

Effects of Temperature and Relative Humidity on Sporulation of *Metarhizium anisopliae* var. *acidum* in Mycosed Cadavers of *Schistocerca gregaria*

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Received September 1, 2000; accepted August 9, 2001

The effects of relative humidity (RH) and temperature on the sporulation of *Metarhizium anisopliae* var. *acidum* on mycosed cadavers of desert locust, *Schistocerca gregaria*, were assessed in the laboratory. Quantitative assessments of conidial production over 10 days under constant conditions showed that sporulation was optimized at RH >96% and at temperatures between 20 and 30°C. Under both these conditions >10⁹ conidia/cadaver were produced. At 25°C, conidial yield was maximized under conditions in which cadavers remained in contact with damp substrate. Relatively little sporulation occurred at 15°C (<3 × 10⁷ conidia/cadaver) and 40°C (<4 × 10⁶ conidia/cadaver) and no sporulation occurred at 10 or 45°C. Following incubation, conidial yield was closely related to the water content of locust cadavers. In separate tests, locust cadavers were incubated for 10 days under diurnally fluctuating temperature and RH that comprised favorable (25°C/100% RH) alternating with unfavorable (40°C/80% RH) conditions for sporulation. In this case, fewer conidia were produced compared with cadavers that were incubated under the favorable conditions for an equal period cumulatively but were not periodically exposed to unfavorable conditions. However, this reduced sporulation observed with the fluctuating condition was not observed when cadavers were similarly incubated under favorable/unfavorable conditions of temperature but were not periodically exposed to the low RH condition. This result implies that sporulation is a dynamic process, dependent not only on periodic exposure to favorable RH but also on the interrelation of this with low RH. Associated tests and the monitoring of changes in cadaver weights imply that the mechanism driving the reduced sporulation under fluctuating RH is the net water balance of cadavers, i.e. the cumulative ability of the fungus/ca-

daver to adsorb water necessary for sporulation at high RH is restricted by water loss associated with intermittent exposure to a low RH. The duration of daily exposure to high humidity appears to be a crucial constraint to the recycling ability of *M. anisopliae* var. *acidum*. © 2001 Academic Press

Key Words: *Metarhizium anisopliae* var. *acidum*; *Schistocerca gregaria*; entomopathogenic fungi; sporulation; horizontal transmission.

INTRODUCTION

A mycoinsecticide based on the entomopathogenic fungus *Metarhizium anisopliae* var. *acidum* (= *flavoviride*) (Deuteromycotina: Hyphomycetes) has been developed and is being commercialized under the tradename "Green Muscle" for the control of locusts and grasshoppers (Acrididae) in Africa (Thomas *et al.*, 2000). A similar product has recently undergone large-scale field trials in anticipation of commercialization in Australia (Milner, 2000). Population models using empirically derived horizontal transmission parameters have predicted that, following its application as a mycoinsecticide, secondary cycling of the fungus may contribute significantly to biological control principally by reducing the frequency of treatment required to maintain hosts below a given threshold (Thomas *et al.*, 1995, 1999). However, these models, along with most concerning insect/fungal dynamics (but see Ardisson *et al.*, 1997; Brown and Nordin, 1982; Hajek *et al.*, 1993), use simplifying assumptions about the ability of the pathogen to produce further inoculum in the environment following death of the host. For instance, the models often assume a discrete delay between the insects dying and the cadavers becoming infective.

Whereas it is experimentally possible to infect locusts (Bateman *et al.*, 1993; Fargues *et al.*, 1997) and other insects (e.g., Doberski, 1981; Ferron, 1977;

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James *et al.*, 1998; Milner *et al.*, 1997; Moore, 1973; Ramoska, 1984; Steinkraus and Slaymaker, 1994) with fungal entomopathogens at low ambient humidities (45–70%), sporulation (i.e., the production of infective asexual conidia on the host cadavers during the necrophytic growth phase of the fungus) is highly dependent on suitable climatic conditions, in particular the availability of high environmental moisture (Benz, 1987; Carruthers and Soper, 1987; Ferron *et al.*, 1991; Hajek and St. Leger, 1994; Hall and Papierok, 1982). Factors that determine sporulation therefore regulate a critical step in the transmission of insect pathogens. Given that many areas where the use of microbial control of acridids is considered are characterized by hot and dry conditions, we might anticipate that secondary cycling of mycoinsecticides will be limited by adequate moisture availability for sporulation. An understanding of conditions required for sporulation is therefore necessary to predict where secondary cycling of mycoinsecticides might occur and thus may provide insights into how fungal epizootics are initiated and maintained.

This paper reports on studies quantifying how different combinations of constant temperature and humidity regulate sporulation of *M. anisopliae* var. *acridum* in mycosed locust cadavers in the laboratory. Moreover, because semiarid environments tend to be associated with strong diurnal changes in temperature and humidity, separate tests investigate the consequences of exposure to fluctuating humidity and temperature regimes.

MATERIALS AND METHODS

Locust Inoculation

A conidial suspension was prepared by suspension of a single spore isolate of *M. anisopliae* var. *acridum* (IMI 330189) at ~5% moisture content in peanut oil (Sigma–Aldrich). This was mixed and serially diluted (by means of hemocytometer counts) to a concentration of 5×10^7 conidia ml⁻¹ before being sonicated for 2 min to separate conidial chains. The viability of the conidial suspension was checked on Sabouraud dextrose agar (SDA) lightly inoculated with diluted suspension (~10⁶ conidia/ml). Germination was assessed from 300 conidia by the counting of the number of conidia that produced a germ tube at least as long as the conidium after 24 h at 25°C. The viability was found to be 76.1% in the first study and 82.3% from an equivalent suspension used in the second study.

Immature adult *Schistocerca gregaria* (7–12 days postfledging; 50:50 sex ratio) were individually topically inoculated following the procedure of Prior *et al.* (1995) by the placing of 2 µl of the conidial suspension under the pronotum with a microapplicator (Burkard Ltd., Rickmansworth, UK), providing a dose of 10⁵ conidia/insect. Immediately after inoculation, insects

were maintained in ventilated Perspex cages at 30 ± 1°C. Under these conditions insects were killed 5–8 days later from fungal mycosis (identified by a characteristic red coloration). Cadavers used in the subsequent studies were weighed with a microbalance and, following initial problems of the growth of secondary fungal contaminants, were surface-sterilized for 3 min in a 2.5% sodium hypochlorite solution prior to incubation. All cadavers were used within 24 h of death. The water content (wc) of a sample of freshly dead mycosed locusts was assessed by their being dried in an oven at 80°C until constant weight.

Incubation under Constant Temperature and Relative Humidity

In the first study, sporulation on locust cadavers was assessed following incubation under different combinations of relative humidity (RH) and temperature. RH was controlled with stable saturated solutions of reagent-grade salts (Sigma–Aldrich), which maintain specific vapor pressures (vp) at a given temperature in enclosed environments (Winston and Bates, 1960). Humidity chambers consisted of airtight clear plastic boxes (14 × 8 × 6 cm) containing 100 ml of saturated salt solution to maintain a range of humidity values at each temperature; (NH₄)₂SO₄ was used for 80%, C₄H₄KNaO₆ for 87%, C₄H₄O₆Na₂ for 93%, and KNO₃, KH₂PO₄, or K₂SO₄ for 96%. The use of three different solutions in the latter case was necessary to achieve the RH across the temperature range used. All RH values were experimentally checked with a thermal hygograph (Grant Instruments, Ltd., UK) and found to be within ±1% of the indicated values across the temperature range, although such variations may influence the precise correlations obtained. Distilled water was used for 100% RH. A single locust cadaver was placed in each incubation chamber and was supported 2–3 cm directly above the solution on a 5 × 5-mm aluminum mesh. Following placement of the cadaver, the chamber was sealed with parafilm to prevent subsequent gaseous exchange with the environment. The risk that water exchange between the solution and the cadaver might change the vp was eliminated through the ability of excess salts to dissolve in and out of solution. Ten cadavers were incubated at each RH and the experiment was conducted at 10–45°C at 5°C increments. At 25°C cadavers were also incubated at 100% RH while remaining in contact with a damp substrate. Most treatments were incubated under darkness, although photoperiod was not strictly controlled on the bases that it has relatively little influence on sporulation of *M. anisopliae* (Alves *et al.*, 1984) and that the majority of conidia were produced internally within the host and thus were not subject to ambient light. After 10 days cadavers were removed

and reweighed and the average number of conidia produced was determined (see below).

To quantify sporulation, cadavers were individually placed in universal tubes; 15 ml of Shellsol T (light paraffin oil) was added and the tube was vigorously shaken by hand and stirred with a seeker to break apart the cadaver and encourage conidia into suspension. Heavily sporulated cadavers quickly broke apart and the hydrophilic conidia were readily suspended in oil. However, less heavily sporulated cadavers required cutting into smaller fragments to facilitate conidial extraction. The different approaches were adopted to give a consistent level of extraction and to avoid bias due to differences in the rigidity of the cadavers resulting from different treatments. The resulting well-agitated suspension was filtered through a 75- μm sieve (to remove larger mycelial particles), serially diluted to obtain a concentration of $\sim 10^6$ conidia ml^{-1} (light green color), and sonicated for 2 min prior to being counted in an improved Neubauer hemacytometer, with 2 counts performed per insect. Average conidial yields from each treatment were determined.

Incubation under Fluctuating Temperature and Relative Humidity

In the second study, locust cadavers were incubated individually in the manner described above, but were exposed to diurnally fluctuating temperature and RH. Changes in RH and temperature were achieved by transfer of cadavers between humidity chambers and incubators every 12 h. Cadavers were incubated at 100% RH and $25 \pm 1^\circ\text{C}$, alternating with 80 \pm 1% RH at $40 \pm 1^\circ\text{C}$, over a period of 10 days, and were weighed at the end of each 12-h period. These values, although clearly very simplified, broadly simulate the type of diurnal changes recorded from field sites in the Sahel and South Africa during overcast/wet weather (Arthurs, 2000). In another treatment this incubation procedure was repeated with the same temperature fluctuation, but at constant high RH; i.e. 100% RH at $25 \pm 1^\circ\text{C}$ alternated with $40 \pm 1^\circ\text{C}$. In addition, to investigate the consequences of exposure to such fluctuations for sporulation, cadavers were also incubated independently under constant temperature and humidity regimes that comprised each condition of these fluctuations separately; i.e. 100% RH at $25 \pm 1^\circ\text{C}$, 80 \pm 1% RH at $40 \pm 1^\circ\text{C}$, and 100% RH at $40 \pm 1^\circ\text{C}$ for 5 days (i.e. the cumulative constant components of the fluctuating treatments). Cadavers were also incubated at 100% RH and $25 \pm 1^\circ\text{C}$ for 5 days while remaining in contact with a damp substrate. Fifteen cadavers were used for each treatment and, after the exposure, the average conidial yield per treatment was determined as described above.

Analysis of Data

All conidial counts were \log_{10} transformed and then analyzed with either one- or two-way analysis of variance (ANOVA) and the Student–Newman–Keuls multiple-range test of comparisons of means (SPSS for Windows, 6.1). Means were considered not statistically different at $P > 0.05$. Changes in the weights of cadavers following incubation were \log_{10} transformed and analyzed by one- and two-way ANOVA.

RESULTS

Incubation under Constant Temperature and Relative Humidity

Quantitative assessments of conidial yield from *S. gregaria* cadavers incubated under different combinations of constant temperature and RH are shown in Table 1. A two-way ANOVA revealed that both RH and incubation temperature influenced the number of conidia harvested from cadavers ($F_{4,360} = 86.3$, $P < 0.00001$ and $F_{7,360} = 4.3$, $P < 0.001$, respectively). Furthermore, there was a significant interaction term ($F_{28,360} = 4.2$, $P < 0.001$). This was reflected in the difference between RH values becoming less as the incubation temperature moved away from the optimum. Thus, within the range $20\text{--}35^\circ\text{C}$, conidial yield generally increased with increasing RH and was optimised at 96% RH or above. The optimal temperature for sporulation at 96% RH or above was $20\text{--}30^\circ\text{C}$. At 25°C , conidial yield was maximized under conditions in which cadavers remained in contact with damp substrate, suggesting, at least at this temperature, that the uptake of water was faster from direct sources than from the atmosphere. Relatively little sporulation occurred at 15 and 40°C , and at these temperatures the effect of RH was not significant over the range tested. No sporulation occurred at 10 or 45°C .

The wc of freshly killed mycosed cadavers was found to be $59 \pm 0.02\%$. A two-way ANOVA revealed that the change in the weight (i.e., wc) of cadavers following incubation was strongly influenced by RH ($F_{4,360} = 539.1$, $P < 0.00001$) and to a lesser extent by temperature ($F_{7,360} = 14.4$, $P < 0.001$), with a significant interaction term ($F_{28,360} = 7.8$, $P < 0.001$). The effect of incubation RH on the final wc of cadavers is illustrated in Fig. 1. The RH value that determines whether water is lost or gained from *M. anisopliae* var. *acidum*-infected locusts (i.e., the equilibrium RH) was found to be $\sim 97\%$ over this range of temperatures.

Sporulation under Fluctuating Temperature and Relative Humidity

Average conidial yields obtained from *S. gregaria* cadavers incubated under fluctuating conditions of RH and/or temperature for 10 days, together with those

TABLE 1

Number of *M. anisopliae* var. *acidum* Conidia (\pm SE) Harvested from *S. gregaria* Cadavers Following Incubation under Factorial Conditions of Temperature and Humidity for 10 Days

Temp. ($\pm 1^\circ\text{C}$)	Relative humidity ($\pm 1\%$)					
	80	87	93	96	100	100 ^a
10	x ^b	x	x	x	x	NA
15	(1.1 \pm 0.8) $\times 10^7$ a(a) ²	(2.4 \pm 3) $\times 10^7$ a(a)	(4.8 \pm 1.6) $\times 10^6$ a(a)	(1.5 \pm 0.8) $\times 10^7$ b(a)	(1.4 \pm 0.6) $\times 10^7$ b(a)	NA
20	(5.9 \pm 4.8) $\times 10^7$ b(a)	(3.5 \pm 1.8) $\times 10^8$ b(b)	(3.1 \pm 2.4) $\times 10^8$ b(b)	(1.4 \pm 0.3) $\times 10^9$ d(c)	(1.5 \pm 0.2) $\times 10^9$ d(c)	NA
25	(6.6 \pm 2.1) $\times 10^7$ b(a)	(4.3 \pm 1.2) $\times 10^8$ b(b)	(3.7 \pm 0.4) $\times 10^8$ b(b)	(1.3 \pm 0.3) $\times 10^9$ d(c)	(1.6 \pm 0.4) $\times 10^9$ d(c)	(2.5 \pm 0.1) $\times 10^9$ (d)
30	(5.7 \pm 0.8) $\times 10^7$ b(a)	(2.5 \pm 0.3) $\times 10^8$ b(b)	(3.8 \pm 1.2) $\times 10^8$ b(c)	(1.7 \pm 0.9) $\times 10^9$ d(d)	(2 \pm 0.3) $\times 10^9$ d(d)	NA
35	(3.2 \pm 6.2) $\times 10^7$ b(a)	(9.8 \pm 0.6) $\times 10^7$ b(a)	(2.8 \pm 1.3) $\times 10^8$ b(a)	(4.9 \pm 1.4) $\times 10^8$ c(b)	(7 \pm 1.4) $\times 10^8$ c(c)	NA
40	(2.1 \pm 1.9) $\times 10^6$ a(a)	(1.8 \pm 0.5) $\times 10^6$ a(a)	(1.2 \pm 0.7) $\times 10^6$ a(a)	(2.4 \pm 0.4) $\times 10^6$ a(a)	(3.1 \pm 1) $\times 10^6$ a(a)	NA
45	x	x	x	x	x	NA

^a Cadavers remained in contact with damp substrate at 100% RH.

^b No fungal outgrowth was observed from cadavers.

^c Different letters represent significant ($P < 0.05$) post hoc contrasts according to the Student–Newman–Keuls test; first letters represent differences between temperatures for a given RH (columns); letters in parentheses represent differences between RH for a given temperature (rows).

incubated under each condition independently, are shown in Table 2. A one-way ANOVA revealed that overall incubation treatment significantly influenced conidial yield ($F_{5,84} = 60.7$, $P < 0.0001$). Significant ($P < 0.05$) differences were also observed between the incubation treatments. Fewer conidia were recovered from cadavers exposed to fluctuating temperature and RH than expected based on the level obtained from those exposed independently to the cumulative high RH condition alone, i.e., 8.6×10^7 compared to 4.9×10^8 . This reduced sporulation observed with the fluctuating condition was not observed when cadavers were exposed to changes in temperature but not periodically transferred to the lower RH, suggesting that temperature changes or incubation period were not responsible for the reduced sporulation in the former case. Sporulation was again highest when cadavers remained in contact with a damp substrate at saturated humidity.

Measurements of the wc of cadavers (assessed via changes in weight) during incubation under fluctuating temperature and RH (100% RH at 25°C alternating with 80% RH at 40°C) are shown in Fig. 2. Unfortunately, cadavers incubated under other treatments were not weighed, but the expected final wc of cadavers based on a 10-day incubation period under each regime separately (calculated from weight changes recorded in the previous study) is indicated in the figure.

DISCUSSION

The first experiment shows the requirement for both appropriate temperatures (20–30°C) and high ambient

RH (>96%) for sporulation of *M. anisopliae* var. *acidum*. Under these incubation conditions, $>10^9$ conidia were recovered from cadavers, which typically contained a dense covering of conidia within the host body cavity and a matt of aerial conidia produced on phialides growing through the intersegmental membranes of the cuticle. As a consequence the cuticle became fragile, possibly aided by the production of chitinolytic enzymes (Gabriel, 1968), and cadavers broke up readily and liberated conidia under mechanical disturbance. These findings are in line with the high moisture requirements for sporulation *in vivo* of other deu-

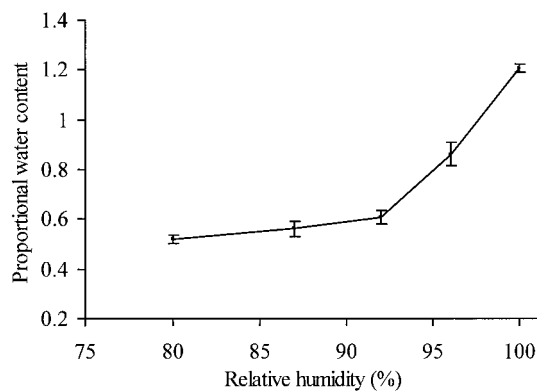


FIG. 1. Proportional change in water content (wc) (\pm SE) of individual *M. anisopliae* var. *acidum*-infected *S. gregaria* cadavers following incubation under different relative humidities (RH) for 10 days. Data are pooled across incubation temperatures. Starting wc = $59 \pm 0.02\%$ body mass. The critical RH determining a net gain or loss of water from fresh cadavers is $\sim 97\%$ over this range of temperatures.

TABLE 2

Number of *M. anisopliae* var. *acidum* Conidia Harvested from *S. gregaria* Cadavers Incubated under Fluctuating Conditions of Humidity (RH) and/or Temperature (T) for 10 Days Together with Those Incubated Independently under Each Condition Separately

RH1-T1 ^a	RH2-T2 ^a	Conidial yield (±SE)
Alternating conditions		
100%/25°C	80%/40°C	$(8.6 \pm 3.9) \times 10^7$ (c) ^e
	100%/40°C	$(5.4 \pm 0.7) \times 10^8$ (b)
Constant conditions		
100%/25°C		$(4.9 \pm 1.8) \times 10^8$ (b)
80%/40°C		$(2.8 \pm 0.6) \times 10^6$ (d)
100%/40°C		$(4.8 \pm 0.5) \times 10^6$ (d)
100%/25°C ^b		$(8.1 \pm 1.5) \times 10^8$ (a)

^a (±1% RH/1°C).

^b Cadavers remained in contact with damp substrate at 100% RH.

^c Different letters represent significant ($P < 0.05$) post hoc contrasts according to the Student–Newman–Keuls test.

teromycete fungi (see Ferron, 1977; Gerson *et al.*, 1979; Luz and Fargues, 1998; Milner and Lutton, 1986; Milner *et al.*, 1997) and in entomophthorean fungi (Brown and Hasibuan, 1995; Glare *et al.*, 1986; Hajek *et al.*, 1990; Millstein *et al.*, 1982; Oduor *et al.*, 1996; Smitley *et al.*, 1986; Steinkraus and Slaymaker, 1994; Wilding, 1969). The current data also suggest that sporulation will be restricted in cooler conditions, even when RH is high. Few data exist on the effects of temperature on sporulation *in vivo* of deuteromycete fungi, although these findings are similar to those assessing sporulation of *M. anisopliae* *in vitro* (Thomas and Jenkins, 1997; Walstad *et al.*, 1970).

Although in the present study sporulation was observed from cadavers incubated in drier conditions and less favorable temperatures, significantly fewer conidia were produced, with little external sporulation, and cuticular integrity tended to be maintained. The conidia associated with such cadavers would almost certainly have a low infectivity under field conditions. Moreover, where few (i.e., $<10^8$ conidia/cadaver) were recovered, conidia often appeared pale and thin-walled under magnification. This was also observed with conidia extracted within the first 24 h of incubation under optimum conditions, suggesting that it may have been associated with early or incomplete development.

The second experiment showed that conidial yield was reduced by the periodic exposure to low humidity, suggesting that sporulation is dependent not only upon the exposure to favorable conditions, but also upon the interrelation of these conditions with those which are unfavorable. Thus, sporulation in cadavers exposed to fluctuating humidity may be less than that expected

based simply on the accumulated exposure to conditions of high RH and favorable temperatures. Associated treatments and the monitoring of changes in cadaver weights imply that the low sporulation in cadavers exposed to fluctuating temperature and humidity was related to the reduced net water balance of the cadavers. Whereas the first experiment showed that the moisture content of cadavers was a strong predictor of sporulation under constant conditions, the second experiment showed that the cumulative ability of the fungus/cadaver to adsorb water at high RH was restricted by intermittent exposure to the lower RH. Water loss from arthropods is proportional to the saturation deficit (Edney, 1957). In this case, water desorption from cadavers at the low RH was more rapid than adsorption at the high RH, resulting in a net cumulative water loss (Fig. 2) and subsequently a reduced conidial yield associated with fluctuations in humidity during incubation (Table 2).

In sum, sporulation of entomopathogenic fungi may be highly dependent on the daily duration of exposure to high RH. This point is supported by the laboratory assays of Fargues and Luz (1998), who investigated the effects of fluctuating moisture and temperature on an isolate of *Beauveria bassiana* in mycosed cadavers of *Rhodnius prolixus*. Near-saturated (97% RH) conditions for >12 h in first-instar and >16 h in both third- and fifth-instar cadavers were required in every 24-h period for sporulation over a 10-day incubation period at favorable temperatures. Moreover, a comparison of the conidial yield from cadavers exposed to fluctuating high/low RH compared with those incubated under continually high RH showed that the former always yielded significantly fewer conidia than that predicted by the ratios of the daily exposure to high RH. This

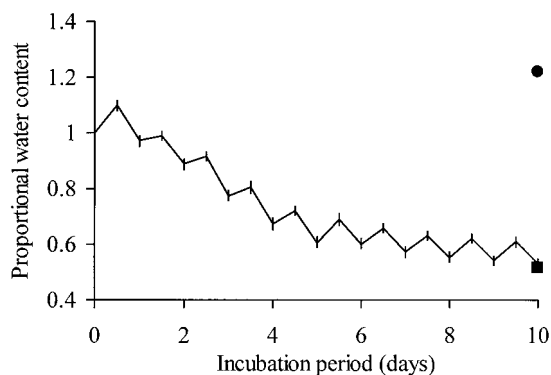


FIG. 2. Changes in proportional water content (wc) (±SE) of individual *M. anisopliae* var. *acidum*-infected *S. gregaria* cadavers during incubation under diurnally fluctuating temperature and RH. Fifteen cadavers were weighed and transferred between temperature/RH treatments comprising 100% RH/25°C and 80% RH/40°C every 12 h. Starting wc = $59 \pm 0.02\%$ body mass. Symbols show the measured final wc of cadavers following 10-day incubation under each condition separately (● = 100% RH and 25°C and ■ = 80% RH and 40°C).

shortfall in conidial production was consistently greater when the low RH value was 43% compared with 75%.

The findings of Fargues and Luz (1998) together with the present studies highlight that fungal entomopathogens may require a period of rehydration under high humidity prior to the initiation of sporulation, and this requirement may prolong interruptions in sporulation under fluctuating moisture conditions. Indeed, there is evidence that exposure to saturation deficits that prevent sporulation commonly causes subsequent lags in the sporulation of entomopathogenic fungi under field conditions. For example, field observations of Gypsy moth, *Lymantria dispar*, infected with *Entomophaga maimaiga* showed that the hourly conidial discharge rate was best correlated with moisture conditions over the preceding 3 h (Hajek and Soper, 1992). A similar lag in peak conidial discharge under fluctuating moisture conditions has been observed with *Erynia* sp. infecting the alfalfa weevil, *Hypera postica*, both in the field (Millstein *et al.*, 1982) and in the laboratory (Millstein *et al.*, 1983). However, high water content of freshly killed insects may eliminate the lag in sporulation associated with older, drier cadavers. For example, the use of logistic regression analysis showed that, along with environmental moisture, the best predictor of sporulation of *Entomophthora grylli* on cadavers of the clearwing grasshopper, *Camnula pellucida*, in the field was cadaver age (fresh cadavers being most likely to sporulate) (Sawyer *et al.*, 1997). The fact that there was no interaction between cadaver age and environmental moisture on the probability of sporulation suggested that the higher starting water content of fresh cadavers was the key variable.

What emerges is that assessments of sporulation rates under favorable RH cannot necessarily be extrapolated to predictions about the infectivity of entomopathogenic fungi in natural environments, which are typified by climatic fluctuations. Against this background, it is unfortunate that relatively few studies quantify sporulation under realistic field conditions; indeed the present studies represented only gross simplifications of field conditions.

From a microbial control perspective, high humidity appears to be the most crucial climatic constraint on sporulation of *M. anisopliae* var. *acidum*, implying that its recycling potential will vary considerably between seasons and ecological zones. For example, in semiarid areas where there is demand for the myco-pesticide product, such as the northern Sahel or southern African Karoo, sporulation will almost certainly be restricted to rainy seasons and then only if air humidity in suitable microhabitats approaches saturation for extended periods, conditions which do not occur in all years. In observations of cadavers of various grasshoppers infected with *M. anisopliae* var. *acidum* in the

Sahel, cadavers readily dried out in the field and sporulation was only ever observed following several days of wet and overcast weather (Arthurs, 2000). However, a number of important acridids, such as *Zonocerus variegates* L., inhabit more humid ecological zones (COPR, 1982), where higher ambient humidity means that rainfall may not be essential. A better understanding of the dynamics involved in the transmission of entomopathogenic fungi in nature will require quantitative investigations into how interactions of temperature and environmental moisture affect sporulation.

ACKNOWLEDGMENTS

Thanks are due to Belinda Luke and Jane Gunn for help in the laboratory and to John Lawton and two anonymous reviewers for helpful reviews of the manuscript.

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