallingford, UK, pp. 253–286.

Zar, J.H., 1999. Biostatistical Analysis, fourth ed. Prentice-Hall, Upper Saddle River, NJ.