

RESEARCH ARTICLE

Influence of substrate and relative humidity on the efficacy of three entomopathogenic fungi for the hide beetle, *Dermestes maculatus* (Coleoptera, Dermestidae)

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(Received 5 November 2010; returned 13 December 2010; accepted 5 January 2011)

Dermestes maculatus De Geer (Coleoptera: Dermestidae) is a carrion feeder that is also a pest of poultry houses, museums, silkworm culture, and many stored foods. The Hypocreales, *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metschnikoff), and *Isaria fumosorosea* Wize, were tested for efficacy against *D. maculatus* larvae on concrete, plastic, leather, and wood surfaces. Only wood had a significant negative effect on efficacy, and *B. bassiana* was the most efficacious species. The conidia of all three species lost viability quickly on wood with various responses on the other surfaces. When beetle larvae were exposed to deposited *B. bassiana* and incubated at 43, 56, 75, or 82% relative humidity, mortality was greatest at the lowest humidity suggesting enhancement of fungal infection by desiccation stress. The results indicate that entomopathogenic Hypocreales, especially *B. bassiana*, may be useful for control of hide beetles when applied at a low dose to surfaces that do not impact the viability of conidia.

Keywords: *Dermestes maculatus*; *Beauveria bassiana*; surfaces; relative humidity

Introduction

Hide beetles, *Dermestes maculatus* De Geer (Coleoptera: Dermestidae), feed on carrion and dry animal products. They are important pests of poultry houses, silkworm culture, museums, and a variety of stored foods (Hinton 1945). In spite of their importance, there are remarkably few published reports related to their control by chemical (Dyte, Ellis, and Lloyd 1965; Lloyd and Dyte 1965; Cloud and Collison 1985; Su and Sheffrahn 1990) or physical (Geden and Carlson 2001; Kaufman, Reasor, Murray, Waldron, and Rutz 2005) means, and there is only one report on the use of a biological agent (Geden and Steinkraus 2003). In the latter case, *Beauveria bassiana* (Balsamo) Vuillemin in three formulations was applied to poultry houses with statistically significant short-term results. Thus, there is a need for further evaluation of biological control agents.

The Hypocreales, *Metarhizium anisopliae* (Metschnikoff), *Isaria fumosorosea* Wize, and *B. bassiana*, are well-established biological control agents. Among fungi, only these species have been investigated as possible microbial controls for stored-product pests. They were selected for this study because they all have mycoinsecticide

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registrations in many countries. Their host ranges are broad, but their potency for the various stored-products target insects varies greatly. Some of the most important pests such as the red flour beetle, *Tribolium castaneum* Herbst, are very tolerant of fungi (Akbar, Lord, Nechols, and Howard 2004; Vassilakos, Athanassiou, Kavalfieratos, and Vayias 2006) while others such as *Rhyzopertha dominica* (Fabricius) (Lord 2005) and *Oryzaephilus surinamensis* (L.) (Throne and Lord 2004) are quite susceptible to fungi, especially *Beauveria bassiana*. Therefore separate screening is needed for each target pest.

Although the conidia of these species are generally considered to be long-lived propagules, it is clear that longevity and efficacy are affected by moisture and contact surfaces. Although moisture is generally considered to be an enhancer of fungal efficacy, several stored-product insects have shown increased susceptibility to *B. bassiana* when exposed to desiccation stress (Lord 2005, 2007a,b). In preliminary tests of fungi against the hide beetle, there was near 100% mortality when atmospheric moisture was near saturation and allowed fungal proliferation, but in subsequent assays with similar doses and more realistic relative humidities (RH) for stored products environments, the mortality was much lower. Therefore humidity effects were assessed in a systematic manner in this study.

Stored-products insects and the materials that are used to control them come into contact with a variety of surfaces such as the concrete of grain bin floors and wood planks in many warehouses. If the surfaces are deleterious to the survival of conidia then the residual activity of mycoinsecticides will be limited. Little attention has been paid to the effect of contact substrates other than soil and leaf surfaces on the longevity of conidia or fungal efficacy. Steinkraus, Geden, and Rutz (1991) reported that mortality of the lesser mealworm, *Alphitobius diaperinus* (Panzer) was greater when *B. bassiana* was applied to polystyrene than on wood. There have been several studies of the persistence of *B. bassiana* in soil and on plant surfaces, but because of the difficulty of quantitatively recovering conidia, the response variable is generally colony-forming units (Inglis, Johnson, and Goettel 1997; Vanninen, Tyni-Juslin, and Hokkanen 2000; Scocco, Gardner, and Shapiro-Ilan 2007), which provide relatively crude estimates of viability loss. Lord (2005) and Magalhães, Lord, Daoust, and Roberts (1987) assessed the persistence of *B. bassiana* conidia on surfaces of plant materials by germination rates. Lord (2005) reported that germination rates declined more rapidly at higher RHs and when conidia were mixed with wheat than when incubated on glass. Magalhães et al. (1987) found no effect of bean, *Phaseolus vulgaris* L., leaves or tubers of the cucurbit, *Ceratosanthes hilariana* Cogniaux, on conidial germination 4 days after deposition.

The purpose of this study was to assess the potential of established mycoinsecticides for development as control agents for *D. maculatus* using frequently encountered substrates and humidities.

Materials and methods

Insects and fungi

Dermestes maculatus larvae were from a culture that was established in 2009 from an infestation at a western Missouri dog food plant. They were maintained on a diet of

ground Purina rice and lamb dog food (Purina, Gray Summit, MO) with rolled oats. Larvae weighing from 5 to 10 mg and 14–18 days of age were used in assays.

The *B. bassiana* was commercially-produced, unformulated conidia of strain GHA (Laverlam, Butte, MT) (ARSEF 6444) that contained 9.4×10^{10} conidia/g. Conidia of *M. anisopliae* strain ESC1 (ATCC 62176), which was registered by EcoScience Corp for control of cockroaches and termites (EPA Registration 129056), and *I. fumosorosea*, strain PFR612 (ARSEF 5462) were produced on potato dextrose agar (PDA) and collected by washing the cultures with 0.2% Tween 80. PFR612 is not registered but was selected for its superior ability to produce collectable conidia relative to most isolates.

The concentrations of suspensions were determined with a hemacytometer and adjusted to final concentrations with 0.2% Tween 80. To confirm viability, the conidia were spread on PDA with a cotton swab, incubated for 18 h at 26°C, and examined microscopically for the presence of germ tubes. Germination rates were at least 88% throughout the study.

Substrate and fungal species bioassays

The three fungus species were compared in two-dose assays with four substrates. One-tenth millilitre of conidial suspensions of 2×10^6 or 2×10^7 conidia/mL in 0.2% Tween or conidia-free Tween 80 solution were placed in 2 cm³ wells of polystyrene assay trays (C-D International, Pitman, NJ) on disks of the substrates that covered the wells bases or directly on the well base that served as the plastic substrate. The doses were 1.5×10^3 , 1.5×10^4 and 0 conidia/mm². The substrates in addition to tray wells were Rockite concrete patching material (Hartline Products, Cleveland, OH), goat leather, and unfinished grade B pine plywood. The conidial suspensions were dried at 30–33°C for ca. 2 h; then beetle larvae were placed individually in wells with a single rolled oat as diet to carry the insects through the assay. The assay trays were covered with perforated adhesive lids. They were incubated at 30°C and 75% RH in darkness for 9 days, after which mortality was evaluated by reaction to probing. Each replicate unit consisted of a 16-well group. The experiment was carried out four times, each iteration being a replicate.

Humidity assay

After determining that *B. bassiana* (GHA strain) was the most efficacious of the tested fungi, it was used to assess the effects of humidity on efficacy. The humidity effects assay was conducted in the same manner as the substrate and fungus species assays except that the dose was increased to 5×10^7 conidia/mL (5×10^6 per well). The humidity for incubation was maintained by the use of plastic storage containers with saturated salt solutions (Greenspan 1977). All assays were incubated at 30°C with 43% RH (2.42 kPa vapor pressure deficit, VPD), 56% RH (1.87 kPa VPD), 75% RH (1.06 kPa VPD), or 82% (0.76 kPa VPD) maintained by saturated K₂CO₃, NaBr, NaCl, and KCl, respectively. The humidities were confirmed with BK Precision 625 (BK Precision, Yorba Linda, CA) and Vaisala HMI41 (Vaisala Oyj, Helsinki) hygrometers. There were six temporal replicates of 16 larvae.

Conidia survival

The effect of substrate on conidia longevity was tested for all three fungi. Conidia were collected and prepared as for the beetle bioassays, and 0.1 mL was placed on 13 mm diameter disks of the four substrates that were used in the beetle assays. In addition, 20 mL borosilicate scintillation vials were used for inert-substrate controls. The liquid was evaporated with light air flow in an incubator at 30°C and 30–40% RH, and the conidia were incubated at 30°C and 75% RH. The conidia on three replicates of each substrate were removed every 24 h by vortexing in 0.2% Tween 80 and plated on PDA with 0.5 g/L chloramphenicol and 0.01% benomyl to allow for a 48 h interval of incubation at 26°C to maximize germination. Germination, as the presence of a germ tube, was assessed from 100 randomly selected conidia/replicate. The experiment was carried out twice with three replicates in each test. The tests did not differ significantly, so the data were merged for analysis.

Statistical analysis

Mortality data were adjusted with Abbott's formula (Abbott 1925). The data for the high dose of 2×10^6 conidia/insect were subjected to two-way ANOVA after arcsine transformation. The variances of data for the low dose were not equal by Levene's test, and those data were not statistically analyzed. Data for RH assays were normally distributed and were not transformed prior to ANOVA. Fisher LSD was used for post hoc means comparisons. Analyses of insect bioassays were done with SigmaStat 3.1 (Systat, Point Richmond, CA). Conidia longevity data were analyzed with the GLIMMIX procedure of SAS (SAS Institute, Cary, NC).

Results

Substrate and fungal species assays

There were significant high dose effects of both the three fungus species ($F_{2,36} = 11.26$, $P < 0.001$) and the substrates ($F_{3,36} = 15.40$, $P < 0.001$; Figure 1), but there was no significant interaction ($F_{6,36} = 0.76$, $P = 0.61$). The mortality of larvae treated with *B. bassiana* was significantly greater than the mortality for larvae treated with *M. anisopliae*, which was significantly greater than that for *I. fumosorosea*-treated larvae. The only significant difference among substrates was lower mortality when the conidia were placed on wood. Control mortality averaged 3.1% and did not exceed 12.5%.

Relative humidity effects

Relative humidity significantly affected mortality ($F_{3,20} = 4.30$, $P = 0.017$; Figure 2). The mortality with the low RH of 43% was greater than that for any other of the RHs. The average control mortality was 4.7% with a maximum of 18.7%.

Conidia survival

The germination rates of all three fungi were reduced to below 50% after 48 h of incubation on all of the substrates (Figure 3). Therefore the 24 h germination was

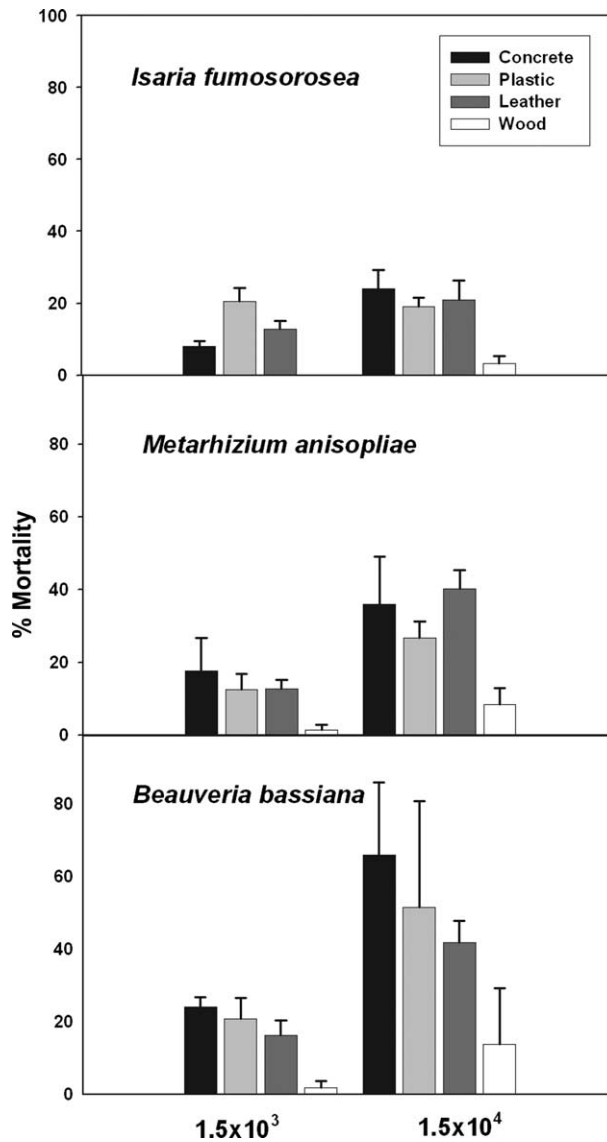


Figure 1. Percentage mortality of *Dermestes maculatus* larvae after exposure to four surfaces that were contaminated with conidia of three fungi at concentrations of 1.5×10^3 and 1.5×10^4 conidia/mm² ($\pm$ SE).

used for comparisons. The 24 h germination of *I. fumosorosea* was significantly greater on concrete than all other substrates except for glass ($F_{4,25} = 3.12$, $P = 0.033$). Similarly, *B. bassiana* germination after 24 h of incubation was greater with concrete than with other substrates except glass and plastic ($F_{4,25} = 8.41$, $P < 0.001$). In contrast, there was significantly greater germination of *M. anisopliae* conidia within 24 h on glass than on all other substrates except leather ($F_{4,25} = 10.79$, $P < 0.001$). Wood clearly had a detrimental effect on the conidia of all species, with germination at 24 h reduced to 0 for *M. anisopliae* and *I. fumosorosea* and 4.7% for *B. bassiana*.

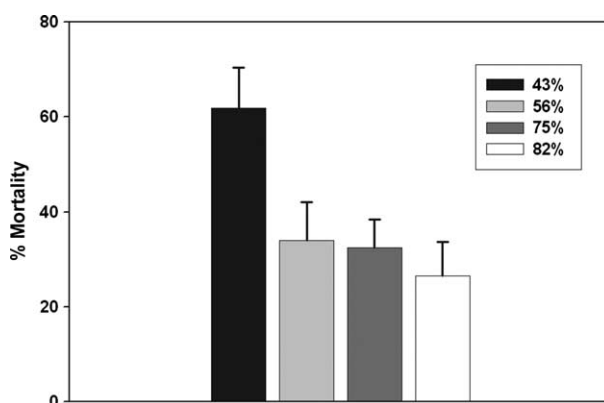


Figure 2. Percentage mortality of *Dermestes maculatus* larvae after exposure to *Beauveria bassiana* at four relative humidities.

Discussion

The results indicate that entomopathogenic Hypocreales, especially *B. bassiana*, may be useful for control of hide beetles when applied at a low dose to surfaces that do not impact the viability of conidia. All three of the fungi tested were pathogenic for *D. maculatus* with *B. bassiana* being the most efficacious. The relatively low mortalities are probably more reflective of a modest challenge than of low pathogenicity. The application was indirect and the doses were low in order to easily detect differences in effect. Furthermore, the residual activity was diminished by time spent in suspension and accelerated loss of conidial viability due to incubation at the relatively high RH of 75%.

While the fungal species used in this study have been assayed for efficacy with numerous specific target pests other than hide beetles, there is only one previous report (Steinkraus et al. 1991) on the effects of substrates other than plant surfaces and soil on the efficacy and none on the survival of their conidia. In concordance with the findings of this study, Steinkraus et al. (1991) found that *B. bassiana* efficacy for *A. diaperinus* was lower on wood than on plastic. It is not surprising that, of the substrates that were tested, wood was the most deleterious to both conidial survival and efficacy. Woody plants must defend themselves against pathogenic fungi and produce a variety of antimicrobial compounds (Iriti and Faoro 2009). Interestingly, Kalsbeek, Pell, and Steenberg (2001) found that the infectivity of conidia of the zygomycete, *Entomophthora schizophorae* Keller and Wilding, persisted longer on pine and wheat than on glass, but the conidia of this species are quite different from those of this study.

A similar apparent anomaly occurred in this study with leather, which was used to approximate carrion hide and which might be expected to harbor antimicrobial compounds. There was no significant difference in the germination rates of any of the three fungi after 24 h of incubation on leather or glass. Perhaps any antimicrobial activity of hides is compromised by the tanning process. Overall, the brevity of conidial persistence was probably due to the fact that the conidia were suspended in Tween 80 solution prior to incubation and thus hydrated. Furthermore, 75% RH is not conducive to the longevity of conidia (Daoust and Roberts 1983; Wraight,

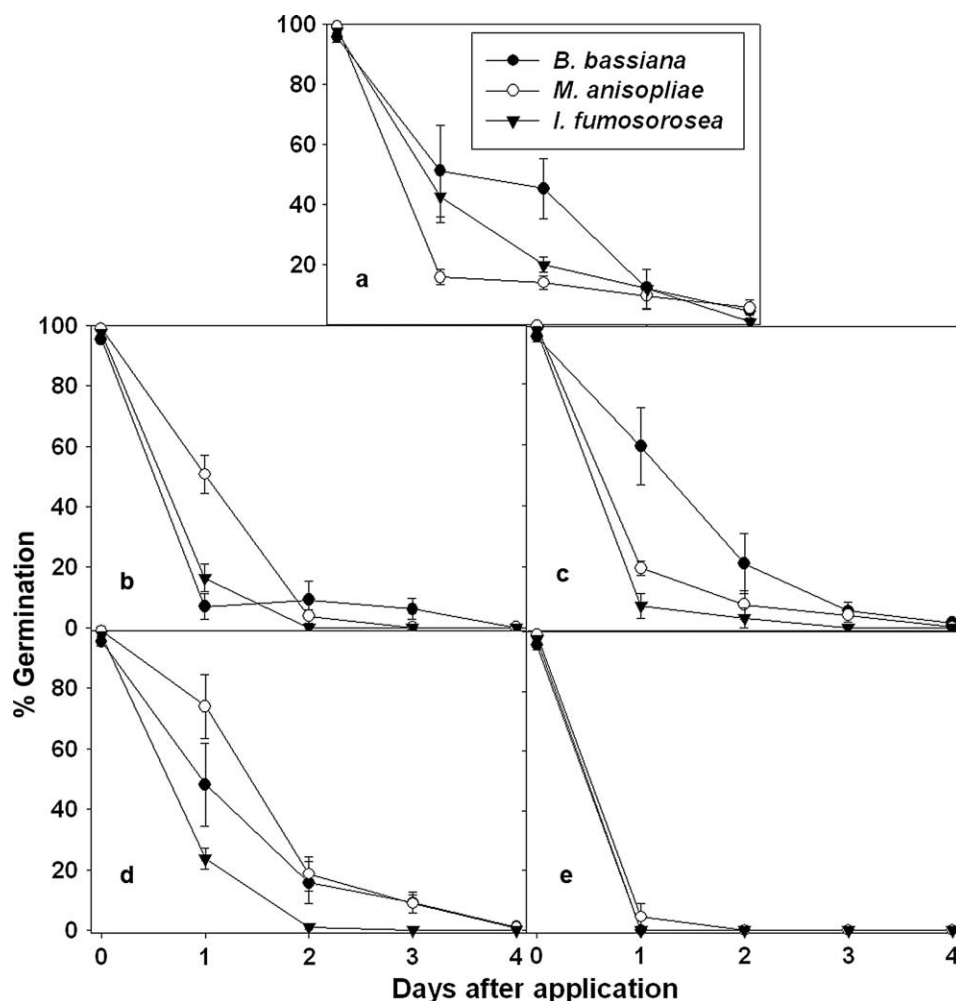


Figure 3. Percentage germination of conidia incubated on five substrates: (a) concrete, (b) leather, (c) plastic, (d) glass, and (e) wood.

Jackson, and de Kock 2001; Hong, Edgington, Ellis, de Muro, and Moore 2005; Lord 2005). Both of these conditions are probably reflective of operational conditions of mycoinsecticide use, in which formulated conidia are suspended in aqueous tank mixes with wetting agents for application and the target insects often occupy relatively moist microclimates.

The conidial preparations used in this study are of two origins and ages. The *B. bassiana* conidia were commercially produced on grain kernels. They have been stored under refrigeration since 1998 but have retained good viability and ability to survive on a variety of substrates. The other two fungi were cultured on PDA and harvested on experiment initiation days. That the 12-year-old, slower germinating conidia of *B. bassiana* killed significantly more larvae than the fresh conidia of the other two species strengthens the case that it has greater potential for hide beetle control than the others.

The results of the bioassays of *B. bassiana* under various humidity regimes extend our previous finding with other target insects. There is a long-standing belief that mycoinsecticides are only efficacious under conditions of high ambient moisture. Indeed, Brower, Smith, Vail, and Flinn (1995) state that fungi have not been developed as microbial control agents of stored-product pests 'because of their dependency upon high ambient humidities'. We have previously found that desiccation stress enhances the susceptibility of some stored-product insect to *B. bassiana* while others do not appear to be so affected (Akbar et al. 2004; Lord 2005, 2007a,b). The results here demonstrate that hide beetle larvae fall into the former category. Stress due to suboptimal ambient moisture induces a variety of poorly understood physiological changes that may be involved in increased susceptibility to fungus. These may include changes in the cuticular chemistry that affect the ability of conidia to attach, germinate, and penetrate, compromise cellular and humoral defenses, and perhaps even altered behavior in a manner that favors fungal infection. Insect mycology might benefit greatly from further studies of fungal infection of desiccation-stressed insects other than those that are associated with stored products.

Acknowledgements

I am grateful to Kris Hartzler for meticulous technical assistance. The statistical analysis by Dr. Mark West is greatly appreciated. I thank Dr. Drauzio Rangel 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 U.S. 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., and 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.
- Brower, J.H., Smith, L., Vail, P.V., and Flinn, P.W. (1995), 'Biological Control', in *Integrated Management of Insects in Stored Products*, eds. Bh. Subramanyam and D.W. Hagstrum, New York: Marcel Dekker, pp. 223–286.
- Cloud, J.A., and Collison, C.H. (1985), 'Laboratory Evaluation of Insecticides for Control of Adult and Larval Hide Beetles, *Dermestes maculatus* DeGeer, from Poultry Houses', *Journal of Agricultural Entomology*, 2, 297–308.
- Daoust, R.A., and Roberts, D.W. (1983), 'Studies on the Prolonged Storage of *Metarhizium anisopliae* Conidia: Effect of Temperature and Relative Humidity on Conidial Viability and Virulence against Mosquitoes', *Journal of Invertebrate Pathology*, 41, 143–150.
- Dyte, C.E., Ellis, V.J., and Lloyd, C.J. (1965), 'Studies on the Contrasting Susceptibilities of The Larvae of Two Hide Beetles (*Dermestes* Spp.; Coleoptera, Dermestidae) to Malathion', *Journal of Stored Products Research*, 1, 223–224.
- Geden, C.J., and Carlson, D.A. (2001), 'Mechanical Barrier for Preventing Climbing by Lesser Mealworm (Coleoptera: Tenebrionidae) and Hide Beetle (Coleoptera: Dermestidae) Larvae in Poultry Houses', *Journal of Economic Entomology*, 94, 1610–1616.
- Geden, C.J., and Steinkraus, D.C. (2003), 'Evaluation of Three Formulations of *Beauveria bassiana* for Control of Lesser Mealworm and Hide Beetle in Georgia Poultry Houses', *Journal of Economic Entomology*, 96, 1602–1607.
- Greenspan, L. (1977), 'Humidity Fixed Points of Binary Saturated Aqueous Solutions', *Journal of Research of the National Bureau of Standards Section A-Physics and Chemistry*, 81, 89–96.

- Hinton, H.E. (1945), *A Monograph of the Beetles Associated with Stored Products*, London: British Museum (Natural History).
- Hong, T.D., Edgington, S., Ellis, R.H., de Muro, M.A., and 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.
- Inglis, G.D., Johnson, D.L., and Goettel, M.S. (1997), 'Effects of Temperature and Sunlight on Mycosis (*Beauveria bassiana*) (Hyphomycetes: Sympodulosporae) of Grasshoppers under Field Conditions', *Environmental Entomology*, 26, 400–409.
- Iriti, M., and Faoro, F. (2009), 'Chemical Diversity and Defense Metabolism: How Plants Cope with Pathogens and Ozone Pollution', *International Journal of Molecular Sciences*, 10, 3371–3399.
- Kalsbeek, V., Pell, J.K., and Steenberg, T. (2001), 'Sporulation by *Entomophthora schizophorae* (Zygomycetes: Entomophthorales) from Housefly Cadavers and the Persistence of Primary Conidia at Constant Temperatures and Relative Humidities', *Journal of Invertebrate Pathology*, 77, 149–157.
- Kaufman, P.E., Reasor, C., Murray, K.D., Waldron, J.K., and Rutz, D.A. (2005), 'Evaluation of a Barrier to Inhibit Lesser Mealworm (Coleoptera: Tenebrionidae) and Dermestidae Movement in High-rise, Caged-layer Poultry Facilities', *Journal of Economic Entomology*, 98, 1744–1749.
- Lloyd, C.J., and Dyte, C.E. (1965), 'The Susceptibility of Larvae of *Dermestes* L. (Coleoptera, Dermestidae) to Some Contact Insecticides', *Journal of Stored Products Research*, 1, 159–167.
- Lord, J.C. (2005), 'Low Humidity, Moderate Temperature, and Desiccant Dust Favor Efficacy of *Beauveria bassiana* (Hyphomycetes: Moniliales) for the Lesser Grain Borer, *Rhyzopertha dominica* (Coleoptera: Bruchidae)', *Biological Control*, 34, 180–186.
- Lord, J.C. (2007a), 'Desiccation Increases the Efficacy of *Beauveria bassiana* for Stored Grain Pest Insects', *Journal of Stored Products Research*, 43, 535–539.
- Lord, J.C. (2007b), 'Enhanced Efficacy of *Beauveria bassiana* for the Red Flour Beetle, *Tribolium castaneum*, with Reduced Moisture', *Journal of Economic Entomology*, 100, 171–175.
- Magalhães, B.P., Lord, J.C., Daoust, R.A., and Roberts, D.W. (1987), '[Effect of *Beauveria bassiana* on *Cerotoma arcuata* (Oliver, 1791) (Coleoptera: Chrysomelidae) after Exposure to the Tubers of *Taiuia* (*Ceratosantes hilariana*)]', *Anais da Sociedade Entomologica do Brasil*, 15, 327–331.
- Scocco, E.A., Gardner, W.A., and Shapiro-Ilan, D.I. (2007), 'Microscopic Evaluation of the Fate of Conidia of Two Entomogenous Fungi in Soil', *Journal of Entomological Science*, 42, 413–414.
- Steinkraus, D.C., Geden, C.J., and Rutz, D.A. (1991), 'Susceptibility of Lesser Mealworm (Coleoptera: Tenebrionidae) to *Beauveria bassiana* (Moniliales: Moniliaceae): Effect of Host Stage, Substrate, Formulation, and Host Passage', *Journal of Economic Entomology*, 28, 314–321.
- Su, N.Y., and Sheffrahn, R.H. (1990), 'Efficacy of Sulfuryl Fluoride against Four Beetle Pests of Museums (Coleoptera: Dermestidae, Anobiidae)', *Journal of Economic Entomology*, 83, 879–882.
- Throne, J.E., and Lord, J.C. (2004), 'Control of Sawtoothed Grain Beetles (Coleoptera: Silvanidae) in Stored Oats Using an Entomopathogenic Fungus in Conjunction with Seed Resistance', *Journal of Economic Entomology*, 97, 1765–1771.
- Vanninen, I., Tyni-Juslin, J., and Hokkanen, H. (2000), 'Persistence of Augmented *Metarhizium anisopliae* and *Beauveria bassiana* in Finnish Agricultural Soils', *BioControl*, 45, 201–222.
- Vassilakos, T.N., Athanassiou, C.G., Kavalieratos, N.G., and Vayias, B.J. (2006), 'Influence of Temperature on the Insecticidal Effect of *Beauveria bassiana* in Combination with Diatomaceous Earth against *Rhyzopertha dominica* and *Sitophilus oryzae* on Stored Wheat', *Biological Control*, 38, 270–281.
- Wraight, S.P., Jackson, M.A., and de Kock, S.L. (2001), 'Production, Stabilization and Formulation of Fungal Biocontrol Agents', in *Fungi as Biocontrol Agents*, eds. T.M. Butt, C. Jackson and N. Magan, Silwood, UK: CAB International.