# Horizontal Transmission of the Entomopathogenic Fungus Metarhizium anisopliae (Imperfect Fungi: Hyphomycetes) and Hydramethylnon Among German Cockroaches (Dictyoptera: Blattellidae)<sup>1</sup>

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ABSTRACT Horizontal transmission of the entomopathogenic fungus, Metarhizium anisopliae (Metchnikoff) Sorokin, and hydramethylnon toxicant among individuals of the German cockroach, Blattella germanica (L.), was evaluated in this study. Transmission of hydramethylnon occurred through the feces. Contaminated feces were toxic to other cockroaches when mixed with standard laboratory diet at different ratios. Lethal time  $(LT_{50})$  of the nymphs increased as the proportion of contaminated feces in the healthy laboratory diet was decreased. When cockroaches were fed a diet consisting of hydramethylnon-contaminated feces and a laboratory diet at ratios of 1:0, 1:1, and 1:5, the mortality reached 100% at days 9, 12, and 17, respectively. The mortality was reduced to approximately 80% at a ratio of 1:10. Fifthstage nymphs exposed to the conidia of M. anisopliae or hydramethylnon toxicant for 6, 12, 24, or 48 h transferred the fungal conidia or the toxicant to healthy nymphs. Rate of mortality increased significantly by increasing the ratio of infected to unexposed cockroaches (i.e., 1:1 ratio > 1:10 ratio), and by increasing the exposure time for the infected cockroaches (48 h versus 6 h) to both M. anisopliae and hydramethylnon. Cockroaches killed by M. anisopliae or hydramethylnon before being presented to healthy cockroaches were less effective in spreading the fungus or toxicant than were live infected cockroaches. When live infected nymphs were mixed with healthy cockroaches, M. anisopliae initially killed cockroaches slightly faster  $(LT_{50}[95\% \text{ CL}] = 10.1 [9.2-11.0] \text{ d})$  than hydramethylnon  $(LT_{50}[95\% \text{ CL}] =$ 12.5 [11.4 - 13.7] d). However, cumulative mortality reached 100% at day 26 for both *M. anisopliae* and hydramethylnon treatments. After mixing healthy nymphs with dead infected cockroaches, the rate of mortality was slower for *M. anisopliae* ( $LT_{50} = 20$  [19.1 - 20.9] d) and hydramethylnon ( $LT_{50} = 14$ [13.4 - 14.7] d) than those recorded in the previous test (that is, mixing liveinfected nymphs with healthy cockroaches). However, the cumulative mortality 28 d after exposure to *M. anisopliae* and hydramethylnon reached 77 and 67%, respectively. Fungal growth was observed on all body parts of

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dead infected nymphs within 14 to 16 d of exposure to M. anisopliae. Dead infected nymphs were not cannibalized suggesting avoidance behavior by healthy nymphs. Consequently, the fungal conidia were not spread as effectively by dead nymphs as with live infected nymphs. Factors affecting performance in light of horizontal transmission of M. anisopliae are discussed.

Key Words Blattella germanica, Metarhizium anisopliae, hydramethylnon

Management of German cockroaches, *Blattella germanica* (L.), has relied primarily on the regular use of chemical insecticides in spray, bait, and dust formulations; however, some efforts have been directed to the use of biological control agents. Because of increasing societal concerns over pesticide residues and exposure of pest control operators to toxicants, the expense associated with the frequent use of insecticides, and the tendency for insecticide-resistant strains of cockroaches to develop, alternative methods for reducing cockroach populations are needed. One potential alternative is the use of biologicallybased insecticides, such as those containing entomopathogenic fungi.

The entomopathogenic fungus, Metarhizium anisopliae (Metchnikoff) Sorokin (Imperfect Fungi: Hyphomycetes), is a common fungal pathogen with a host range of over 200 species (Roberts and Yendol 1971). This fungus has been administered to many species of insects by a number of methods including direct sprays, injection, or by the application of the fungus to soil or plant material (Gunnarsson and Lackie 1985, Huxham et al. 1989, Krueger et al. 1992, Villani et al. 1994). To date, the majority of work evaluating M. anisopliae for biological control of insects has focused on applications involving agriculturally-important insect pests (Butt et al. 1992, Verkleij et al. 1992, Moorhouse et al. 1993). In addition, there are a few studies in which M. anisopliae has been examined as a potential agent in the control of household insects such as termites (Toumanoff 1965, Kramm et al. 1982), ants (Kelley-Tunis et al. 1995), and cockroaches (Gunnarsson and Lackie 1985, Gunner et al. 1991). No detailed information is available on the pathogenicity and horizontal transmission of M. anisopliae regarding B. germanica. Horizontal transmission occurs when a pathogen is transferred from individual to individual either through the integument or natural body openings but not directly from parent to offspring (Canning 1982). The Bio-Path<sup>™</sup> Cockroach Control Chamber, developed by the EcoScience Corp. (Worcester, MA) for the management of insects infesting structures, consisted of fungal conidia of M. anisopliae on a nutrient medium within an inoculation chamber.

Hydramethylnon, an amidinohydrazone insecticide, inhibits mitochondrial electron transport (Hollingshaus 1987) and causes delayed mortality by disruption of respiratory energy production (Silverman et al. 1986). The efficacy of hydramethylnon, in a bait form, has been documented against indoor cockroach populations (Reierson et al. 1982, Bennett and Runstrom 1984, Milio et al. 1986, Patterson and Koehler 1989, Appel 1990, Reid et al. 1990). Hydramethylnon has a delayed action, with no symptoms of intoxication until 24 h after exposure (Silverman and Shapas 1986). Insecticides with delayed activity are important for the control of ants (Su et al. 1980, Banks et al. 1981, Klotz and Reid 1993), termites (Su et al. 1982), and cockroaches (Silverman et al. 1991) because toxicant transfer among insects is enhanced.

Here, we report results of laboratory studies that determined the factors affecting the horizontal transmission of conidia of M. anisopliae and hydramethylnon toxicant among individuals of B. germanica. Our objectives were to (1) determine the passage of hydramethylnon through contaminated feces, (2) quantify the effect of horizontal transmission on pathogenecity and toxicity of M. anisopliae and hydramethylnon to cockroaches exposed to the fungus or the toxicant for a fixed time period, and (3) determine the potential for horizontal transmission of M. anisopliae and hydramethylon between live or dead infected/toxified cockroaches and healthy insects.

## **Materials and Methods**

**Test insects.** An insecticide-susceptible (JWax) strain of *B. germanica* (Koehler and Patterson 1986) was used in this study. This strain was isolated from the field (in the late 1930's) before the introduction of synthetic organic insecticides. Cockroaches were maintained in culture on a laboratory diet consisting of Wayne rodent blox (Continental Grain, Chicago, IL) and exposed to a photo period of 12:12 (L:D) h at  $26 \pm 1^{\circ}$ C and  $55 \pm 2\%$  RH. Test insects were fourth- or fifth-instar nymphs that were obtained by establishing large groups of late third- or fourth-instar nymphs, respectively, with food, water and harborage. On a daily basis, individuals found in these groups which had molted either to the fourth- or fifth-instar nymphs were distinguished from their cohorts by their white appearance (that is, before changing their color within 12 h). All experiments, outlined below, were conducted at  $24 \pm 1^{\circ}$ C and 55-58% RH.

**Fungus and toxicant.** The conidia of *M. anisopliae* were obtained from Bio-Path<sup>TM</sup> Cockroach Control Chambers. Within each chamber, a nutrientcontaining agar (50 ml), inoculated with *M. anisopliae*, formed a mat of hyphae and conidia on the ceiling of the chamber. A sterile polystyrene pad was placed on the floor of the chamber (Gunner 1991) to maintain a sufficiently high humidity. The opposing surfaces of the chamber were separated by a space of 2 to 3 mm through which the cockroaches were forced to pass through as they entered and/or exited a chamber, thus exposing the cockroaches to fungal conidia. Approximately  $1 \times 10^9$  colony forming units were present within each Bio-Path<sup>TM</sup> chamber. Horizontal transmission and pathogenicity of *M. anisopliae* were compared with hydramethylnon, with delayed toxicant activity, formulated at a concentration of 1.65% in the commercial COMBAT bait base (COMBAT Roach Killing System, The Clorox Company, Pleasanton, CA).

**Passage of toxicant through cockroach feces.** Approximately 500 2-d old fifth-instar *B. germanic* nymphs were held in a plastic box  $(26 \times 20 \times 10 \text{ cm})$  provisioned with hydramethylnon bait, standard laboratory diet, a 6-cm water vial, and a  $10\text{-cm}^2$  cardboard tent harborage. The inner walls of the box were coated with a thin film of petrolatum and mineral oil (1:3) which, together with a ventilated-lid, confined the cockroaches. Three days after starting the test, the laboratory diet was removed to increase the rate of cockroach

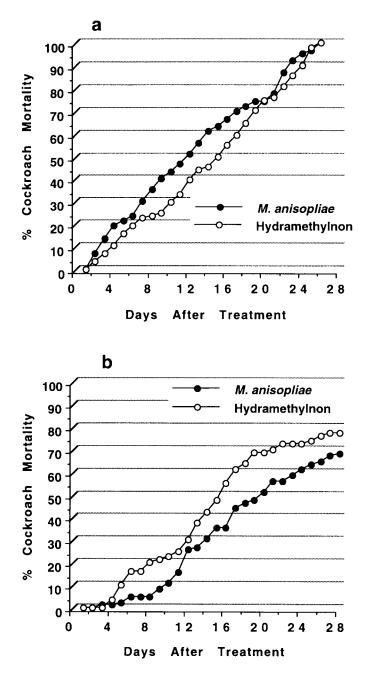


Fig. 2. Percent mortality of nymphal cockroaches after mixing (a) live or (b) dead infected or toxified cockroaches with healthy insects.

died (that is, 100% mortality) before the inoculation/bait chambers were removed, leaving dead infected cockroaches, feces, lab diet, water, and harborage. Twenty healthy fifth-instar nymphs were then mixed with the dead cockroaches. Experiments were replicated 4 times in both tests, and nymphal mortality was assessed daily for 28 d. Twelve dead cockroaches from the *M. anisopliae* treatment were observed under the microscope to confirm the external presence or absence and location of *M. anisopliae*.

**Statistical analysis.** Lethal times (LT<sub>50</sub>s) obtained from each experiment were estimated by regression of the cumulative probit mortality versus the log10 of time (d) (SAS Institute 1990). A criterion of non overlapping 95% confidence limits (CL) of LT<sub>50</sub> was used to define significant differences between treatments.

#### **Results and Discussion**

**Passage of hydramethylnon toxicant through cockroach feces.** Fifthinstar nymphs that fed on hydramethylnon bait produced feces that was toxic to healthy cockroaches. Similar results were reported by Silverman et al. (1991).  $LT_{50}$ , in this test, increased significantly (i.e., 95% CL do not overlap) as the proportion of contaminated feces in the laboratory diet was decreased (Table 1). The mortality of cockroaches as a function of time after exposure is shown in Fig. 1. At 21 d, mortality of nymphs fed only the rodent blox or the non-contaminated feces was 2% and 7% respectively. When cockroaches were fed contaminated feces at ratios of 1:0, 1:1, and 1:5 (feces : rodent blox), the mortality rate reached 100% at day 9, 12, and 17, respectively. At a 1:10 ratio, hydramethylnon provided 80% mortality within 21 d. The toxic effect in this study was attributable to hydramethylnon in the feces.

The mortality produced by hydramethylnon-contaminated feces indicated horizontal transmission. The delayed action of hydramethylnon and its low rate of metabolism by insects are probably responsible for its activity against individuals that do not feed directly on the toxic bait (Silverman et al. 1991). Aggregation pheromone, secreted with the hydramethylnon-contaminated feces, may mark a potential attractive food source for *B. germanica* (Ishii and Kuwahara 1967). Also, the composition of the bait base used in this test may have affected the level of mortality by influencing hydramethylnon excretion or feces palatability. Although horizontal transmission occurred when the sole food source was mixed with contaminated feces, it may not have occurred when the food source was unadulterated.

**Forced exposure test.**  $LT_{50}$  for *B. germanica* nymphs following forced exposure to the conidia of *M. anisopliae* or after feeding on hydramethylnon are shown in Table 2. Mortality increased significantly (P < 0.05, 95% CL do not overlap) as the proportion of infected or toxified cockroaches to unexposed cockroaches increased (that is, higher mortality at 1:1 ratio than at a 1:10 ratio) and with increased exposure time (that is, higher mortality after 48 h of exposure than after 6 h) (Table 2). When fifth-instar nymphs were exposed to *M. anisopliae* for 6 h and then mixed with unexposed nymphs, the 1:1 ratio resulted in 56.3% mortality by the end of the test period ( $LT_{50} = 34.3$  d), while only 20.5% of the test population died in tests at the 1:10 ( $LT_{50} = 53.7$  d) (Table 2). When

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Ratio of contaminated feces to laboratory diet (wt/wt)	n*	$X^{2**}$	Slope ± SE	$LT_{50}^{\dagger} (95\% \text{ CL})$
1:0	540	13.2	$4.0 \pm 0.4$	3.4 (2.9 - 3.9)
1:1	780	25.8	$3.5 \pm 0.4$	5.5 (4.9 - 6.3)
1:5	960	37.3	$2.8 \pm 0.3$	8.2 (7.1 - 9.4)
1:10	1260	16.0	$2.4\pm0.2$	11.9 (11.1 - 12.9)

Table 1. Lethal time to 50% mortality $(LT_{50})$ of German cockroach
nymphs following exposure to hydramethylnon-contaminated
feces or a laboratory diet + contaminated feces.

\* Number of observations included in the regression analysis.

\*\* Values of the chi-square, fiducial limits were calculated using a t value of  $\geq$  1.96.

 $\dagger$  Lethal time (days) before death of 50% of German cockroach nymphs following exposure to hydramethylnon contaminated-feces or a laboratory diet + contaminated-feces. LT<sub>50</sub>s with overlapping 95% confidence limits are not significantly different.

nymphs exposed to *M. anisopliae* for 48 h were mixed with unexposed cockroaches at a 1:1 ratio, mortality among the healthy cockroaches increased more rapidly and all cockroaches were dead by day 16 ( $LT_{50} = 12.8$  d). At the 1:10 ratio, only 23.9% mortality was reached 28 d after exposure ( $LT_{50} = 44.0$  d). Similar results were obtained with hydramethylnon (Table 2). Mortality in the control treatments, at various exposure times and ratios, ranged from 0 to 6.3%.

Brief exposure to M. anisopliae consistently provided faster kill than hydramethylnon. Significant differences in activity between M. anisopliae and hydramethylnon occurred at each ratio of exposed to unexposed cockroaches for 6 and 12 h, except at a 1:1 ratio for 12 h exposure time (Table 2). Differences in activity between treatments occurred only at the 1:1 ratio after exposure of "donor" cockroaches to the fungus or the toxicant for 24 h; in the 48 h exposure time treatment, differences in activities occurred at 1:1 and 1:10 ratios.

The presence of M. anisopliae spores on the integument of infected cockroaches may have resulted in an increase in the rate at which the contaminated individuals were groomed by other individuals. In our study, fungal growth was observed on dead cockroaches in all treatments, but the most fungal growth occurred in the 48 h exposure time. Kramm et al. (1982) noted that healthy termites which groomed M. anisopliae-infected termite for 15 sec experienced considerable mortality, and when the grooming time was increased to 4 min, the previously unexposed termites died as if they were continuously exposed to the infected individual.

Mixing live or dead infected cockroaches with healthy insects. In the first test, where live infected cockroaches were mixed with healthy insects, 20 to 25% cockroach mortality was reached at day 4 with hydramethylnon and at day 7 for *M. anisopliae*. When the remaining 75 to 80% of live infected nymphs were mixed with healthy nymphs, the conidia of *M. anisopliae* 

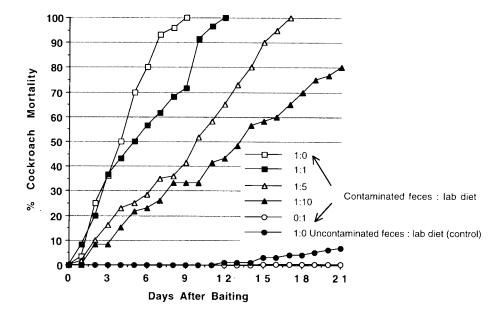


Fig. 1. Mortality of nymphal German cockroaches fed hydramethylnoncontaminated feces.

resulted in significantly (P < 0.05) faster (95% CL do not overlap) kill of cockroaches ( $LT_{50}$  [95% XL] = 10.1 [9.2 - 11.0] d, slope ± SE = 2.8 ± 0.2,  $X^2$  = 102.3) than hydramethylnon ( $LT_{50}$  [95% CL] = 12.5 [11.4 - 13.7] d, slope ± SE = 3.2 ± 0.3,  $X^2$  = 146.9) (Fig. 2a). However, the cumulative cockroach mortality for both *M. anisopliae* and hydramethylnon reached 100% at day 26; mortality reached 5% in the control treatment.

Dead nymphs used in the second test (that is, mixing dead-infected cockroaches with healthy insects) were obtained at day 9 with hydramethylnon, and at day 13 with *M. anisopliae*.  $LT_{50}$  values resulting from mixing healthy and dead infected nymphs were higher than in the previous test. The  $LT_{50}$  (95% CL) was 20 (19.1 - 20.9) d for *M. anisopliae* (slope ± SE = 3.4 ± 0.2,  $X^2$  = 10.8) and 14 (13.4 - 14.7) d for hydramethylnon (slope ± SE = 2.8 ± 0.1,  $X^2$  = 19.9). The cumulative cockroach mortality at 28 d was 67 and 77% after exposure to dead infected nymphs exposed to *M. anisopliae* and hydramethylnon, respectively (Fig. 2b); mortality reached 2.5% in the control treatment.

Transmission of the fungus or toxicant in cockroach populations may be enhanced by the behavior of live cockroaches. Cockroaches killed by *M. anisopliae* or hydramethylnon before being mixed with healthy cockroaches were less effective in spreading the fungus or toxicant than were live infected cockroaches. The reduction in the frequency of contact between exposed and unexposed cockroaches may be an important factor affecting the transmission of the fungus or toxicant.

Exposure Time, h	Treatment	Ratio of exposed to unexposed nymphs	n*	$X^{2**}$	Slope ± SE	LT <sub>50</sub> † (95% CL)	% mortality at 28d‡
9	M. anisopliae	$1:1 \\ 1:5 \\ 1:10$	$\begin{array}{c} 448 \\ 1344 \\ 2464 \end{array}$	$\begin{array}{c} 4.0\\ 10.8\\ 4.5\end{array}$	$3.8 \pm 0.5$ $2.9 \pm 0.2$ $3.1 \pm 0.3$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	56.3 60.4 20.5
	Hydramethylnon	1:1 1:5 1:10	$\begin{array}{c} 448 \\ 1344 \\ 2464 \end{array}$	7.8 4.4 4.4	$\begin{array}{c} 2.1 \pm 0.3 \\ 2.3 \pm 0.3 \\ 1.7 \pm 0.2 \end{array}$	35.3 (28.0 - 52.8) 43.2 (36.2 - 56.1) 124.4 (82.4 - 248.0)	56.3 35.4 13.6
12	M. anisopliae	1:1 1:5 1:10	448 1344 2464	14.7 12.4 7.5	$2.4 \pm 0.4$ $2.4 \pm 0.2$ $2.5 \pm 0.2$	$\begin{array}{rrrr} 31.0 & (25.7 & -42.2) \\ 29.8 & (26.4 & -34.1) \\ 58.1 & (47.7 & -77.4) \end{array}$	62.5 54.2 21.6
	Hydramethylnon	1:1 1:5 1:10	$\begin{array}{c} 448 \\ 1344 \\ 2464 \end{array}$	14.7 7.0 4.2	$\begin{array}{c} 1.2 \pm 0.2 \\ 1.9 \pm 0.2 \\ 1.5 \pm 0.2 \end{array}$	$\begin{array}{c} 59.7 \ (37.3 - 165.6) \\ 52.2 \ (41.5 - 73.7) \\ 130.2 \ (86.2 - 253.1) \end{array}$	$\begin{array}{c} 43.8\\ 35.4\\ 13.6\end{array}$
24	M. anisopliae	1:1 1:5 1:10	$320 \\ 1344 \\ 2464$	$\begin{array}{c} 6.8\\ 96.4^{*}\\ 11.5\end{array}$	$14.3 \pm 1.7 \\ 7.2 \pm 0.8 \\ 5.0 \pm 0.5$	$\begin{array}{rrrr} 14.7 \ (14.1 & -15.2) \\ 20.5 \ (19.3 & -21.9) \\ 36.6 \ (33.4 & -41.5) \end{array}$	100.0 (20 d) 97.9 23.9
	Hydramethylnon	1:1 1:5 1:10	$288 \\ 1344 \\ 2464$	$30.1 \\ 166.2^{*} \\ 9.9$	$2.4 \pm 0.5$ $2.2 \pm 0.4$ $2.5 \pm 0.2$	$\begin{array}{rrrrr} 10.1 \left( \begin{array}{ccc} 8.0 & - & 13.3 \right) \\ 27.4 \left( 21.3 & - & 43.9 \right) \\ 45.0 \left( 39.1 & - & 54.5 \right) \end{array}$	100.0 (18 d) 72.9 34.1
48	M. anisopliae	1:1 1:5 1:10	$272 \\ 1200 \\ 2464$	$\begin{array}{c} 28.3 \\ 779.9^{*} \\ 14.3 \end{array}$	$2.2 \pm 0.5$ $4.0 \pm 1.6$ $3.0 \pm 0.3$	$\begin{array}{rrrr} 12.8 (& 9.9 - & 19.8) \\ 19.9 (14.3 - & 66.6) \\ 44.0 (38.5 - & 53.0) \end{array}$	100.0 (17 d) 100.0 (25 d) 23.9
	Hydramethylnon	1:1 1:5 1:10	$272 \\ 1296 \\ 2464$	20.4 161.6* 14.2	$1.7 \pm 0.3$ $2.1 \pm 0.4$ $2.1 \pm 0.1$	$\begin{array}{c} 5.5 \left( \begin{array}{c} 4.2 \\ 23.6 \left( 18.5 \\ -36.6 \right) \\ 79.0 \left( 59.3 \\ -119.1 \right) \end{array}$	100.0 (17 d) 100.0 (27 d) 37.5

caused by systematic departure from the model; the value of 2.11 was used in computing fiducial limits. \*\* Sin

+ Lethal time (days) before 50% of German cockroach nymphs died following exposure of M. anisopliae or hydramethylnon. LT50 with overlapping 95% confidence limits are not significantly different.

 $\ddagger$  Numbers in parentheses indicate the number of days in which 100% mortality was reached.

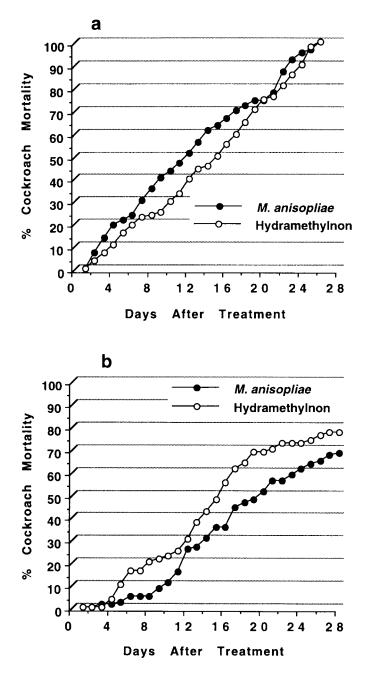


Fig. 2. Percent mortality of nymphal cockroaches after mixing (a) live or (b) dead infected or toxified cockroaches with healthy insects.

Fungal growth was observed on all body parts of dead nymphs within 14-16 days of exposure to *M. anisopliae*. The fungal mycelium sporulated on the body of dead nymphs; nymphs were not cannibalized, suggesting an avoidance behavior by unexposed nymphs. Consequently, the fungal conidia were not spread as effectively as with live infected cockroaches. Similar observations were reported by Kramm et al. (1982) in *Reticulitermes* sp. termites. In addition, the design of the inoculation chamber may affect the amount of conidia received by an individual cockroach. The inoculation chamber used in our tests was placed in such a manner that the fungus was on the floor of the chamber and not on the ceiling as in the original Bio-Path<sup>™</sup> chamber. Therefore, the cockroaches were infected, when they contacted the fungus in the chamber or, during grooming, from spores initially acquired on their legs (including tarsi), mouth parts, antennae, and abdominal sternites. This varies from the Bio-Path<sup>™</sup> design where cockroaches are infected from spores initially acquired on their abdominal tergites. To maximize infectivity of the fungus, the geometry of the chamber of *M. anisopliae* could be improved so that the fungus grows on the bottom, top, and/or lateral sides of the chamber.

The process of host invasion, host specificity, virulence, the stability and persistence of entomopathogenic fungi in the environment, and the cause of death by deuteromycete infection in insects have been reviewed by Ferron (1978), Andreadis (1987) and Ignoffo (1988). Metarhizium anisopliae produces enzymes which degrade cuticular lipids. These enzymes assist penetration of the fungus through the cuticle (Gillespie and Claydon 1989, Gupta et al. 1991, Leger et al. 1991). Huxham et al. (1989) stated that the Destruxins A and B, cyclic depsipeptides produced by *M. anisopliae*, inhibited or reduced the activity of hemocytes (the freely circulating cells associated with the immune response) of the American cockroach Periplaneta americana (L.) and the locust Schistocerca gregaris Forsk. The conidia germinate after attachment of the fungus to the cockroach integument. Subsequently, the germ tube of germinating conidia penetrate the cuticle of the cockroach until they reach the internal body cavity (hemocoel), thereby killing the cockroach. Gunnarsson and Lackie (1985) have also shown that P. americana exhibited a defense reaction (nodule formation) to the injected suspension of conidiospores of *M. anisopliae*. However, no mention of the potential of the fungus for cockroach control was made.

From this study, it is clear that horizontal transmission of M. anisopliae or hydramethylnon toxicant bait form may occur among B. germanica. Both hydramethylnon and M. anisopliae have good potential for use in integrated control of B. germanica. However, this potential is dependent on a number of critical variables. In this study, infestation of a large proportion of the population seems to have a profound impact on the performance of this fungal pathogen and hydramethylnon for control of B. germanica. Transmission is density dependent in that the larger the proportion of infected individuals, the greater the chance for an epizootic causing a population decline (Andreadis 1987). Density of the fungus, the amount of conidia acquired by the individual cockroach, and the viability of the spores over time in storage or the field environment are other important variables. Walstad et al. (1970) stated that spores of M. anisopliae stored at 21°C lost all viability after only a few months, whereas spores stored at  $8^{\circ}$ C remained viable for at least one year. Future studies should be directed toward screening a more virulent strain of *M*. *anisopliae* that better recognizes the cockroach cuticle as a stimulus to attach and germinate.

Age and composition of the bait base and the culture medium, or the food attractants available within the bait/inoculation chamber are critical variables affecting the performance of both *M. anisopliae* and hydramethylnon for cockroach control. Gunner et al. (1991) showed that the effectiveness of the inoculation chamber in reducing cockroach populations was identical between fresh chambers and chambers 3 to 6 wk old. They stated that the addition of a cockroach attractant to the inoculation chamber increased cockroach mortality relative to chambers without the attractant. Because no attractants are present in the Bio-Path<sup>TM</sup> Cockroach Chamber, the effectiveness of the fungus in killing the cockroaches might be improved by including attractants such as an aggregation pheromone, banana extract, molasses, or laboratory diet.

Entomopathogenic fungi have rigid environmental requirements, in particular with respect to relative humidity, for conidial production, viability, and host infection. The fungus *M. anisopliae*, however, can reproduce over a wide range in temperature (10 to  $40^{\circ}$ C), and relative humidity above 92.5% (Walstad et al. 1970). Brief exposure to < 75% relative humidity reduces infectivity. Presently, there is a need for research to develop moisture-retaining formulations to allow fungal growth at sub-optimal humidity and to target those cockroaches foraging in such an environment.

Results on horizontal transmission and pathogenicity of the fungal conidia of M. anisopliae within a population are important for the development of strategies using this fungus for the control of B. germanica in the field. The research discussed here was conducted under a manageable and observable laboratory environment (constant temperature and relative humidity), on an insecticide-susceptible strain of B. germanica, and in a small testing arena. Thus, there is a need for more quantitative field studies with large populations, resistant strains, and under a range of environmental conditions. Additional research will hopefully improve the understanding of the direct interactions of this naturally occurring fungus and cockroaches in an urban environment, and will also assess the contribution of this fungus to German cockroach management programs.

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