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Control of rice weevil (*Sitophilus oryzae* L., Coleoptera: Curculionidae) with various formulations of *Metarhizium anisopliae*

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Abstract

Formulations of *Metarhizium anisopliae* conidia with oven ash, chalk powder, charcoal and wheat flour at a ratio of 1:4 (W/W) were prepared. Formulations containing charcoal and oven ash had a conidial viability half-life of 4.1–4.3 months at $20 \pm 1°C$. In comparison, unformulated conidia held under the same conditions had a viability half-life of 0.9 month. Bioassays carried out at $28 \pm 2°C$ and $70 \pm 5\%$ RH indicated that the treatments with charcoal and oven ash formulations at a rate of 2.0% or 2.8 mg/cm^2 of treated area resulted in 73.3–86.7% mortality of adult *Sitophilus oryzae* after 7 days when treatments were applied before or after pest infestations. Mortality in F1 adults was 28.9–47.5% when exposed, upon emergence, to the same formulations previously applied against their parents. Treatments applied before pest infestation with charcoal or oven ash formulation reduced damage rates to wheat grains to 0.5% compared to the control (6.0%). Development time of *S. oryzae* was prolonged 4–8 days when the grains were treated before pest infestation with fungal formulations in charcoal or oven ash. A synergistic effect between the fungus and the most effective dusts, charcoal or oven ash, for the "after pest infestation" treatments was obtained during bioassays for evaluation of *S. oryzae* adult mortality. Additional experiments are required to explain the mechanism of this synergism.

Keywords: Wheat grains; *Sitophilus oryzae*; *Metarhizium anisopliae*; Fungal formulation; Dust carriers

1. Introduction

Rice weevils (*Sitophilus oryzae*) are considered a primary stored-grain insects in warm climate areas. They cause significant losses to stored grains, especially cereals, at conditions favorable to their development (25–35°C and low RH). Integrated management of these insects is considered the most effective approach for their control. Potential for the use of entomopathogenic fungi to control *S. oryzae* and other stored grain insects has been recently reported (Padin et al., 1996, 1997, 2002; Rice and Cogburn, 1999; Dal-Bello et al., 2001; Stathers et al., 2002; Wakefield et al., 2002). *Beauveria bassiana* has proven highly effective against the major stored-grain insects: *S. oryzae*, *Rhyzopertha dominica*, and *Tribolium castaneum* (Padin et al., 1996, 1997, 2002; Rice and Cogburn, 1999; Dal-Bello et al., 2001). *Metarhizium anisopliae* is less frequently reported for the control of stored-grain insects although it has been used to actively control other insect species infestations (Prior and Greathead, 1989; Rath, 1992; Zimmermann, 1993; Quarles, 1995; Milner et al., 1996; Lomer et al., 1997; Ludwig and Oetting, 1998; Batta, 2003). Mixtures of *M. anisopliae* conidial suspensions with that of *B. bassiana* has been used against *S. oryzae* on wheat grains (Dal-Bello et al., 2001). However, other investigators have demonstrated *M. anisopliae*'s lack of efficacy for *S. oryzae* or other stored-grain insects when used alone (Padin et al., 1996; Moino et al., 1998). The use of diatomaceous earth as an effective dust for suppressing several stored-grain beetles especially red flour beetles, *T. castaneum*, has been studied (Subramanyan et al., 1994; Quarles and Winn, 1996; Dowdy, 1999). However, enhancing the effectiveness of *M. anisopliae* for *S. oryzae* or other stored-grain insects by combining its

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conidia with various types of dusts has not been investigated. The objectives of this research were: (i) to assess viability of the *M. anisopliae* conidia over time in prepared fungal formulations with four types of dust carriers and (ii) to assess the efficacy of prepared fungal formulations for the control of rice weevils and the damage they cause to stored wheat grains.

2. Materials and methods

2.1. Insect rearing

Adults of *S. oryzae* (strain RW1) collected from infested stored wheat grains were reared on healthy wheat grains (CV: Anbar) held in cloth mesh covered plastic pots (15 cm diameter by 20 cm high) at $28\pm2^{\circ}C$, $70\pm5\%$ RH, and 16:8 L:D cycle. Newly emerged adults (males and females) were used in the experiments.

2.2. Fungal formulations

Five fungal formulations were prepared using strain Meta. 1 of *M. anisopliae* (obtained from the Galillee Regional Research and Development Center in Eilapoun, Israel). Each formulation contained fungal conidia and a dust carrier at 1:4 ratio (W/W). The dust carriers that were obtained from local sources are wheat flour (milled durum wheat grains of CV: Anbar), oven ash (completely burned paper sheets), chalk powder (finely ground board chalks) and charcoal (finely ground coal pieces). Comparative treatments were unformulated conidia and fungus-free dust carriers. Conidia harvested from 14-day-old cultures of *M. anisopliae* grown on oat meal agar medium (OMA) plates were thoroughly mixed with the carrier in screw capped bottles. The formulations contained 5.2×10^{8} conidia/g determined by dilution of 1 g of the formulation in 50 ml of sterile distilled water held in screw-capped vials followed by vigorous shaking before counting by hemacytometer. The same stock fungal conidia were used in preparing the formulations to ensure that no differences were present among the individual formulations. The initial water content measured on a dry weight basis was 6% for unformulated conidia and 3–4% for the formulations, according to the type of dust carrier.

2.3. Conidial viability

Suspensions of 0.5% (W/W) of formulations or 0.1% (W/W) of unformulated conidia in sterile distilled water were prepared and then diluted 100-fold. Twenty-five microliters of diluted suspensions were spread onto the surface of glass slides held in Petri dishes under humid conditions and scored for germination after 24 h of incubation at $25\pm2^{\circ}C$. No wetting agent or agar medium for coating the slides was used in this test. The assessment of conidial viability was determined each week for a 22-week period. The formulated and unformulated conidia were stored during the study in tightly closed screw capped bottles under a constant temperature of $20\pm1^{\circ}C$. This was to exclude the effect of variable environmental conditions on conidial viability during the assessment. Three replicates representing three glass slides per formulated or unformulated conidia were used and three counts were performed on each. The mean percent conidial germination was calculated. Log-linear regression analyses of viability (% conidial germination) versus time were performed to determine the viability half-life of each formulated and unformulated conidia treatment.

2.4. Bioassays

The application of fungal treatments was carried out by adding 0.2 g of each formulation containing 1.04×10^{8} conidia to 200 wheat grains (mean weight = 10.02 g) held in a small, cloth-mesh covered plastic can (9.5 cm diameter, height 5 cm and area 70.85 cm^2). This resulted in an application rate of 2.8 mg/cm^2 of treated area or 2.0% (W/W of formulation to wheat grains). As control treatments, 0.16 g of each fungus-free dust and 0.04 g of unformulated conidia were applied versus 0.2 g of prepared formulation. Ten newly emerged adults (five males and five females) of *S. oryzae* were introduced using a small brush into each replicate can. Infested cans were then incubated at $28\pm2^{\circ}C$, $70\pm5\%$ RH, and 16:8 L:D cycle. Three replicate cans per treatment and 1–2-week-old preparations of formulated and unformulated conidia were used in all tests.

2.4.1. Adult insect mortality and wheat grain damage

To assess efficacy against *S. oryzae*, treatments were applied either before or after pest infestations. Accordingly, introduction of insects was done either on the same day of the treatment or 14 days before the treatment. The 14-day period was sufficient for the insect to cause damage to the grains. Dead and living adults were counted 7 days after the treatment and used to the percentage of adult mortality. The damage rate to wheat grains by the insect was assessed with the treatments applied before pest infestation. The number of damaged grains was counted 21 days after the treatment and percentage of damage was calculated for each replicate can.

2.4.2. Development time and mortality of F1 adults

To assess treatment effects on subsequent *S. oryzae* development time, all adults surviving treatment were removed from cans 14 days following treatment. The 14-day period was sufficient for egg deposition and subsequent hatching. All replicate cans were then incubated as indicated above until peak emergence of F1 adults. Development time was defined as the time period from egg deposition to peak emergence of F1 adults. The effect of the previously applied treatments on the mortality of emerged F1 adults was also assessed. Dead and living F1 adults were counted 7 days after treatment and peak emergence, and the percentage of adult mortality was calculated.

2.5. Statistical analyses

Means of percentage of adult mortality, percentage of damaged grains, and development time of the insect were statistically analyzed using ANOVA followed by Tukey-HSD test.

3. Results

3.1. Conidial viability

Mean percentage of conidial germination of *M. anisopliae* decreased over a 22-week period of regular observation for all tested formulations (Fig. 1). The log-linear regression analyses of the mean percent conidial germination versus time indicate that the time period for 50% conidial germination were 17.7, 18.6, 12.4, 6.0, and 3.7 weeks for oven ash + conidia, charcoal + conidia, chalk powder + conidia, wheat flour + conidia, and unformulated conidia, respectively (the half-life time was calculated from regression equations in Fig. 1). It is concluded that the time periods for 50% reduction in conidial viability was the longest for conidia formulated in oven ash or charcoal and the shortest for the unformulated conidia.

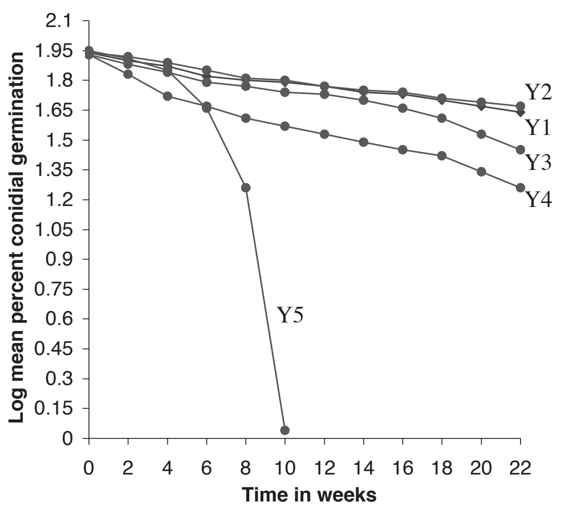

Fig. 1. Log mean percent conidial germination on time of the formulated conidia of *M. anisopliae* (strain Meta 1) in oven ash ($Y1$), charcoal ($Y2$), chalk powder ($Y3$), wheat flour ($Y4$), and unformulated conidia ($Y5$). Log-linear regression equations for the above relationships are: $Y1 = 1.911935 - 0.012017$ Time, $r^2 = 0.98$; $Y2 = 1.931427 - 0.012491$ Time, $r^2 = 0.99$; $Y3 = 1.915121 - 0.017480$ Time, $r^2 = 0.96$; $Y4 = 1.854614 - 0.026027$ Time, $r^2 = 0.97$; $Y5 = 2.281319 - 0.157289$ Time, $r^2 = 0.73$.

3.2. Effect of fungal treatments on adult insect mortality and wheat grain damage

Treatments with conidia formulated in charcoal or oven ash and applied either before or after pest infestations resulted in the highest adult mortalities of *S. oryzae* compared to other types of formulations and ash alone treatments (Table 1). Mean mortalities were 73.3% and 80.0% when treatments with the two formulations were applied before pest infestation, and 73.3% and 86.7% when treatments were applied after pest infestation. These mortalities were significantly higher (at $P<0.05$) compared with the other formulations and ash alone treatments when applied after pest infestations, but not significantly higher when applied before pest infestation (Table 1). This indicates that a synergistic effect was obtained for the first case, but mere additivity for the second case. Treatments with the conidial formulations in charcoal and oven ash resulted in the lowest damage rates to grains by *S. oryzae* when applied before pest infestation (0.5% for both formulations) (Table 2). Significant differences (at $P<0.05$) were obtained when the treatments with the two formulations were compared to treatments with wheat flour only, wheat flour + conidia or chalk powder only, but not with charcoal or oven ash alone treatments (Table 2). This indicates an additive effect of the treatment. It is important to mention that adult mortalities of

Table 1
Effect of fungal treatment applied either before or after pest infestations on the mortality of *S. oryzae* adults on wheat grains (CV: Anbar) 7 days after treatment (28 ± 2°C, 70 ± 5% RH, and 16:8 L:D cycle)

Treatments	Mean percent adult mortality (±SEM)[a]	
	Treatment before pest infestation	Treatment after pest infestation
Wheat flour only	13.3 ± 4.3a	6.7 ± 2.6a
Chalk powder only	26.7 ± 6.9ab	13.3 ± 4.2ab
Charcoal only	46.7 ± 9.2ab	33.3 ± 7.5abc
Oven ash only	46.7 ± 8.8ab	40.0 ± 8.1bc
Wheat flour + conidia	33.3 ± 7.8ab	40.0 ± 8.4bc
Chalk powder + conidia	46.7 ± 8.9ab	46.7 ± 9.1bc
Charcoal + conidia	73.3 ± 7.7b	73.3 ± 7.6bcd
Oven ash + conidia	80.0 ± 9.3b	86.7 ± 10.8bcde
Unformulated conidia	26.7 ± 7.0ab	26.7 ± 6.8abc

[a] Means of percent adult mortality within the column followed by different letters are significantly different at $P<0.05$ according to Tukey-HSD test.

Table 2
Effect of fungal treatment applied before pest infestation on damage caused by *S. oryzae* on wheat grains 21 days after treatment (28±2°C, 70±5% RH, and 16:8 L:D cycle)

Treatments	Mean percent damage to wheat grains (±SEM)[a]
Wheat flour only	6.0±1.4bc
Chalk powder only	3.3±0.5b
Charcoal only	1.2±0.4ab
Oven ash only	1.2±0.3ab
Wheat flour+conidia	4.2±1.5bc
Chalk powder+conidia	2.5±0.7ab
Charcoal+conidia	0.5±0.1a
Oven ash+conidia	0.5±0.2a
Unformulated conidia	2.3±0.9ab

[a] Means of percent damage to grains within the column followed by different letters are significantly different at $P<0.05$ according to Tukey-HSD test.

Table 3
Effect of fungal treatment applied before pest infestation on development time of *S. oryzae* on wheat grains at 28±2°C, 70±5% RH, and 16:8 L:D cycle

Treatments	Mean development time in days on wheat grains (±SEM)[a]
Wheat flour only	32.3±1.7a
Chalk powder only	34.3±1.6a
Charcoal only	37.0±1.1ab
Oven ash only	36.6±1.3ab
Wheat flour+conidia	33.0±1.8a
Chalk powder+conidia	35.3±1.7a
Charcoal+conidia	41.3±2.8ab
Oven ash+conidia	45.0±1.9b
Unformulated conidia	35.0±1.8a

[a] Development time was calculated from the infestation date (egg deposition) until the peak emergence of adults. Means of development time within the column followed by different letters are significantly different at $P<0.05$ according to Tukey-HSD test.

70.5±6.9% and 79.7±8.3% were obtained when 4-month-old preparations of conidial formulations in charcoal and oven ash were applied, respectively, against *S. oryzae* adults for the "before pest infestation" treatments. These mortalities are similar to that obtained in Table 1 when 1- to 2-week-old preparations of the same treatments (before pest infestation technique) were used.

3.3. *Effect of fungal treatments on the development time and mortality of F1 adults*

The development time of *S. oryzae* at 28±2°C and 70±5% RH increased from 36.6 days on wheat grains treated with oven ash alone to 45.0 days on the grains treated with oven ash+conidia (Table 3). This difference was significant (at $P<0.05$) when compared to the unformulated conidia or other ash alone treatments (Table 3). The highest mortality in F1 adults (47.5%) occurred with oven ash+conidia treatment followed by the mortality with charcoal+conidia treatment (28.9%) (Table 4). Adult mortality caused by the first type of treatment was significantly different (at $P<0.05$) from other treatments including that of oven ash alone. This indicates a synergistic effect of this treatment on F1-adult mortality. This effect was similar to that obtained by the same treatment when applied against the parents of F1-adults.

Table 4
Effect of fungal treatment applied after pest infestation on F1 adult mortality of *S. oryzae* on wheat grains 7 days after peak emergence (28±2°C, 70±5% RH, and 16:8 L:D cycle)

Treatments	Mean percent F1 adult mortality (±SEM)[a]
Wheat flour only	3.3±1.1a
Chalk powder only	5.6±2.4a
Charcoal only	16.2±1.4a
Oven ash only	17.3±1.5a
Wheat flour+conidia	7.9±2.1a
Chalk powder+conidia	14.8±1.7a
Charcoal+conidia	28.9±6.9ab
Oven ash+conidia	47.5±5.2b
Unformulated conidia	7.5±2.0a

[a] Means of percent F1 adult mortality within the column followed by different letters are significantly different at $P<0.05$ according to Tukey-HSD test.

4. Discussion

Results suggest that some of the dust carriers can prolong the shelf-life of *M. anisopliae* conidia, but further experiments should be conducted to prove this suggestion since no previous studies have been carried out in this respect. Depending on the initial water content of the conidia formulated with various dusts (3–4% on dry weight basis) or unformulated (6% on dry weight basis), some or all dusts used in the formulations may serve as slow desiccants. The results also suggest that the conidia with less water content, such as conidia combined with the dusts, were more stable than the unformulated conidia, given that the formulated and unformulated conidia used in the assays were obtained from a common lot of the same fungal culture. Although conidial viability of the fungus was reduced by 50% after 4.1–4.3 months with the most effective dusts (charcoal and oven ash), no significant losses in the fungal efficacy resulted as adult mortality of *S. oryzae* remained 70–80% when 4-month-old formulations were tested against the insect. This result indicates that the shelf life of the fungus was not affected during the time it was mixed with the two dusts. This is in agreement with the limits of permissible loss in conidial viability which are acceptable in less regulated countries and is reflected by a non-significant loss in fungal efficacy. The short half-life of unformulated *M. anisopliae* conidia (<1 month) obtained here was similar to that found by other investigators under similar storage conditions (Daoust et al., 1982, 1983; Daoust and Roberts, 1983; Burges, 1998).

Results obtained here show that adult mortality of *S. oryzae* was high. It ranged from 73.3% to 86.7% with *M. anisopliae* conidia formulated in charcoal and oven ash, and applied either before or after pest infestations. These results contrast with the results obtained by Dal-Bello et al. (2001), who reported that the treatment of *S. oryzae* on wheat grains with *M. anisopliae* alone was not effective, but produced 50% adult mortality of *S. oryzae* after 30 days of treatment with a conidial suspension mixture of *M. anisopliae* and *B. bassiana.* The treatment of *S. oryzae* with a conidial powder of *B. bassiana* alone resulted in >80% mortality of the adults reared on three types of food substrates for 21 days following the treatment (Rice and Cogburn, 1999). Also, the treatment of stored wheat grains with formulated *B. bassiana* in milled rice significantly reduced the total grain weight loss due to *S. oryzae* infestation during 4 months of storage (Padin et al., 2002). Comparing the above results with our results may indicate that different isolates of the same fungus can have different infectivity for *S. oryzae*. Thus, it is conceivable that our isolate of *M. anisopliae* is effective, but Dal-Bello's isolate is not equally effective or less effective. The same can be mentioned in comparing species of fungi such as *Metarhizium* vs. *Beauveria*.

Our results on treatment of *S. oryzae* with the dust carriers alone indicated moderate levels of adult mortality (33.3–46.7%) after 7 days of the treatment, the most effective dusts being charcoal and oven ash. Comparable mortality levels were obtained by certain investigators when they used diatomaceous earth dust for treatment of stored-grain beetles especially red flour beetles, *T. castaneum* (Subramanyan et al., 1994; Quarles and Winn, 1996; Dowdy, 1999). Higher adult mortalities of *T. castaneum* (up to 100%) were obtained when combination treatments of heat (50°C for 30 min.) with the most effective diatomaceous earth formulation were applied (Dowdy, 1999). Our combination treatments of *M. anisopliae* conidia with the most effective dusts of charcoal and oven ash resulted in comparably higher adult mortalities of *S. oryzae*. Synergistic effect between the two types of dusts and *M. anisopliae* for *S. oryzae* adult mortality (e.g. after pest infestation treatments) was statistically obtained in this work. This is in agreement with the synergism obtained by Padin et al. (2002) for *B. bassiana* and milled rice (as desiccant dust) against the stored-grain beetles.

In conclusion, overall results obtained here demonstrated that *M. anisopliae* was not effective for control of rice weevils on stored wheat grains when used alone as it did not cause more than 26.7% adult mortality in all bioassays even at high doses. Dust carriers significantly contributed to efficacy when combined with the fungus, so that additive or synergistic effect between them was produced. Additional experiments will be required to explain the mechanism by which this increased efficacy is produced. All of the mortalities among *S. oryzae* adults that were observed were taken at a 7-day incubation period. It should be interesting to see what would happen with a 10-, 20- or 30-day incubation as other studies especially on *B. bassiana* efficacy were carried out over an extended period. The residual effectiveness of our fungal formulations against *S. oryzae* on wheat grains was demonstrated by delayed development of the insect and mortality obtained in F1-adults. Integration of using fungal formulations in charcoal or oven ash into the insect management plans is possible after confirmation of the results at a large scale in stored grains facilities.

References

Batta, Y.A., 2003. Production and testing of novel formulations of the entomopathogenic fungus *Metarhizium anisopliae* (Metch.) Sorokin (Deuteromycotina: Hyphomycetes). Crop Protection 22 (2), 415–422.

Burges, D.H., 1998. Formulation of mycoinsecticides. In: Burges, D.H. (Ed.), Formulation of Microbial Pesticides. Kluwer, Dordrecht, pp. 132–185.

Dal-Bello, G., Padin, S., Lopez-Lastra, C., Fabrizio, M., 2001. Laboratory evaluation of chemical–biological control of the rice weevil (*Sitophilus oryzae* L.) in stored grains. Journal of Stored Products Research 37 (1), 77–84.

Daoust, R.A., Roberts, D.W., 1983. Studies on 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.

Daoust, R.A., Ward, M.G., Roberts, D.W., 1982. Effect of formulation on the virulence of *Metarhizium anisopliae* conidia against mosquito larva. Journal of Invertebrate Pathology 40, 228–236.

Daoust, R.A., Ward, M.G., Roberts, D.W., 1983. Effect of formulation on the viability of *Metarhizium anisopliae* conidia. Journal of Invertebrate Pathology 41, 151–160.

Dowdy, A.K., 1999. Mortality of red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae) exposed to high temperature and diatomaceous earth combinations. Journal of Stored Products Research 35, 175–182.

Lomer, G.J., Prior, C., Kooyman, C., 1997. Development of *Metarhizium* spp. for the control of grasshoppers and locusts. In: Goettel, M.S., Johnson, D.L. (Eds.), Microbial Control of Grasshoppers and Locusts, Vol. 171. Mem. Entomol. Soc., Canada, pp. 265–286.

Ludwig, S.W., Oetting, R.D., 1998. Control of thrips (*Frankliniella occidentalis*) and the fungus (*Bradysia* spp.) in potting media. SNA Research Conference Proceedings 43, 166–167.

Milner, R.J., Miller, L., Lutton, G.G., Driver, F., 1996. Biological control of black field cricket *Teleogryllus commodus* walker (Orthoptera: Gryllidae) using fungal pathogen *Metarhizium anisopliae* (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes). Plant Protection 11, 9–13.

Moino Jr., A., Alves, S.B., Pereira, R.M., 1998. Efficacy of *Beauveria bassiana* (Balsamo) Vuillemin isolates for control of stored-grain pests. Journal of Applied Entomology 122 (6), 301–305.

Padin, S.B., Bello, G.M., Vasicek, A.L., Dal-Bello, G., 1996. Bioinsecticide potential of entomopathogenic fungi for stored-grain pests. Revista de la Facultad de Agronomia Universidad de Buenos Aires 15 (1), 1–7.

Padin, S.B., Dal-Bello, G., Vasicek, A.L., 1997. Pathogenicity of *Beauveria bassiana* for adults of *Tribolium castaneum* (Coleoptera, Tenebrionidae) in stored-grains. Entomophaga 42 (4), 569–574.

Padin, S., Dal-Bello, G., Fabrizio, M., 2002. Grain loss caused by *Tribolium castaneum*, *Sitophilus oryzae,* and *Acanthoscelides obtecus* in stored durum wheat and beans treated with *Beauveria bassiana.* Journal of Stored Products Research 38 (1), 69–74.

Prior, C., Greathead, D.J., 1989. Biological control of locusts: the potential for the exploitation of pathogens. Plant Protection Bulletin 37, 37–48.

Quarles, W., 1995. New technologies for termite control. IPM Practitioner 17, 1–9.

Quarles, W., Winn, P.S., 1996. Diatomaceous earth and stored product pests. IPM Practitioner 18, 1–10.

Rath, A.C., 1992. *Metarhizium anisopliae* for control of Tasmanian pasture scarab *Adoryphorus couloni*. In: Jackson, T.A., Glare, T.R. (Eds.), Use of Pathogens in Scarab Pest Management. Intercept Ltd, Andover, UK, pp. 217–228.

Rice, W.C., Cogburn, R.R., 1999. Activity of the entomopathogenic fungus *Beauveria bassiana* (Deuteromycota: Hyphomycetes) against three coleopteran pests of stored-grain. Journal of Economic Entomology 92 (3), 691–694.

Stathers, T., Zd'arkova, E., Wakefield, M., Lukas, J., Hubert, J., 2002. Entomopathogenic fungi in grain storage: biological control of pest insects and mites with special reference to entomophthorales. In: Zd'arkova, E., Lukas, J., Hubert, J. (Eds.), Proceedings of the Second Meeting of Working Group—4: Biocontrol of Arthropod Pests in the Stored-Products, Prague, Czech Republic, 30–31 May, pp. 100–109.

Subramanyan, B.H., Swanson, C.L., Madamanchi, N., Norwood, S., 1994. Effectiveness of Insecto®, a new diatomaceous earth formulation, in suppressing several stored-grain insect species. In: Highley, E., Wright, E.J., Banks, H.J., Champ, B.R. (Eds.), Proceedings of the Sixth International Working Conference on Stored-Product Protection, Canberra, Australia, pp. 650–659.

Wakefield, M., Cox, P.D., Wildey, K.B., Price, N.R., Moore, D., Bell, B.A., Zd'-arkova, E., Lukas, J., Hubert, J., 2002. The use of entomopathogenic fungi for stored-product insect and mite control: biological control of pest insects and mites with special reference to entomophthorales. In: Zd'arkova, E., Lukas, J., Hubert, J. (Eds.), Proceedings of the Second Meeting of Working Groupe—4: Biocontrol of Arthropod Pests in the Stored-Products, Prague, Czech Republic, 30–31 May, pp. 110–115.

Zimmermann, G., 1993. The entomopathogenic fungus *Metarhizium anisopliae* and its potential as a biocontrol agent. Pesticide Science 37, 375–379.