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SUSCEPTIBILITY OF TWO GRASSHOPPER SPECIES TO SOME STRAINS OF THE ENTOMOPATHOGENIC FUNGUS *METARHIZIUM* (DEUTEROMYCOTINA: HYPHOMYCETES) AND THE EFFECT OF HUMIDITY

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Abstract

The susceptibility of two grasshopper species to aqueous and oil formulations of some strains of two *Metarhizium* species were compared. Both grasshoppers showed similar responses to increasing concentrations of the pathogens. However, *Schistocerca gregaria* was more susceptible to *M. flavoviride* IMI 330189 than *Zonocerus variegatus*. The effect of *M. anisophliae* IMI 168777/ii on the two grasshoppers in terms of LC_{50}s was similar, there being no overlaps in fiducial limits. Incubation humidity had a significant effect on mortality of infected grasshoppers, with median-lethal-times decreasing with increasing concentration of spore suspension. Differences in percentage mycosis on cadavers killed under different humidities were not significant ($P < 0.05$) indicating that infection occurred at all humidities studied.

Résumé

ADU-MENSAH, J.: *Prédisposition de deux espèces de sauterelle à quelques souches de fongus entomopathogène* Metarhizium *(Deuteromycotina: Hyphomycetes) et l'effet d'humidité.* La prédisposition de deux espèces de sauterelle aux formulations aqueuses et huileuses de quelques souches de deux espèces de *Metarhizium* étaient comparées. Les deux sauterelles montraient des réactions semblables aux concentrations augmentant des pathogénies, toutefois, *Schistocerca gregaria* était plus prédisposée à *M. flavoviride* IMI 330189 que *Zonocerus variegatus*. L'effet de *M. anisopliae* IMI 168777/ii sur les deux sauterelles en fonction de LC_{50}s était semblable, n'ayant pas d'empiètement sur les limites fiduciaires. Humidité d'incubation evait un effet considérable sur la mortalité des sauterelles infectées, avec les temps-mortel-médiane diminuant avec l'augmentation de concentration de spore en suspension. Les différences en pourcentage de mycose sur les cadavres tués sous des humidités différentes n'étaient pas considérables, indiquant que l'infection avait lieu à tous les humidités étudiés.

Introduction

Locusts and grasshoppers are the most serious pests of agriculture in the Sahelian region of Africa (Geddes, 1990). Long-term use of chemical insecticides for control has resulted in the development of resistance, new pest outbreaks, as well as public concerns for the environment. Several strains of *Metarhizium* sp. are important pathogens of locusts and grasshoppers with a potential as biopesticides for an environmentally-friendly control measure. A selection process for these strains would be a bioassay procedure where subtle differences in infectivity would be established. Several standard bioassay procedures have been employed for both microbial agents and chemical insecticides. The selection of an application method for fungal pathogens is influenced by both the pathogen and the host species, and most meaningful results are obtained in assays where the pathogen is applied in the same way as used in the field (Hall, 1982). Attempts to simulate field conditions in the labora-

tory have evolved several techniques. For example, Gillespie (1984) bioassayed thrips and mites on leaf discs that have previously been sprayed with *Verticillium lecanii* while Daoust, Ward & Roberts (1982) applied conidia formulations to the water surface in petri-dishes containing mosquito larvae.

Infection of susceptible host insects following field application of myco-insecticides has for a long time been considered as dependent on weather conditions particularly relative humidity and temperature. However, results with *Beauveria* indicate that infection may proceed independently of ambient r.h. (Ferron, 1977; Marcandier & Khachatourians, 1987).

Experimental

Freeze-dried spores of *M. flavoviride* IMI 330189, IMI 324673 and *M. anisopliae* IMI 168777/ii were obtained from the International Mycological Institute and, subsequently, maintained on Molisch's agar. The spores of *M. flavoviride* IIBC 191-609, isolated from a field infected with *Zonocerus variegatus*, was supplied from International Institute of Tropical Agriculture Bio-control Centre at Cotonou, Benin, which also supplied parent stocks of *Schistocerca gregaria* and field collected *Z. variegatus*. Both insects were maintained as cultures in the laboratory.

Conida of the fungal isolates *M. flavoviride* IMI 330189 and *M. anisopliae* IMI 168777/ii were harvested from 15-day-old growths to prepare aqueous conidial suspensions of different concentrations between 10^4 and 10^8 conidia ml^{-1} in 100 ml Kilner jars. Ten insects were immersed singly in a suspension of known concentration for 20 s. Insects were allowed to drip dry for 30 min and vortexed 20 times in sample tubes containing 20 ml distilled water. The mean spore concentration of resulting suspensions and the dose delivered to an insect was calculated.

Three replicates of 20 adult grasshoppers 5-7 days old post emergence (sex ratio 1:1) were immersed individually in known concentrations for 20 s; control insects were dipped in distilled water. Oil formulations of *M. flavoviride* IMI 3301891, IMI 324672 and IIBC 191-609 were prepared in peanut oil to 1.1×10^8 conidia ml^{-1}, and 1 μl (equivalent to 10^5 conidia/insect) was placed on the abdomen; control insects were given pure oil. Incubation conditions in both cases were 28 ± 2 °C, 40% ± 6% r.h. and 12 h light. Insects were examined daily, and dead insects were incubated on moist tissue paper in Petri-dishes for possible external sporulation. Probit analyses were done after corrections with Abbott's formular (Abbott, 1925) where corrected mortality M = 100 (x-y/x) and x = survival in control insects, y = survival in treated insects. LC_{50}s Median-Lethal-Times (MLT) and other parameters were produced by Probit Regression Analyses (Finney, 1971).

Different humidity chambers were provided in perspex tanks with glass sheet covers sealed air tight with vaseline. The ratio of free water surface area to volume of air above it was maintained at ≤ 25:1 to reduce the time required to attain equilibrium r.h. (Martin, 1962). Saturated salt solutions were prepared at 30 °C and poured into the chambers for equilibration at 28 ± 2 °C. The following salts were used to produce humidities as required in the chambers: NaH_2PO_4, NaCl, $MgCl_2$ and KOH, for 90, 75, 35 and 20 per cent r.h., respectively, assessed with a Vaisala humidity and temperatue indicator (HMI 31-UT). Humidity in tanks with vials containing insects, as well as build up after opening and closing for removal of dead insects, were also monitored. Each adult of *S. gregaria* was inoculated with 10^5 conidia from an oil formulation of the fungus *M. flavoviride* IMI 330189. Twenty inoculated insects were placed in each chamber and sealed with vaseline, control insects were given pure oil. Daily records of mortality were taken and cadavers were examined daily for external sporulation for 5 days, after which they were transferred unto moist tissue paper in Petri-dishes.

Results

The relationship between concentration of aqueous fungal suspensions and dose recovered from insect cuticles was represented by the equation:

TABLE 1

LC_{50}s and associated parameters for infectivity of aqueous formulations of M. flavouiride *IMI 330189 against* S. gregaria *and* L. variegatus *derived from probit regression analysis*

Days after		*Fiducial limits*		*Regression equations*	
inoculation	LC_{50}	*Lower*	*Upper*	*Intercept*	*Slope*
S. gregaria					
4	9.4×10^7a	3.4×10^7	2.6×10^8	2.12	0.36a
5	1.4×10^6b	1.9×10^5	7.7×10^6	1.23	0.61b
6	2.5×10^5c	1.5×10^5	4.1×10^5	0.02	0.92c
7	too low to be computed				
Z. variegatus					
4	1.1×10^8a	5.0×10^5	2.5×10^8	-1.15	0.38a
5	1.5×10^7d	8.1×10^6	2.7×10^7	0.67	0.60b
6	1.2×10^6e	8.7×10^5	1.7×10^6	-1.68	1.10c
7	6.5×10^5f	5.2×10^5	8.1×10^5	-6.67	2.01d

LC_{50}s of the same day results follwed by same letters not significantly different, there being no overlaps in fiducial limits; for slopes, $P > 0.05$

TABLE 2

LC_{50}s and associated parameters for infectivity of aqueous formulations of M. anisopliae, *IMI 168777/ii against* S. gregaria *and* Z. variegatus *derived from probit regression analysis*

Days after		*Fiducial limits*		*Regression equqtions*	
inoculation	LC_{50}	*Lower*	*Upper*	*Intercept*	*Slope*
S. gregaria					
4	1.8×10^8a	9.0×10^7	3.5×10^8	-0.41	0.66a
5	9.2×10^6b	1.3×10^6	6.7×10^7	-1.27	0.90b
6	2.1×10^6c	2.6×10^5	1.7×10^7	-1.10	0.96c
7	4.6×10^5d	3.1×10^5	6.9×10^5	-1.24	1.10d
Z. variegatus					
4	2.9×10^8a	1.0×10^8	8.5×10^8	0.00	0.59a
5	1.6×10^7b	7.2×10^5	3.5×10^8	-1.71	0.93b
6	8.1×10^6c	4.0×10^5	1.6×10^8	0.16	0.70c
7	2.5×10^6d	2.2×10^5	2.9×10^7	0.36	0.72d

LC_{50}s of the same day results follwed by same letters not significantly different, there being no overlaps in fiducial limits; for slopes, $P > 0.05$

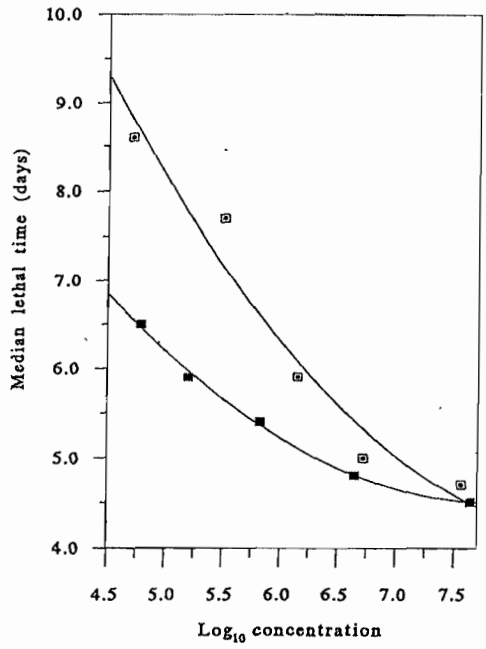

Fig. 1. Relationship between close and median-lethal-time for *M. flaroviride* IMI 330189 (▣) and *M. anisopliae* IMI 168777/ii for *Schistocerca gregaria* (●)

$Log_{10} D = -0.077 + 0.755 \log_{10} C$ ($r^2 = 0.98$; $P = 0.001$)

where D = dose recovered, and C = concentration of suspension.

The response slopes for *S. gregaria* and *Z. variegatus* to *M. flavoviride* IMI 330189 from the 4th to the 7th day after inoculation were not significantly different ($P > 0.05$) (Table 1). LC_{50} for *S. gregaria* after 5 and 6 days of inoculation were significantly lower than that for *Z. variegatus*, there being no overlaps in fiducial limits. For *M. anisopliae* IMI 168777/ii, response slopes for the two grasshopper species as well as LC_{50}s were not significantly different having overlaps in fiducial limits (Table 2). Median-lethal-times (MLT) were inversely related to spore concentration in the aqueous formulations with greater reductions at the lower ends of the concentration spectrum (Fig. 1). For oil formulations, the three strains of *M. flavoviride* against *S. gregaria* produced MLT which were not significantly different ($P > 0.05$) (Table 3) while for *Z. variegatus* strain IIBC 191-609 produced significantly lower MLT ($P \leq 0.05$) than the other two strains. All strains produced

TABLE 3

Median lethal times and parameter estimates of mortality caused by three strains of M. flavoviride *to* S gregaria *and* Z. variegatus

Strain	*MLT (days)*	*c.v.m*[a]	*n.m.m*[b]
S. gregaria			
IMI330189	5.4 ± 0.3a	0.29 ± 0.06	0.0
IIBC191-609	5.8 ± 0.4a	0.30 ± 0.01	0.0
IMI324673	5.7 ± 0.1a	0.30 ± 0.07	0.0
Z. variegatus			
IMI330189	7.0 ±0.1a	0.49 ± 0.10	3.7 ± 1.3
IIBC191-609	5.4 ± 0.2b	0.38 ± 0.03	1.7 ± 1.1
IMI324673	7.2 ± 0.3a	0.36 ± 0.02	8.3 ± 3.4

MLTs for each grasshopper species followed by same letters not significantly different ($P > 0.05$).
a = coefficient of variation of mortality; b = non mycosis mortality

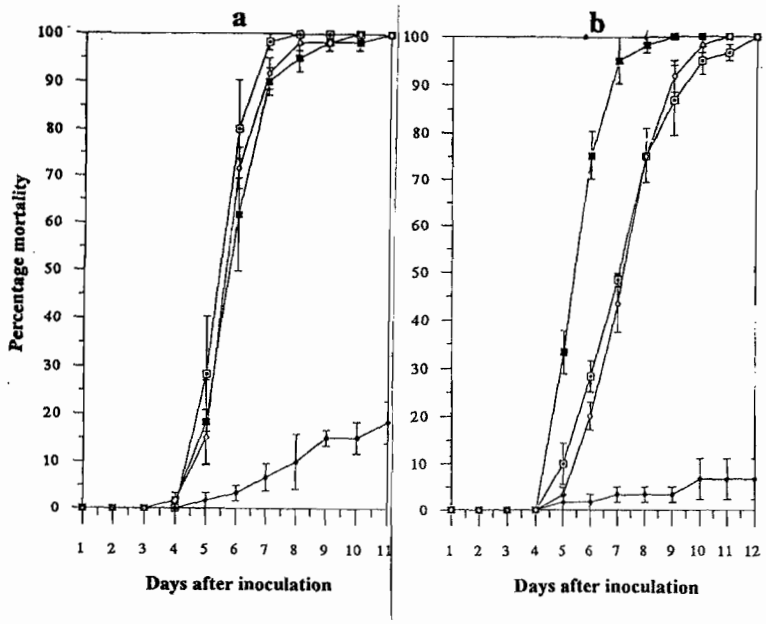

Fig. 2. Mortality-time curves for *Schistocerca gregaria* (a) and *Zonocerus variegatus* (b) after innoculation with 10^5 conida per insect of *Metarhizium flavoride* IMI330189 IMI324673 ( ) I9I-609 ( ) andcontrol ( )

100 per cent mortality in their hosts within 12 days of inoculation (Fig. 2) with external sporulation on all cadavers of *S. gregaria*. In *Z. variegatus*, however, non-mycosis mortality between 1.7 and 8.3 per cent were recorded in all strains. Lower coefficients of variation of mortality (c.v.m.) were obtained in *S. gregaria* than in *Z. variegatus*.

Incubation humidity had a significant effect ($P < 0.05$) on mortality of infected insects from 3-6 days after incubation (Fig. 3). MLT decreased with increasing humidity, that of 20% r.h. significantly higher than all the others (Table 4). Cadavers in tanks with humidity ≤ 75% sporulated externally only after transference unto moist tissue paper in Petri-dishes. Total percentage sporulation on cadavers ranged from 20.8 to 37.5 with no significant differences between treatments ($P > 0.05$).

Discussion

Both species of fungi elicited similar responses to increasing conidial concentrations from both grasshoppers; however, *M. flavoviride* IMI 330189 was more virulent to *S. gregaria* than *Z. variegatus*, while for *M. anisopliae* IMI 168777/ii virulence to both grasshoppers was similar. The relationship between MLT and spore concentration was asymptotic and that beyond a certain point any extra conidial pickup by the host insect may not contribute much, if at all, to the total biological effect. This confirms the results of Bateman *et al.*, (1993) using oil formulations. The higher virulence of *M. flavoviride* I91-609 to *Z. variegatus* than *S. gregaria* contributes to the debate about the relative merits of old and new associations for classical biological control (Hokkanen & Pimentel, 1984); however, it seems that the most useful guideline in the search for and selection of pathogens is to look at isolates from the target insect or its taxonomic relatives (Moore & Prior, 1993). Low percentage mortality without mycosis noted in *Z. variegatus* may still reflect death due to the fungus; however, fungal isolates which show a high n.m.m. may indicate the inclusion of toxins in pathogenesis (Roberts, 1980). Lower coefficients of variation of mortality of *S. gregaria* compared to *Z. variegatus* probably reflects the more uniform population structure of the former compared with the more vari-

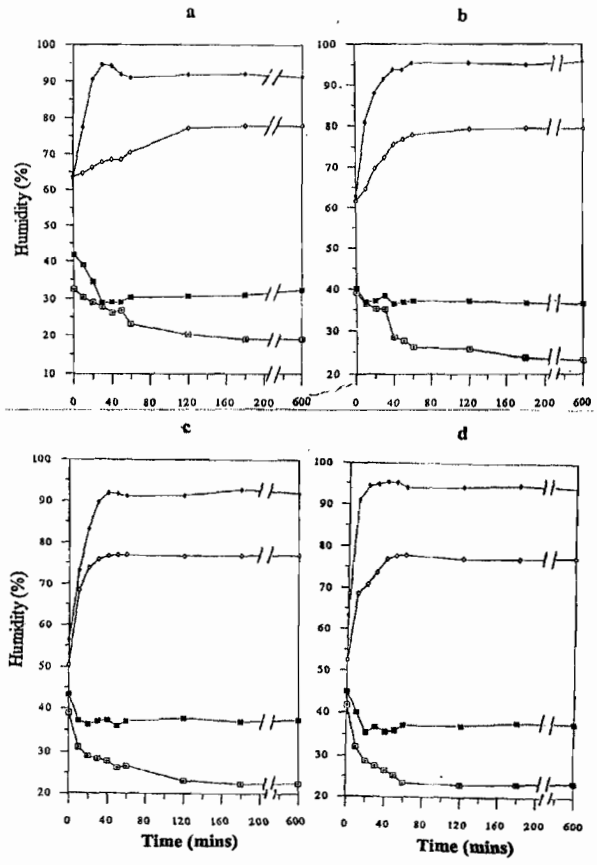

Fig. 3. Mortality-time curves for (a) infertility of *Metarhizium flavoride* IMI 330189 to *Schistocerca gregaria* and (b) control insects at 20% ( ), 35% ( ) 75% ( ) and 90% ( ) humidity

able population of the latter collected from the field. In-breeding depression and loss of genetic variation are common features in laboratory insect colonies (Berlocker & Friedman, 1981).

Immersion of test insects in aqueous conidial suspensions was analogous to a drench application which may only be possible in knapsack sprays. However, for the purpose of a comparative assessment of virulence, the method could be considered a standard procedure and highly effective in transmission of the fungus. The direct application of conidia to the insect cuticle frequently results in more rapid infection than application where secondary pickup occurs from surfaces and/ or food as demonstrated by Wegensteiner & Fuhrer (1988) in *Hylobius abeities*.

Although higher humidities caused faster rates of mortality, all insects died within 9 days regardless of humidity. This is similar to the infection of the European corn borer (*Ostrinia hubilalis*) by *B. bassiana* and *M. anisopliae* (Riba & Marcandier, 1984). The significant difference between the probit mortality slopes for 20% r.h. and the others may

TABLE 4

Median lethal times and sassociateed parameters of Schistocerca gregaria *incubated under different humidities*

Humidity (%)	*Probit line equation*		*MLT (days)*	*Per cent mycosis*
	Intercept	*Slope*		
20	-0.11	6.60a	5.6 ±0.2a	22.9± 5.5
31	-0.38	7.70b	4.7 ±0.4b	37.5± 7.2
74	-0.50	8.65b	4.3 ±0.4bc	35.4± 7.5
94	-0.31	9.23b	3.5 ±0.1c	20.8± 5.5

Numbers in a column followed by same letters not significantly different ($P > 0.05$)

have resulted from changes in the physiology of the fungus and/or to that of the insects under different humidities. For example, certain anomalies in germination of Moniliales spores between 30 % r.h. and 45 % r.h. have been reported (Marcandier & Khachatourians, 1987).

The relationship between r.h. and survival of Acrididae has not been studied in detail but the high levels of control mortality at near saturation suggests a poor survival rate of *S. gregaria* at high humidity. Thus, stress as a factor could have accounted for the high mortality recorded. However, sporulation on cadavers, which is a proof of mycosis provided further evidence that infection occurred at all humidities. Similar results were reported on the infection by *B. bassiana* of the red locust (Schaefer, 1936), *Melanoplus sanguinipes* (Marcandier & Khachatourians, 1987) and of *Acanthoscelides obtectus* Say (Ferron, 1977). However, the degree of sporulation in the present study was higher and important in disease transmision among susceptible hosts. Although near saturation humidity was necessary for external mycelia growth, the same cannot be said of the use of fungal preparations as a bio-insecticide. It is possible that the microclimate of the boundary layer of air around the insect cuticle may supply the water needed for the initial stages of the infection process, particularly the inter-segmental membranes which are probable important penetration sites (Prior, Jollands & Patourel, 1988).

Reference

ABBOTT, W. S. (1925) A method of computing the effectiveness of an insecticide. *J. econ. Ent.* **18**, 265-267.

BATEMAN, R. P., CAREY, M., MOORE, D. & PRIOR, C. (1993) The enhanced infectivity of *Metarhizium flavoviride* in oil formulation to desert locusts at low humidities. *Ann. appl. Biol.* **122**, 145-152.

BERLOCKER, S. H. & FRIEDMAN, S. (1981) Loss of genetic variation in laboratory colonies of *Phormia regina*. *Entomologia exp. appl.* **30**, 205-208.

DAOUST, R. A., WARD, M. G. & ROBERTS, D. W. (1982) Effect of formulation on the virulence of *Metarhizium anisopliae* conidia against mosquito larvae. *J. Invert. Pathol.* **7**, 31-32.

FERRON, P. (1977) Influence of relative humidity on the development of fungal infection caused by *Beauveria bassiana* (*Fungi imperfecti*) in imagines of *Acanthoscelides obtectus* (Co.: Bruchidae). *Entomophaga* **22**, 393-396.

FINNERY, D. J. (1971) *Probit Analysis*, 3rd edn. Cambridge: Cambridge University Press.

GEDDES, A. M. W. (1990) The relative importance of crop pests in Sub Saharan Africa. *Nat. Resour. Inst. Bull.* **36**, 69 pp.

GILLESPIE, A. T. (1984) The Potential of Entomogenous Fungi to Control Glasshouse Pests and Brown Planthoppers of Rice. *(PhD. Thesis)*, University of Southampton.

HAJEK, A. E., HUMBER, R. A. & GRIGGS, M. H. (1990) Decline in virulence of Entomophthora maimaga (Zygomycetes: Entomophthorales) with repeated *in vitro* subculture. *J. Invert. Pathol.* **56**, 91-97.

HALL, R. A. (1982) Deuteromycetes: virulence and bioassay design. *Proceedings of the 3rd International Colloquium of Invertebrate Pathology*, pp. 191-196.

HOKANEN & PIMENTEL (1984) New approach for selecting biological control agents. *Can. Entomologist* **116**, 1109-1121.

MARCANDIER, S. & KHACHATOURIANS, G. G. (1987) Susceptibility of the migratory grasshopper, *Melanoplus sanguinipes* (Fab.) (Orthoptera: Acrididae), to *Beauveria bassiana* (Bals.) Vuillemin: Influence of Relative Humidity. *Can. Entomologist.* **119**, 901-907.

MARTIN, S. (1962) The control of conditioning atmospheres by saturated salt solutions. *J. scienti. Instrum.* **39**, 370-372.

MOORE, D. & PRIOR, C. (1993) The potential of mycoinsecticides. *Biocontrol News Inf.* **14**, 31-40.

MOOREHOUSE, E. R. (1990) The Potential of the Entomgenous Fungi *Metarhizium anisopliae* as a Microbial Control Agent of the Black Vine Weevil *Otiorhynchus sulcatus*. (PhD. Thesis.) University of Bath.

PRIOR, C., JOLLANDS, P., LE PATOUREL, G. (1988) Infectivity of oil and water formulations of *Beauveria bassiana* (Dueteromycotina: Hyphomycetes) to the cocoa weevil pest *Pantorhytes plutus* (Coleoptera: curculionidae). *J. Invert. Pathol.* **52**, 66-72.

RIBA, G. & MARCANDIER, S. (1984) Influence de

l'humidite relative sur l'agressivite et la viabilite des souches de *Beauveria bassiana* (Bals.) Vuillemin et de *Metarhizium anisopliae* (Metsch.) Sorokin, hyphomycetes pathogenes de la pyrale du mais, *Ostrinia nubilalis* Hbn. *Agronimie* **4**, 189-194.

ROBERTS, D.W. (1980) Toxins of entomopathogenic fungi. In *Microbial Control of insects, Mites* and *Plant Diseases 1970-1980.* (ed H. D. Burges), pp. 441-464. New York: Academic Press.

SCHAEFER, E. E. (1936) The white fungus disease (*Beauveria bassiana*) among red locusts in S. Africa and some observations on the grey fungus disease (*Empusa grylli*). *South African Department of Science Bulletin* **160**, 5-28.

WEGENSTEINER, R. & FUHRER, E. (1988) The efficiency of *Beauveria bassiana* (Bals.) Vuill. against *Hylobius abeitis* L. (Col.: Curculionidae). *Entomophaga* **33**, 339-438.

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