Neotropical Entomology

ISSN: 1519-566X

journal homepage: www.scielo.br/ne

BIOLOGICAL CONTROL

Differential Susceptibility of Adults and Nymphs of *Blattella germanica* (L.) (Blattodea: Blattellidae) to Infection by *Metarhizium anisopliae* and Assessment of Delivery Strategies

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Keywords
German cockroach, microbial control, entomopathogenic fungi

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Edited by Madelaine Venzon – EPAMIG

Received 29 January 2010 and accepted 15 December 2010

Abstract

Microbial insecticides for cockroach control, such as those containing entomopathogenic fungi, may be an alternative to reduce contamination by chemicals in housing and food storage environments. Virulence of isolate ESALQ1037 belonging to the *Metarhizium anisopliae* complex against nymphs and adults of *Blattella germanica* (L.), and its infectivity following exposure of insects to a contaminated surface or to *M. anisopliae*-bait were determined under laboratory conditions. Estimated LD_{50} 15 d following topical inoculation was 2.69 x 10^5 conidia per adult, whereas for nymphs the maximum mortality was lower than 50%. Baits amended with *M. anisopliae* conidia had no repellent effect on targets; adult mortality was inferior to 25%, and nymphs were not susceptible. All conidia found in the digestive tract of *M. anisopliae*-bait fed cockroaches were unviable, and bait-treated insects that succumbed to fungal infection showed a typical mycelial growth on mouthparts and front legs, but not on the hind body parts. As opposed to baits, the use of a *M. anisopliae* powdery formulation for surface treatment was effective in attaining high mortality rates of *B. germanica*. Both nymphs and adults were infected when this delivery strategy was used, and mycelia growth occurred all over the body surface. Our results suggest that the development of powders or similar formulations of *M. anisopliae* to control *B. germanica* may provide faster and better results than some of the strategies based on baits currently available.

Introduction

Fungi are organisms that act through contact, penetrating the cuticle through mechanical force and enzymatic action to colonize the insect's hemocoel (Gupta *et al* 1991, St. Leger *et al* 1991). The entomopathogenic fungus *Metarhizium anisopliae* has a broad geographical distribution, and is easily isolated from soil samples and several arthropods. Fungal isolates within the *M. anisopliae* complex are known to infect more than 200 species of insects from different orders (Zimmermann 1993). *Metarhizium anisopliae* has been widely applied in agriculture and is regarded as an important pest control agent worldwide in several agroecosystems (Faria & Wraight 2007, Alves *et al* 2008, Li *et al* 2010).

The use of conventional insecticides in housing environments and in food storage areas may provoke human and pet contamination (Wickham 1995, Cochran 1999). Biological strategies for cockroach control could reduce such risks and provide a longer-term effect. In spite

of considerable advances in studies with *M. anisopliae* in agricultural settings, there are few references to its use in urban areas. Regarding cockroaches, a few studies have evaluated the potential of fungal isolates towards *Blattella germanica* (L.) (Steenberg *et al* 1997, Pachamuthu *et al* 1999), their horizontal transmission capability (Kaakeh *et al* 1996, Quesada-Moraga *et al* 2004) and their association with chemical insecticides (Kaakeh *et al* 1997, Pachamuthu & Kamble 2000, Zurek *et al* 2002). Behavioral characteristics of cockroaches, such as aggregation (Kaakeh *et al* 1996) and hiding preference for spots with high humidity, may favor the horizontal transmission of the fungus. However, few of the applied studies have focused on control strategies regarding fungus delivery systems in urban environments.

Efficient treatments are important for horizontal transmission of the pathogen from diseased to healthy insects (Kaakeh *et al* 1996, Quesada-Moraga *et al* 2009), causing epizootics in field populations and a longer-term control effect. Unfortunately, the differential susceptibility of *B. germanica* nymphs and adults to fungal pathogens and different delivery strategies have not been tested. Therefore, the objective of the present study was to evaluate the differential susceptibility of *B. germanica* nymphs and adults to infections by *M. anisopliae*, as well as to assess the infectivity of this pathogen when applied as baits or as a powdery formulation for surface treatment.

Material and Methods

Insects

Insects were reared in glass containers (55 x 25 x 30 cm) covered with voil in order to keep them from escaping and to allow for ventilation. Corrugated cardboard tube shelters were arranged in the bottom of the recipient, which was maintained at 27 ± 2ºC, 60 ± 10% RH, in a dark room. Water in plastic drinking bottles and commercial dog-food (Purina Dog Chow®, Nestlé Brasil Ltda., São Paulo, SP, Brazil) were provided weekly. The recipients were cleaned on a monthly basis, when adults would be eliminated and only the immature would be kept in the rearing container.

Production of M. anisopliae *conidia*

The isolate ESALQ1037 was originally isolated from fire ant adults (*Solenopsis invicta* Buren) from Porto Alegre, state of Rio Grande do Sul, Brazil. This isolate is maintained in the fungal collection of the Laboratório de Patologia de Insetos e Controle Microbiano at ESALQ – USP. Stored conidia were transferred to Petri dishes with complete media (0.36 g KH_2PO_4; 1.05 g Na_2HPO_4; 0.6 g $MgSO_4$; 1.0 g KCl; 1.58 g $NaNO_3$; 10 g anhydrous glucose; 5 g yeast extract; 20 g agar; 1000 ml distilled water) and Petri dishes were incubated for a 7-day period at 26 ± 1ºC and 12h photophase for conidial production. Conidia were then scraped off with a sterile metal spatula and stored at -30ºC, constituting the inoculum used in all the experiments. At the time of bioassay preparation, the stored conidia were used for production of fresh conidia on solid media as previously described.

Dose-response of B. germanica *to* M. anisopliae

Virulence of *M. anisopliae* towards nymphs and adults of *B. germanica* was evaluated by topical applications. For the adults, each treatment consisted of 48 insects, divided into three replicates with eight males and eight females each. Only highly active and mobile adults were selected for this experiment, avoiding the slow moving and moribund insects. For the immature stage, 20 to 22 d-old unsexed nymphs were used, in three replicates of 16 nymphs for each treatment. Nymphs at this age were at the third instar and were between 5 mm and 7 mm long. In order to obtain nymphs of known age, females with darkened and well developed oothecae were anesthetized with CO_2 and the final portion of the abdomen was pressed with tweezers for oothecae release. The oothecae were kept into plastic cups with moistened cotton and were maintained at 27 ± 1ºC, in complete darkness until nymph eclosion.

Conidial suspensions were prepared by adding conidia to sterile water with 0.1 ml Tween 20® L^{-1} and standardized following counts using a Neubauer chamber. Conidia suspensions were sonicated and filtered through a cloth to remove mycelial particles and conidia clusters previous to their use. However, for the highest concentration ($2x10^9$ conidia ml^{-1}), conidia were visibly clumped when in suspension. Doses of 2×10^4, 6.3×10^4, 2×10^5, 6.3×10^5 and 2×10^6 conidia/insect were evaluated for both adults and nymphs. The control group received only sterile water with Tween 20®.

Cockroaches were anesthetized with CO_2 for 20 s, and each insect was inoculated with 1 µl of the conidial suspension in the thorax (sterna region) with a glass micro syringe coupled to an automatic micro applicator (Burkard Manufacturing Co. Ltd., Rickmansworth, UK). Following inoculation of a group of eight insects, conidial suspension was removed from the micro syringe and agitated for better homogenization. Insects were transferred to plastic cups (6 x 10 cm) containing water and food, and kept at 27 ± 1ºC, relative humidity above 90% and complete dark. Visual count was done every three days and the number of dead insects in each replicate was scored. The cadavers were transferred to plates with moistened cotton for the confirmation of mortality by the fungus. Both experiments were repeated twice on different dates.

Metarhizium anisopliae *infectivity following exposure to contaminated surfaces*

Infectivity was assessed for adults and nymphs by exposing insects to *M. anisopliae*-dusted surfaces. Insect selection and incubation conditions were as described earlier. Conidia collected directly from the 7 d-old cultures were mixed with mineral powder talc ($Al_2Si_2O_5(OH)_4$) at concentrations of 1%, 5% and 10%, whereas insects in the control were exposed only to talc. Estimation of the number of conidia in powder formulation was performed, suspending 0.001 g of fresh conidia in sterile water with 0.1 ml L^{-1} of Tween 20® and counting the number of conidia using a Neubauer chamber after serial dilution. Mixtures were then made by adding conidia to mineral talc (w/w) and shaking the powder into hermetic plastic bags. In each treatment, six 15 cm x 15 cm ceramic tiles were treated with 1.3 mg of the *M. anisopliae*-powder per cm^2, homogeneously spread with a brush. Doses of 6.5 x 10^5, 3.25 x 10^6 and 6.5 x 10^6 conidia cm^{-2} were obtained on the tiles.

Groups of 16 insects were anesthetized with CO_2 and transferred to a plastic cup, which was placed upside down onto a treated tile. The surface of exposure of insects to the treated surface correspond the diameter (10 cm diameter) of the plastic cup. The inner edge of each cup received a thin layer of vaseline in order to prevent cockroaches from climbing it and to force them to contact the contaminated surface. Insects were kept confined for 10 min in contact with the treated surface and were induced to walk to an untreated tile placed beside the treated one at the end of the exposure period. Insects were then transferred to cups by turning untreated tiles 180 degrees, which also prevented contamination of the cup and insects by excess *M. anisopliae*-amended powder from the treated surface. Water and food were provided, and treated insects were kept under 27 ± 1ºC in complete darkness until the end of the experiment. Under these conditions, RH inside recipients was above 90%.

The evaluations were performed every three days during 15 days. The cadavers were transferred to plates with moistened cotton for the confirmation of mortality by the fungus. The viability of *M. anisopliae* conidia was evaluated for the powdery mix (1%) at 1, 72 and 120 h post-storage at 27 ± 1ºC and RH above 90%. Four replicates of 100 mg each of the talc mix was added to 9.9 ml of distilled water and then shaken for two minutes. After successive dilutions, a 0.1ml conidial suspension was placed onto potato dextrose agar medium (PDA – Difco Laboratories, Detroit, MI, USA) and the plates incubated at 27 ± 1ºC and 12h photophase. Following a 120h period, the number of colony forming units (CFU) was counted. The number of CFU 1h after powdery preparation was considered as the initial conidial viability.

Infectivity following exposure to M. anisopliae*-based bait*

Infectivity was also assessed for adults and nymphs through exposure of an attractive *M. anisopliae*-amended bait prepared with milk powder and yeast extract (5:1), to which *M. anisopliae* conidia was added at the concentrations (w/w) of 1% (5 x 10^8 conidia g^{-1}), 5% (2.5 x 10^9 conidia g^{-1}) and 10% (5 x 10^9 conidia g^{-1}). Estimation of the number of conidia in bait was performed by counting a conidia sample using a Neubauer chamber after serial dilutions as earlier described. Two hundred and fifty milligrams of each bait was pressed into plastic feeding tubes (adapted P200 pipette tips 0.5 cm diameter x 3 cm long), which were just large enough for the introduction of the insect's head and front legs into them. Six feeding tubes were used for each treatment. Each replicate, consisting of one plastic cup holding 16 insects, received one feeding tube, which remained as the only food source for the first 72 h. Afterwards, dogfood and water were provided. The control treatment consisted of baits without fungal structures. All treatments were kept under 27±1ºC and RH above 90% in complete darkness until the end of the experiment.

The evaluations were performed every three days for 15 days. The cadavers were transferred to plates with moistened cotton for the confirmation of mortality by the fungus. In order to find out whether there was a repellent effect due to the presence of conidia, the average consumption of food by adults was estimated by calculating the weight loss of the feeding tubes plus bait for all treatments before and after a 72h period. Viability was determined as explained earlier.

Viability of M. anisopliae *conidia in the digestive tract of* B. germanica

A group of 40 unsexed *B. germanica* adults, divided into four replicates of 10 insects each, was confined in plastic cups. Each container received 1 g of *M. anisopliae*-amended bait (1%) through a feeding tube. After 48h, insects were killed with CO_2 and dissected on a Petri dish with distilled water. For each insect treated with the bait, the digestive tract was entirely removed through a transversal cut of the abdomen and transferred to a glass slide. Twenty digestive tracts were evaluated directly under a microscope, checking for the presence of fungal structures and their developmental stages. For the remaining 20 insects within the same group, the contents of the digestive tract were diluted in 2 ml of distilled water and transferred to a PDA medium with streptomycin (0.5g L^{-1}). The Petri dishes were incubated (27 ± 1ºC and 12h photophase) for 24h and 48h, and the percentage of conidial germination was determined.

Statistical analyses

Mortality percentages within the 15-day period following topic inoculation were corrected by Abbott (1925) and submitted to Probit analyses for determination of the dose-response curve and estimates of LD_{50} for adults. Survival analysis was applied to infectivity data on contaminated surface data in order to estimate the mean and median survival times (ST_{50}) and their 95% fiducial limits ($FL_{95\%}$). The Statistical Package for Social Science 11.0 for Windows (SPSS, 2002) was used to compute nonparametric estimates of the survivor function by the Kaplan-Meier method, and the Log-Rank (Montel-Cox) test with $\alpha = 5\%$ probability for pairwise comparisons among treatments. Mortality percentages caused by different conidia doses on each inoculation methods were submitted to analysis of variance and the averages compared by the Tukey´s test ($P < 0.05$), using the SAS 8.02 software (SAS Institute, 1999-2001).

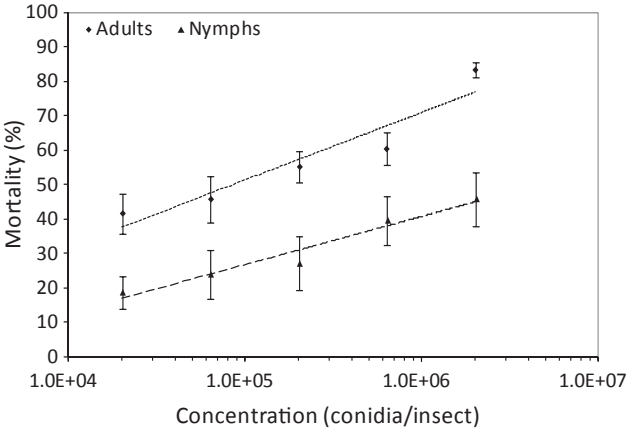

Fig 1 Mortality of *Blattella germanica* adults and nymphs exposed to different conidia concentrations of *Metarhizium anisopliae* isolate ESALQ1037 at 15 days after topical application (27 ± 1ºC, RH > 90% and scotophase of 24h). Bars depict mean ± SE (standard error).

Results

Dose-response of B. germanica *to* M. anisopliae

Conidia used in the experiments showed over 95% viability. The insect mortality increased with the increase in the concentration of the inoculated conidia. Mortality percentages for adults and nymphs treated with the lowest and highest doses ranged from 41.7% to 83.3% and from 18.7% to 45.8%, respectively (Fig 1). Cadavers presented mycelial growth and conidiation in the intersegmental regions when submitted to high humidity conditions. For non-treated insects, mortality averages were always lower than 10% and symptoms of disease were not observed. The LD_{50} obtained for adults was 2.69 x 10^5 conidia per individual, with limits between 5.43 x 10^4 and 8.06 x 10^5 conidia (C.I. 90%; $t = 5.23$; $\chi^2 = 5.02$). LD_{50} for nymphs was not calculated, since the highest mortality percentage was lower than 50%.

Metarhizium anisopliae *infectivity following exposure to contaminated surfaces*

Mortality of *B. germanica* nymphs and adults was higher when submitted to surfaces contaminated with the pathogen (Table 1). No symptoms of disease were observed for untreated insects. Mortality percentage for nymphs was up to 59% at nine days post-application

Table 1 Cumulative mortality (%) of *Blattella germanica* nymphs and adults exposed to different doses of *Metarhizium anisopliae* (ESALQ1037) presented as baits or powder formulation (27 ± 1ºC, RH > 90%, 24h scotophase).

M. anisopliae-amended powder (conidia cm^{-2})	Nymphs		Adults	
	Days after inoculation			
	9 days[1]	15 days[1]	9 days[1]	15 days[1]
Control	1.1 ± 1.0 a	1.1 ± 1.0 a	7.3 ± 3.0 a	16.7 ± 5.7 a
6.50 x 10^5	59.4 ± 9.5 b	60.4 ± 7.6 b	55.2 ± 6.5 b	86.5 ± 4.4 b
3.25 x 10^6	82.3 ± 5.4 b	83.3 ± 5.5 b	93.7 ± 2.3 c	98.9 ± 1.0 c
6.50 x 10^6	73.9 ± 7.5 b	76.1 ± 7.3 b	96.9 ± 1.4 c	100.0 ± 0.0 c
M. anisopliae-amended bait (conidia g^{-1})	9 days [ns]	15 days [ns]	9 days [ns]	15 days
Control	1.1 ± 1.0	2.1 ± 1.3	3.1 ± 2.1	8.3 ± 2.1 a
5.0 x 10^8	1.1 ± 1.0	1.1 ± 1.0	12.6 ± 3.6	21.8 ± 6.2 ab
2.5 x 10^9	0.0 ± 0.0	0.0 ± 0.0	8.3 ± 3.1	12.5 ± 3.6 ab
5.0 x 10^9	0.0 ± 0.0	1.1 ± 1.0	9.4 ± 1.4	28.1 ± 4.7 b

For each formulation, mean (± standard error) followed by the same letter in the columns are not significantly different (Tukey test $P > 0.05$); [ns]non significant

[1]transformed data ($\sqrt{x+1}$)

and differed from that obtained in the control ($F = 71.12$; df = 3, 20; $P < 0.001$). Adult mortality in treated samples was also higher than that of untreated samples in the same period ($F = 280.85$; df = 3, 20; $P < 0.001$). Fifteen days post-application, adult mortality was over 85% for the lowest concentration, and ≥ 98.9% for the highest concentration ($F = 440.50$; df = 3, 20; $P < 0.001$), whereas the highest mortality rate for nymphs was 83.3% ($F = 98.30$; df = 3, 20; $P < 0.001$). Besides, this dusting technique resulted in faster mortality of treated insects when compared to the use of baits.

Mean survival times (ST_{50}) were lower for adults and nymphs exposed to a surface treated when compared to the untreated insects (Table 2). Surfaces treated with 3.25×10^6 or 6.5×10^6 conidia cm^{-2} of *M. anisopliae* reduced the mean and median adult survival time when compared to the lowest conidial concentration tested (equivalent to 6.5×10^5 conidia cm^{-2}) ($\chi^2 = 57.15$, $P < 0.001$ and $\chi^2 = 72.38$, $P < 0.001$, respectively). Differences in survival were also observed for nymphs exposed to 3.25×10^6 or 6.5×10^6 conidia cm^{-2} when compared to nymphs exposed to 6.5×10^5 conidia cm^{-2} ($\chi^2 = 17.01$, $P < 0.001$ and $\chi^2 = 7.37$, $P = 0.007$, respectively). However, no differences in survival were observed between adults and nymphs exposed to 3.25×10^6 ($\chi^2 = 3.36$, $P = 0.07$) or 6.5×10^6 conidia cm^{-2} ($\chi^2 = 0.53$, $P = 0.47$).

Infectivity following exposure to the M. anisopliae*-based bait*

Baits amended with *M. anisopliae* did not repelled cockroaches when compared to a fungus-free diet, even at the highest conidial concentration. The average ingestion of *M. anisopliae*-amended bait by adults (0.12 g) was the same as that recorded for conidia-free baits (0.12 g) during the 72h feeding period. Survival of *B. germanica* nymphs treated with *M. anisopliae*-amended bait was not affected, but mortality rates of adults ranged from 12% to 28% at 15 days post-application, even though no differences were observed among treatments ($F = 4.72$; df = 3, 20; $P = 0.012$) (Table 1).

Evaluation of M. anisopliae *conidial viability in the digestive tract of* B. germanica

Forty-eight hours after exposure to *M. anisopliae*-treated baits, non-germinated conidia were present in all three portions of the adult digestive tract. Clusters of non-germinated conidia were observed in the hindgut 48h following bait ingestion. Likewise, conidia recovered from the lumen of the digestive tract did not germinate onto PDA medium. Non-germinated conidia were also recovered in fecal pellets. There was no reduction in conidial viability both for the *M. anisopliae*-amended bait and *M. anisopliae*-amended powder after 72h storage at room temperature. Reduction in conidial viability was lower after 120h storage in the powder formulation (from $2.15 \pm 0.13 \times 10^8$ CFU g^{-1} to $1.28 \pm 0.06 \times 10^8$ CFU g^{-1}) as compared to the bait treatment (from $1.57 \pm 0.08 \times 10^8$ CFU g^{-1} to $1.67 \pm 0.24 \times 10^6$ CFU g^{-1}).

Discussion

Adults of *B. germanica* are more susceptible to *M. anisopliae* infection than nymphs, since mortality of nymphs was always lower than that observed for adults at the same doses. Furthermore, delivery of the pathogen by a dusting technique was more efficient than the use of baits. The differential susceptibility could be related to the molting of the cuticle by the nymphal stage, which is particularly relevant when the pathogen is inoculated immediately prior to ecdysis or when the time

Table 2 Mean and median survival times of *Blattella germanica* nymphs and adults exposed to *Metarhizium anisopliae* (ESALQ1037) powder formulations (27 ± 1ºC, RH > 90%, 24h scotophase).

M. anisopliae-amended powder (conidia cm^{-2})	Survival time (days)			
	Mean[1] ± SE	FL (95%)	Median ± SE	FL (95%)
Control (nymphs)	14.9 ± 0.1	14.7 – 15.1	ND[2]	ND
Control (adults)	14.2 ± 0.3	13.6 – 14.7	ND	ND
6.50×10^5 (nymphs)	8.7 ± 0.5	7.7 – 9.8	6.0 ± 1.2	3.7 – 8.3
3.25×10^6 (nymphs)	5.7 ± 0.5	4.6 – 6.5	ND	ND
6.50×10^6 (nymphs)	6.5 ± 0.5	5.4 – 7.5	ND	ND
6.50×10^5 (adults)	9.8 ± 0.4	9.1 – 10.5	9.0 ± 0.8	7.4 – 10.6
3.25×10^6 (adults)	5.9 ± 0.2	5.5 – 6.4	6.0 ± 0.1	5.7 – 6.3
6.50×10^6 (adults)	5.6 ± 0.2	5.2 – 6.0	6.0 ± 0.1	5.8 – 6.2

Mean and median survival times (± standard error) were determined by the Kaplan-Meier method. FL – fiducial limits.
[1]Estimation is limited to the largest survival time if it is censored.
[2]ND = not determined.

interval between ecdysis is small (Vey & Fargues 1977, Vestergaard *et al* 1995, Ekesi & Maniania 2000). Although not all adults had the same physiological age, we believe that the differences observed in mortality between adult and nymphs was not substantially affected by this factor. Insects that were not exposed to *M. anisopliae* also showed similar mortality rates during the bioassays. Although the cuticle of cockroaches constitutes an important physical barrier, making the penetration by *M. anisopliae* a difficult step, high mortality rates were recorded when adults were exposed to the powdery formulation.

Previous studies also proved the efficiency of different *M. anisopliae* isolates in the control of the German cockroach (Kaakeh *et al* 1996, Kaakeh *et al* 1997, Quesada-Moraga *et al* 2004). The mortality ranges and LD_{50} value for *B. germanica* adults are similar to those obtained for isolate ESC-1 within the *M. anisopliae* complex (Pachamuthu *et al* 1999). However, high doses and long periods were required to *M. anisopliae* to cause satisfactory levels of mortality. One of the main limitations of the infection chamber commercially available in the USA in the 1990s was the long period required for the fungus to control the insect population and its limited shelf-life (Gunner *et al* 1991, Kaakeh *et al* 1996). The chamber contained an agar plate to keep a humid environment to allow for fungus infection, and a water source in the formulation to attract insects.

Infected cockroaches may carry the fungus back to their hideouts and transmit the disease (Quarles 1995). The success in *B. germanica* control with *M. anisopliae* is related, among other factors, to the number of conidia adhered to each individual (Kaakeh *et al* 1997). The powdery formulation favored the adhesion of a larger conidial load to cockroaches cuticle exposed to indoor surfaces treated with *M. anisopliae*. We believe that conidia that stuck to a body area in which cleaning by the insect was difficult, resulting in the higher mortality observed. Quesada-Moraga *et al* (2004) reported LT_{50} varying from 5.3 d to 14.8 d for topical inoculation of 4 µl of suspensions containing 4.2×10^9 and 4.2×10^8 conidia ml^{-1}, respectively. In the present study, for both the nymphal and adult stages exposed to surfaces treated with the powder formulation containing between 5×10^9 and 5×10^8 conidia g^{-1}, the mean survival time range was lower.

Insects exposed to the powdery formulation had their body surface visibly contaminated by spores as compared to those exposed to *M. anisopliae*-amended baits. Intersegmental regions of the thorax and abdomen and regions such as legs are favorable for conidia adherence and certainly more difficult to clean. Also, quantitative and temporal differences in adhesion and germination were related to pathogenicity (Butt *et al* 1995).

Our data showed that the delivery of conidia by baits is not as efficient as the dusting of surfaces. Firstly, although infection may also occur via natural openings, such as the mouth, *per os* penetration seems not to be a relevant way of infection. Secondly, cockroaches exposed to dusted surfaces are more likely to acquire much higher quantities of the infective stages of the pathogen than by feeding on baits. Lastly, we observed that *per os* administration of conidia resulted in their inactivation inside the digestive tract of *B. germanica*.

It has been shown that ingested conidia of *M. anisopliae* are inhibited once they pass through the gut of termites (Siderhurst *et al* 2005, Yanagawa & Shimizu 2007, Chouvenc *et al* 2009) and locusts (Dillon & Charnley 1986). On the other hand, high mortality of *Culex quinquefasciatus* Say larvae was reported shortly following exposure to *M. anisopliae* conidia, suggesting the release of toxins in the digestive tract (Alves *et al* 2002). Such an action was not observed in our study. Interestingly, the few insects that succumbed to infection in treatments with *M. anisopliae*-amended baits showed exterior signs of fungal growth, around initially the head and front legs (regions that contacted the bait). Cadaver colonization by the fungus in this case was slow and symptoms of the disease appeared later and non-homogeneously along the insect's body, probably because opportunistic bacteria occurred simultaneously. Fungal penetration probably took place through the cuticle at these regions as previously reported for grasshoppers (Jeffs *et al* 1997), and regions that contact the bait were first colonized. In the present study with treatments with *M. anisopliae*-amended powder, exterior signs of fungal growth were faster and homogeneous among dead insects.

Although *M. anisopliae* is considered a strong control agent of *B. germanica*, the period of time necessary to attain high mortality percentages raises doubts regarding its practical application. We demonstrated in here that nymphs of *B. germanica* are less susceptible to infection by *M. anisopliae* than are adults, and that appropriate formulation and delivery strategies may increase the pathogen effectiveness in controlling cockroaches. The development of formulations that yield higher infection and mortality rates is crucial in making this entomopathogen accepted for applied control of cockroaches, and an association with specific materials that may improve conidia stability, or with chemical products, could be interesting options. Conidia delivery strategies may also favor fungal infection of all insect developmental stages. Besides, the development of formulations capable of providing better adherence of fungal structures to the cuticle of insects and maintaining high conidial viability for longer periods is urgently needed.

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