Cuticle Fatty Acid Composition and Differential Susceptibility of Three Species of Cockroaches to the Entomopathogenic Fungi *Metarhizium anisopliae* (Ascomycota, Hypocreales)

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ABSTRACT Differences in free fatty acids (FFAs) chemical composition of insects may be responsible for susceptibility or resistance to fungal infection. Determination of FFAs found in cuticular lipids can effectively contribute to the knowledge concerning insect defense mechanisms. In this study, we have evaluated the susceptibility of three species of cockroaches to the entomopathogenic fungi *Metarhizium anisopliae* (Metschnikoff) Sorokin by topical application. Mortality due to *M. anisopliae* was highly significant on adults and nymphs of *Blattella germanica* L. (Blattodea: Blattellidae). However, mortality was faster in adults than in nymphs. Adults of *Blatta orientalis* L. (Blattodea: Blattidae) were not susceptible to the fungus, and nymphs of *Blaptica dubia* Serville (Blattodea: Blaberidae) were more susceptible to the fungus than adults. The composition of cuticular FFAs in the three species of cockroaches was also studied. The analysis indicated that all of the fatty acids were mostly straight-chain, long-chain, saturated or unsaturated. Cuticular lipids of three species of cockroaches contained 19 FFAs, ranging from $C_{14:0}$ to $C_{24:0}$. The predominant fatty acids found in the three studied species of cockroaches were oleic, linoleic, palmitic, and stearic acid. Only in adults of *Bl. orientalis*, myristoleic acid, γ-linolenic acid, arachidic acid, dihomolinoleic acid, and behenic acid were identified. Lignoceric acid was detected only in nymphs of *Bl. orientalis*. Heneicosylic acid and docosahexaenoic acid were identified in adults of *Ba. dubia*.

KEY WORDS fatty acid, cuticle, cockroach, entomopathogenic fungi, *Metarhizium anisopliae*

Introduction

The development of insecticide resistance in the cockroaches is a serious problem in control of these insects. New insights were focused on the effects of the use of the entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin (Ascomycota, Hypocreales) against *Blattella germanica* L. (Blattodea: Blattellidae) and *Periplaneta americana* (L.), evaluating their virulence and host range (Murali Mohan et al. 1999, Quesada Moraga et al. 2004, Lopes and Alves 2011). Previously, authors reported studies on the evaluation of *B. bassiana* effects on *B. germanica* (Pachamuthu et al. 1999, Zurek et al.

2002); other reports were related to virulence, transmission, and differential susceptibility of nymphs and adults of *B. germanica* to *M. anisopliae* (Quesada Moraga et al. 2004, Lopes and Alves 2011). These studies showed differences in mortality between different species of cockroaches and between nymphs and adults of the same species. Differences between treatments and fungal species tested against cockroaches may be due to the surface structure and the chemical composition of the host cuticle. Such differences could affect the attachment of fungal propagules to the cuticle (Boucias and Pendland 1984). The insect cuticle is the first barrier against attack of pathogens, and adhesion and penetration of the cuticle by conidia are key limiting factors in the colonization of the insect. The presence of antifungal compounds in the cuticle, as well as the efficiency of cellular and humoral defense reactions, are related with susceptibility or resistance to fungal invasion of insects (Vilçinskas and Götz 1999). Moreover, cuticle composition influences conidial germination and hyphal growth of fungi, resulting in differential susceptibility of insects (Boucias and Latgé 1988, Wang et al. 2005).

Free cuticular lipids of insects change in composition and quantity, depending on the species and their developmental stage (Gołębiowski et al. 2011). Whereas the protein and chitin composition of the insect procuticle

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appears to be similar in all groups, the epicuticular components are extremely heterogeneous and, therefore, they have the potential to respond to different pathogen, particularly in insects (Gołębiowski et al. 2011). Although the lipid composition of cuticles of some insects is already known (Lockey 1988), there is not enough knowledge about this composition in cockroach species.

The aims of this study are to increase knowledge about fatty acids of cuticle of several species of cockroaches and to evaluate differential susceptibility of nymphs and adults of *B. germanica*, *Blatta orientalis* L. (Blattodea: Blattidae), and *Blaptica dubia* Serville (Blattodea: Blaberidae) to a native isolate of *M. anisopliae*.

Materials and Methods

Insects. Nymphs and adults of *B. germanica*, *Bl. orientalis*, and *Ba. dubia* were collected from households and at the zoological park in La Plata, Buenos Aires, Argentina, between July 2010 and September 2011. Cockroaches were confined in plastic boxes with lids (30 by 25 by 20 cm^3) and provided with cardboard shelters. They were fed with commercial dog food (Purina Dog Chow, Nestlé Argentina S.A., Buenos Aires) and tap water ad libitum. The cockroaches were maintained in a rearing room at $26 \pm 2°C$, $56 \pm 10\%$ relative humidity (RH), and a photoperiod of 12:12 (L:D) h.

Fungal Cultures. *Metarhizium anisopliae sensu lato* (Metschnikoff) Sorokin was originally isolated from an unidentified Cercopidae (Hemiptera) collected in 2004 from Los Hornos, Buenos Aires province, Argentina. The strain was deposited at the CEPAVE (Centro de Estudios Parasitológicos y de Vectores) entomopathogenic fungal culture collection (La Plata, Argentina) under the accession number CEP 085. This isolation was cultured on Sabouraud dextrose agar + 1% yeast extract (SDAY 1%) medium and incubated at $25 \pm 1°C$ and a photoperiod of 12:12 (L:D) h. Conidia were harvested from 15-d-old cultures. They were scraped with a sterile looper and collected into sterile plastic tubes (45 cm^3) containing 5 ml of 0.01% (v/v) Tween 80 (Merck, México). A suspension of conidia was vortexed for 5 min. Concentrations of conidia were adjusted to 10^6, 10^7, 10^8, and 10^9 conidia/ml using a Neubauer chamber. For every bioassay, the conidia were re-isolated from *Tenebrio molitor* (Coleptera: Tenebrionidae)-infected larvae. The viability of the conidia used in the treatments was checked, and conidia used in each experiment showed over 95% viability.

Median Lethal Doses (LD_{50}) and Median Lethal Time (LT_{50}) Assessment for Nymphs and Adults of Cockroaches. Virulence of *M. anisopliae* toward nymphs and adults of *Bl. orientalis*, *B. germanica*, and *Ba. dubia* was evaluated by topical applications. For this experiment, carbon dioxide was used to immobilize insects. The doses of conidia are adjusted accorded to the weight of adults and third instars of nymphs. The weight of adults and nymphs ranged between 67.4–16.5 mg for *B. germanica*, 329–52.6 mg for *Bl. orientalis*, and 2267–65 mg for *Ba. dubia*.

Cockroaches were treated on ventral abdominal region with 6 µl of the suspension of fungi with a semiautomatic micropipette. Based on the results of a preliminary experiment, four conidial suspension were applied at a final dose 6×10^3, 6×10^4, 6×10^5, and 6×10^6 conidia per cockroach and tested on nymphs and adults separately. However, due to the high difference in weights between species of cockroaches from preliminary assays, the *Ba. dubia* and *Bl. orientalis* adults were treated with different final dosages 2×10^5, 2×10^6, 2×10^7, and 2×10^8 conidia per cockroach and were applied 150 µl of the suspension on the ventral abdomen segment with a semiautomatic micropipette. The conidial suspension was vortexed for 30 s to keep it stable. Controls were treated with 6 µl or 150 µl of Tween 80, 0.01% (v/v), respectively. A total of 150 nymphs and 150 adults were monitored in three repetitions of the experiments for each species of cockroaches. Three repetitions were performed at different times, with 10 nymphs and 10 adults per treatment. Afterwards, cockroaches were transferred to plastic cups (250 cm^3) at $25 \pm 1°C$ and $70 \pm 5\%$ RH. Five nymphs of cockroaches were placed per container, and adults were placed individually in plastic cup. Food and water were placed inside the containers and changed every two days. Mortality was monitored daily up to 20 d posttreatment. Dead cockroaches were removed daily and placed on slides inside Petri dishes (100 mm diameter) containing filter paper moistened with sterile distilled water. Insects were superficially sterilized with 70% ethanol followed by a second bath in sterile distilled water, and then a third bath with antibiotic 40,000 units/ml chloramphenicol (Parafarm, Argentina) and 80,000 units/ml streptomycin (Parafarm, Argentina), and the last bath was done using sterile distilled water, for 10 s. Cadavers were placed on wet filter paper disks into sterile 100-mm Petri dishes sealed with Parafilm and maintained in an incubator chamber at $25 \pm 1°C$ and $70 \pm 5\%$ RH. Emergence of mycelia was monitored for a total of 8 d. Infected insects with evidence of external fungal growth were examined under a stereomicroscope, and the fungus was re-isolated in culture medium to confirm the cause of infection. Fungal structures were stained with 0.01% (w/v) lactophenol and cotton blue 1% (w/v). Fungal slide preparations were observed under an Olympus microscope with phase contrast to verify the fungal species. For the LT_{50}, a 10^9 conidia/ml suspension was prepared and tested on nymphs and adults under the same conditions explained above.

Extraction of Cuticular Lipids. Cockroaches were anesthetized with carbon dioxide exposed for 10 s. They were dissected under a stereomicroscope (Zeiss Stemi DV4) with 0.9% saline. Five adults and five third-instar nymphs of each species were used to determine fatty acid from the cuticle. Fat body residues from the cuticle were removed and discarded, with saline sterile in cotton. Sampling processing consisted of total cuticular lipids extraction using the Folch procedure (Folch 1957) using the Folch mix (chloroform/methanol 2:1 v/v) in a ratio of 20:1 w/v respect to the sample mass. Partition was performed with 20% v/v

distilled water. The upper methanolic phase was discarded. The remaining chloroformic phase was evaporated to dryness under a nitrogen stream. The dry extract was then saponified using a 10% solution of potassium hydroxide in methanol for 1 h at 80°C using an electrical heating block to remove sterols, pigments, and other unsaponifiable lipids whose presence interferes with fatty acids analysis method. Unsaponifiable material was extracted with petroleum ether. Potassium soaps of fatty acids contained in the sample were then acidified using concentrated hydrochloric acid, a process that releases fatty acids. Free fatty acids were treated with 10% boron trifluoride in methanol, in a nitrogen atmosphere and using an electric hot plate for 30 min at 80°C. This procedure converts fatty acids in methyl ester derivatives more volatile than fatty acids, necessary condition for the subsequent analysis by gas chromatography. The methyl esters were extracted using petroleum ether.

Gas Chromatography. Fatty acid composition was determined by gas chromatography using a capillary column 50 m in length, 0.25 mm internal diameter, and 0.1 μm film thickness (Chrompack CP SIL 88) on a gas–liquid chromatograph Hewlett Packard 6890 equipped with a flame ionization detector (FID). The analysis conditions were—1) initial temperature of 185°C for 3 min, 2) heating ramp rate of 3°C per minute up to 230°C, and 3) maintaining temperature of 230°C for 25 min. The fatty acids profile was obtained by comparing the relative retention times with commercial standards (NuCheck prep.) analyzed previously in the same column.

Statistical Analysis. To determine significant differences among the mortality of cockroaches between treatments ANOVA was performed. An angular transformation of the data was done, using the arc cosine of the square root of the mortality percentage before the analysis, to stabilize the variance error (Zar 1996). Tukey's test ($P < 0.05$) was performed when significant differences appeared among the treatments. Statistical calculations were performed using Statgraphics package. Analyses were performed using the Probit version 1.5 Stastistical Software (USEPA) to determine the dose–response curve and to estimate LD_{50} for cockroaches and their respective 95% confidence limits (CL). Median lethal time values were calculated with their respective 95% CL by using the statistical software for correlated data developed by Throne et al. (1995). Differences between values were considered significant ($P < 0.05$) if the respective 95% CL did not overlap. To compare among treatments, an ANOVA with a multiple-level range test was conducted.

Results

Determining LD_{50} and LT_{50} to Three Species of Cockroaches. Mortality due to *M. anisopliae* was highly significant for adults ($F = 12.42$; df = 4; $P = 0.0007$) and nymphs ($F = 6.32$; df = 4; $P = 0.0084$) of *B. germanica* (Fig.1). Mortality was faster in adults than in nymphs, and nymphs showed an LD_{50} greater

than adults. LT_{50} for adults was lower than nymphs (Table 1).

Nymphal mortality of *Bl. orientalis* was highly significant compared with the control ($F = 5.82$; df = 4; $P = 0.01$; Fig. 2). However, adults of *Bl. orientalis* were not susceptible to the fungus (Table 1). Consequently, nymphs showed LD_{50} greater than nymphs of *B. germanica* and *Ba. dubia* (Table 1). However, LT_{50} was lower than nymphs of *B. germanica*.

Mortality due to *M. anisopliae* was highly significant for adults ($F = 33.63$; df = 4; $P = 0.0000$) and nymphs ($F = 43.33$; df = 4; $P = 0.0000$) of *Ba. dubia* (Figs. 2 and 3). Nymphs of *Ba. dubia* were more susceptible to the fungus than adults (Table 1). The results showed that there were highly significant differences among treatments of nymphs of *B. germanica* and *Bl. orientalis* with adults of *Ba. dubia* ($F = 2.8$; df = 12; $P = 0.004$), and such differences were influenced by concentration of fungal inoculum.

Cuticular Fatty Acid of Nymphs and Adults. The analysis of fatty acids methyl esters by gas chromatography indicated that all of the fatty acids were mostly straight-chain, long-chain, saturated or unsaturated (Table 2). The content of fatty acids present on the cuticle of cockroach species consisted of chains of 14 to 24 carbon atoms. There was a marked dominance of fatty acids containing 16 to 18 carbon atoms. The predominant fatty acids found in the three studied species of cockroaches were oleic acid ($C_{18:1\ n9c}$), linoleic acid ($C_{18:2\ n6}$), palmitic acid ($C_{16:0}$), and stearic acid ($C_{18:0}$; Table 2; Fig. 4). Oleic acid ($C_{18:1\ n9\ c}$) was detected with a highest percentage in all samples. It varied from 30 to 60% of the total of the present fatty acid. Only in adults of *Bl. orientalis*, myristoleic acid ($C_{14:1\ n5}$), γ-linolenic acid ($C_{18:3\ n6}$), arachidic acid ($C_{20:0}$), dihomolinoleic acid ($C_{20:2}$), and behenic acid ($C_{22:0}$) were detected (Fig. 5A). Lignoceric acid ($C_{24:0}$) was detected only in nymphs *Bl. orientalis* (Fig. 5D). Heneicosylic acid ($C_{21:0}$) and docosahexaenoic acid (DHA; $C_{22:6\ ω-3}$) presence was confirmed in adults of *Ba. dubia* (Fig. 5B). Differences in the fatty acids composition between species and development stage are presented in Figures 4 and 5.

Discussion

Our results revealed a relation between dose- and time-dependent mortality confirmed by previous reports (López and Alves 2011) in *B. germanica*. Our paper is the first study that describes the susceptibility of adults and nymphs of *Bl. orientalis* and *Ba. dubia* to *M. anisopliae* in the laboratory. Firstly, it was possible to estimate the susceptibility of nymphs of *Bl. orientalis* and nymphs and adults of *B. germanica* and *Ba. dubia* to the fungus *M. anisopliae* through LT_{50} and LD_{50}. Secondly, the adults of *Bl. orientalis* were not susceptible to this isolate of entomopathogenic fungus. Nymphs and adults of *Ba. dubia* and adults of *B. germanica* had overwhelming 90% mortality, while in nymphs of *B. germanica* and *Bl. orientalis*, it was not higher than 60%. Results shown that susceptibility to *M. anisopliae* have variation between species and
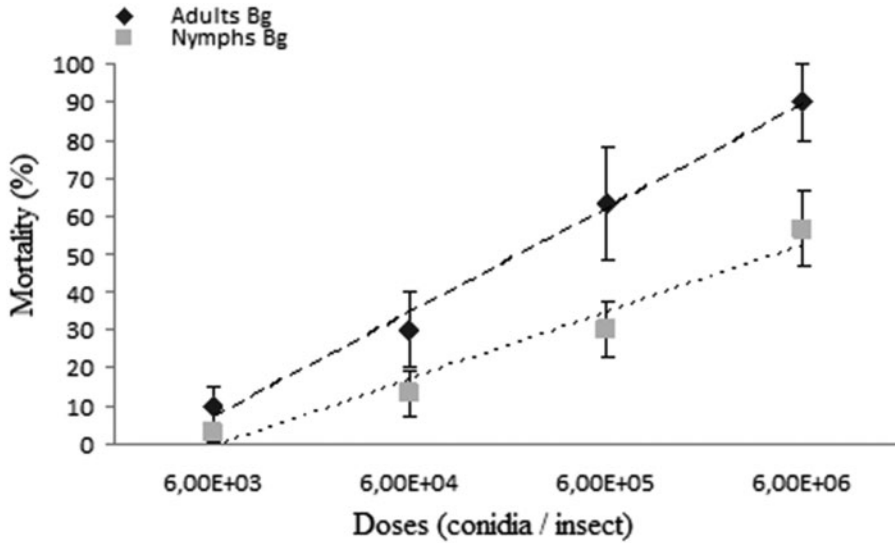
Fig. 1. Mortality of adults and nymphs of *B. germanica* exposed to different conidia concentration of *M. anisopliae* at 20 d after topical application. Bars depict mean ± SE.

Table 1. Median lethal dose (LD$_{50}$) and median lethal time (LT$_{50}$) of adults and nymphs of cockroaches treated with *Metarhizium anisopliae* CEP085

Species	Stage[a]	n	Slope ± SE	LD$_{50}$[b]	95% CL	X^2	LT$_{50}$[c]	95% CL
Bl. orientalis	A	150	–	N/S	–	–	–	–
Ba. dubia	A	150	1.76 ± 0.33	7.1×10^6	4×10^6–1.2×10^7	0.96	6.5	4.4–11.8
B. germanica	A	150	0.92 ± 0.17	2.7×10^5	1.1×10^4–6×10^5	0.01	5.6	4.5–7.5
Bl. orientalis	N	150	0.73 ± 0.27	5.5×10^6	1.6×10^6–1.5×10^8	1.22	6.8	5.1–10.6
Ba. dubia	N	150	1.15 ± 0.17	1.8×10^5	9.6×10^4–3.2×10^5	0.48	4	1.3–12.8
B. germanica	N	150	0.65 ± 0.14	3.4×10^6	1.2×10^6–2×10^7	0.04	10.6	9–12.8

LD$_{50}$ and LT$_{50}$ and respective 95% CL based on four concentrations of 10^6, 10^7, 10^8, and 10^9 conidia/ml were calculated 20 d after treatment.

[a] A, adults; N, nymphs.
[b] LD$_{50}$ expressed in conidia per cockroaches; N/S, no susceptibility.
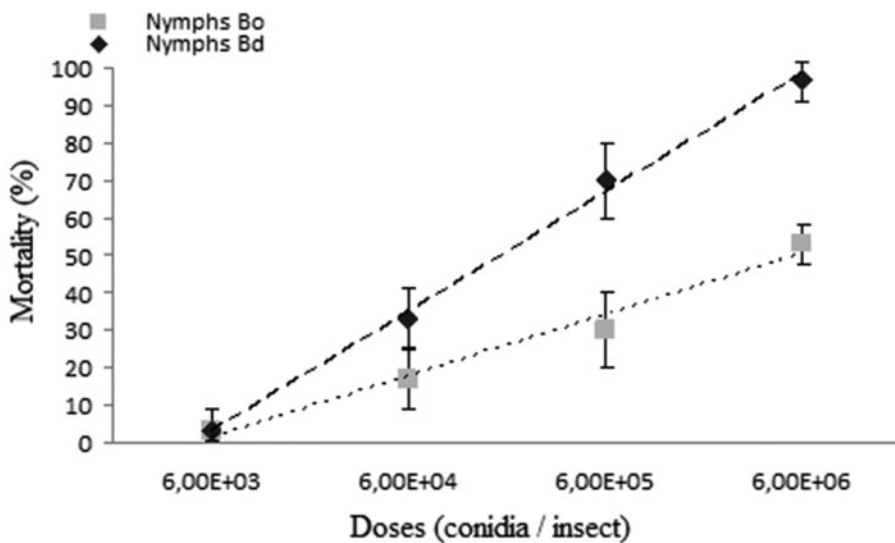[c] LT$_{50}$ expressed in days.

Fig. 2. Mortality of nymphs of two species of cockroaches (*Bl. orientalis and Ba. dubia*) exposed to different conidia concentration of *M. anisopliae* at 20 d after topical application. Bars depict mean ± SE.
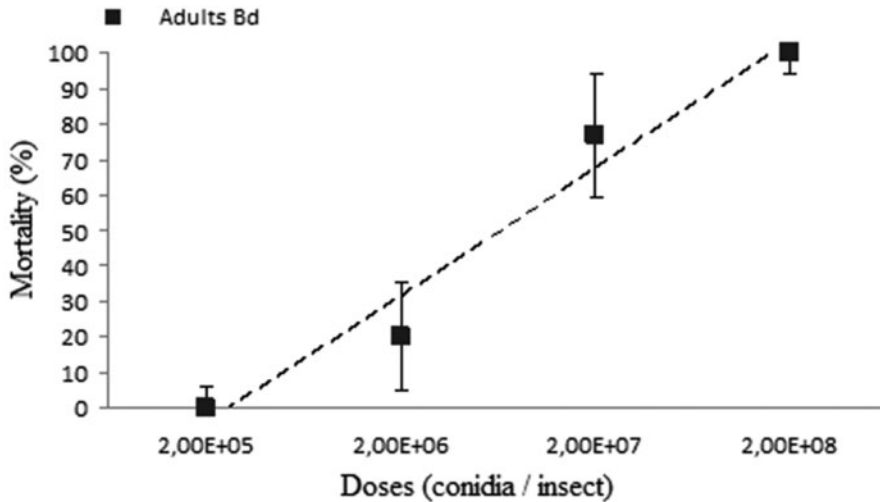
Fig. 3. Mortality of adults of *Ba. dubia* exposed to different conidia concentration of *M. anisopliae* at 20 d after topical application. Bars depict mean ± SE.

Table 2. Fatty acid contents in the cuticle lipids of the nymphs and adults of cockroach

Fatty acids	*Bl. orientalis*[a]		*Ba. dubia*[a]		*B. germanica*[a]	
	Nymphs	Adults	Nymphs	Adults	Nymphs	Adults
$C_{14:0}$	N/D	0.4 ± 0.1	N/D	N/D	N/D	0.7 ± 0.1
$C_{14:1\,n5}$	N/D	1.4 ± 0.8	N/D	N/D	N/D	N/D
$C_{15:0}$	0.8	0.4 ± 0.2	1.4	N/D	1.8	3.2 ± 0.3
$C_{15:1n9}$	0.4	N/D	N/D	N/D	N/D	0.6 ± 0.2
$C_{16:0}$	25.0	11 ± 5.3	21.2	8.2 ± 1.4	36.3	22.5 ± 1.5
$C_{16:1\,n7}$	2.2	1.1 ± 1	4.9	1.1 ± 0.7	N/D	3.3 ± 1.3
$C_{17:0}$	0.8	0.2 ± 0.1	N/D	0.3 ± 0.1	N/D	N/D
$C_{18:0}$	8.7	7.2 ± 1.4	5.4	9.8 ± 2.3	25.9	8.3 ± 4
$C_{18:1n9\,c}$	42.8	55.3 ± 7.3	42.6	59 ± 6	29.9	43.2 ± 0.2
$C_{18:1\,n9\,t}$	N/D	1.3 ± 1	N/D	3.4 ± 0.3	N/D	2.4 ± 1
$C_{18:2\,n6}$	16.7	18.2 ± 3.7	23.1	16.1 ± 2	6.1	14.9 ± 2
$C_{18:3\,n3}$	0.9	1 ± 0.1	1.4	1.4 ± 0.2	N/D	0.9 ± 0.1
$C_{18:3\,n6}$	N/D	0.4 ± 0.2	N/D	N/D	N/D	N/D
$C_{20:0}$	N/D	0.4 ± 0.3	N/D	N/D	N/D	N/D
$C_{20:2}$	N/D	0.2 ± 0.1	N/D	N/D	N/D	N/D
$C_{21:0}$	N/D	N/D	N/D	0.2 ± 0.1	N/D	N/D
$C_{22:0}$	N/D	1.5 ± 0.6	N/D	N/D	N/D	N/D
$C_{22:6\,\omega3}$	N/D	N/D	N/D	0.5 ± 0.3	N/D	N/D
$C_{24:0}$	1.7	N/D	N/D	N/D	N/D	N/D
Sum	100	100	100	100	100	100

[a] % w/w, relative content of fatty acids (%). Data are presented as the means ± SD of five separate analyses performed on different samples.

developmental insect stages, as it was previously reported for *B. germanica* (López and Alves 2011) and for *P. americana* (Hernández-Ramírez et al. 2007). Adults of *B. germanica* were more susceptible to *M. anisopliae* infection than nymphs when topical applications were used. Our results of treatment by topical applications agreed with results recorded by Lopes and Alves (2011). This differential susceptibility at various life stages can be attributed to interaction between the insect integument being penetrated by the fungus and ecdysis of nymph stages. Ecdysis has been reported to be an important factor in insect resistance to fungal infection, particularly when the time interval

between successive molting is short (Ekesi and Maniania 2000, Lopes and Alves 2011). Several studies had been reported about the lipid cuticular composition of insect species. These researches demonstrated the importance of cuticular components on microorganisms' action (Saito and Aoki 1983, James et al. 2003, Gołębiowski et al. 2011). Insect epicuticle is a waxy layer with fatty acids, lipids, and sterols (Anderson, 2004), and total fatty acid composition change during the insect development period. Cuticular fatty acids have a profound effect on fungal spore germination and differentiation: they can be toxic, fungistatic, or occasionally, for some pathogenic species, stimulatory (Gillespie et al. 2000, Boguś et al. 2010). In our studies, the cuticular lipids analysis of the three species of cockroaches showed that most of the extracted fatty acids had chains of 14–24 carbon atoms. Among these chains, the amount of fatty acids with the highest percentage detected had chains of 15–18 carbon atoms (Table 2). Fatty acids with <14 carbon atoms were not detected. Fatty acids with alkyl chains of >18 carbon atoms are rarely detected in insect cuticular lipids (Gołębiowski et al. 2008). In our studies, only fatty acids of >18 carbon atoms were detected in adults of *Ba. dubia* and nymphs and adults of *Bl. orientalis*. In *Ba. dubia* were observed $C_{21:0}$ and $C_{22:6}$. In adults and nymphs of *Bl. orientalis* were detected $C_{20:0}$ and $C_{20:2}$; and $C_{22:0}$ and $C_{24:0}$, respectively. However, these percentages were low in comparison with the total fatty acids.

The presence of fatty acids with 1, 2, or 3 double bonds on the epicuticular fatty acid structure of insects has frequently been reported in the literature. For example, $C_{16:1}$, $C_{18:1}$, and $C_{18:2}$ fatty acids were found in the lipids isolated from adults and larvae of *Lasyioderma serricorne* (Baker et al. 1979), and the same profile was detected in the lipids of *Fannia canicularis* larvae and adults (Kerwin 1984). Unsaturated fatty acids detected in our study were $C_{14:1n5}$, $C_{15:1n9}$,
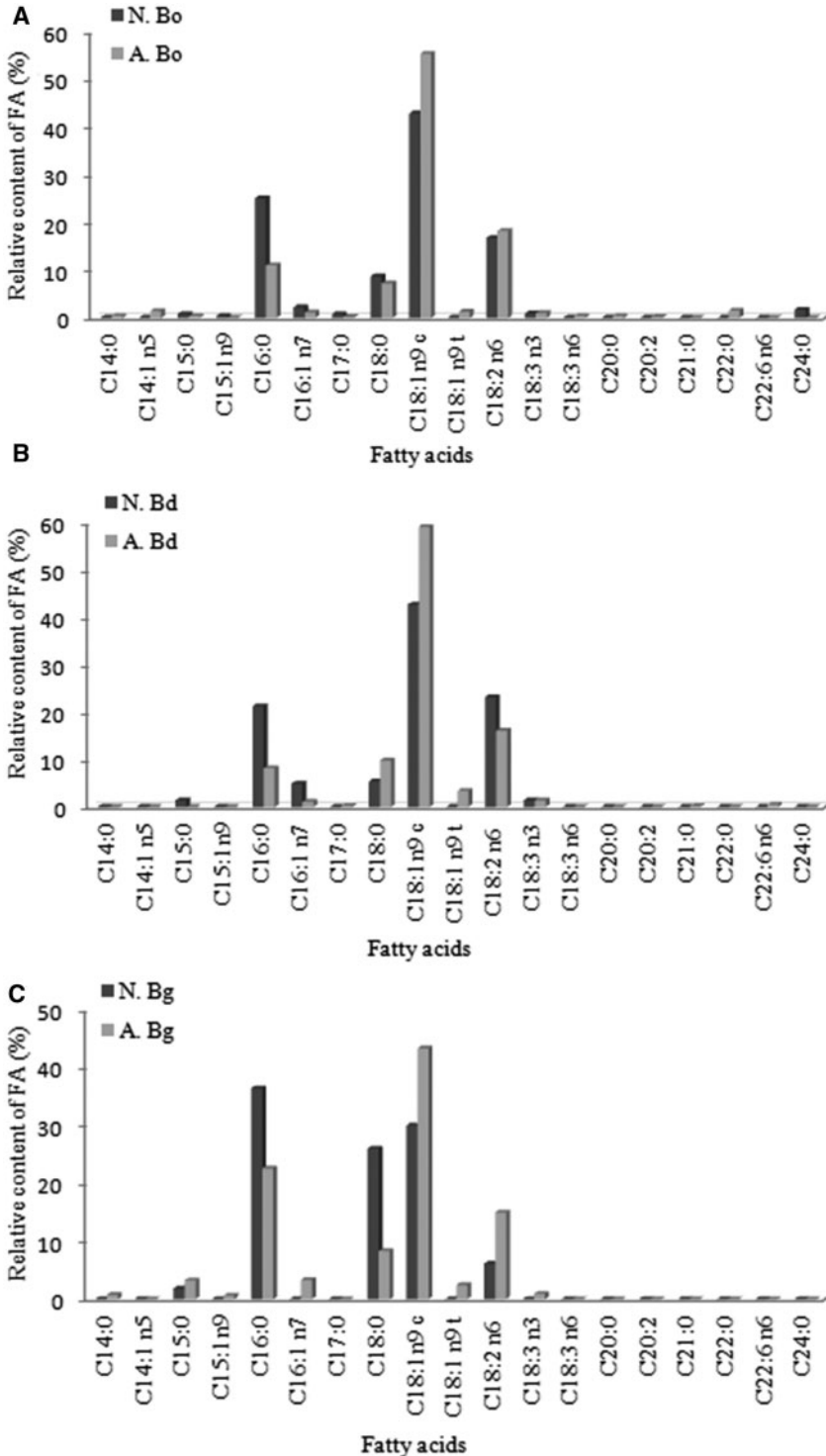
Fig. 4. Composition of free FAs in the cuticular lipid extracts of *Bl. orientalis* (A), *Ba. dubia* (B), and *B. germanica* (C). Myristic acid ($C_{14:0}$), myristoleic acid ($C_{14:1n5}$), pentadecylic acid ($C_{15:0}$), Cis-10-pentadecanoic acid ($C_{15:1n9}$), palmitic acid ($C_{16:0}$), palmitoleic acid ($C_{16:1n7}$), margaric acid ($C_{17:0}$), stearic acid ($C_{18:0}$), oleic acid ($C_{18:1n9c}$), elaidic acid ($C_{18:1n9t}$), linoleic acid ($C_{18:2n6}$), α-linolenic acid ($C_{18:3n3}$), γ-linolenic acid ($C_{18:3n6}$), arachidic acid ($C_{20:0}$), dihomolinoleic acid ($C_{20:2}$), heneicosylic acid ($C_{21:0}$), behenic acid ($C_{22:0}$), DHA ($C_{22:6\omega3}$), lignoceric acid ($C_{24:0}$).
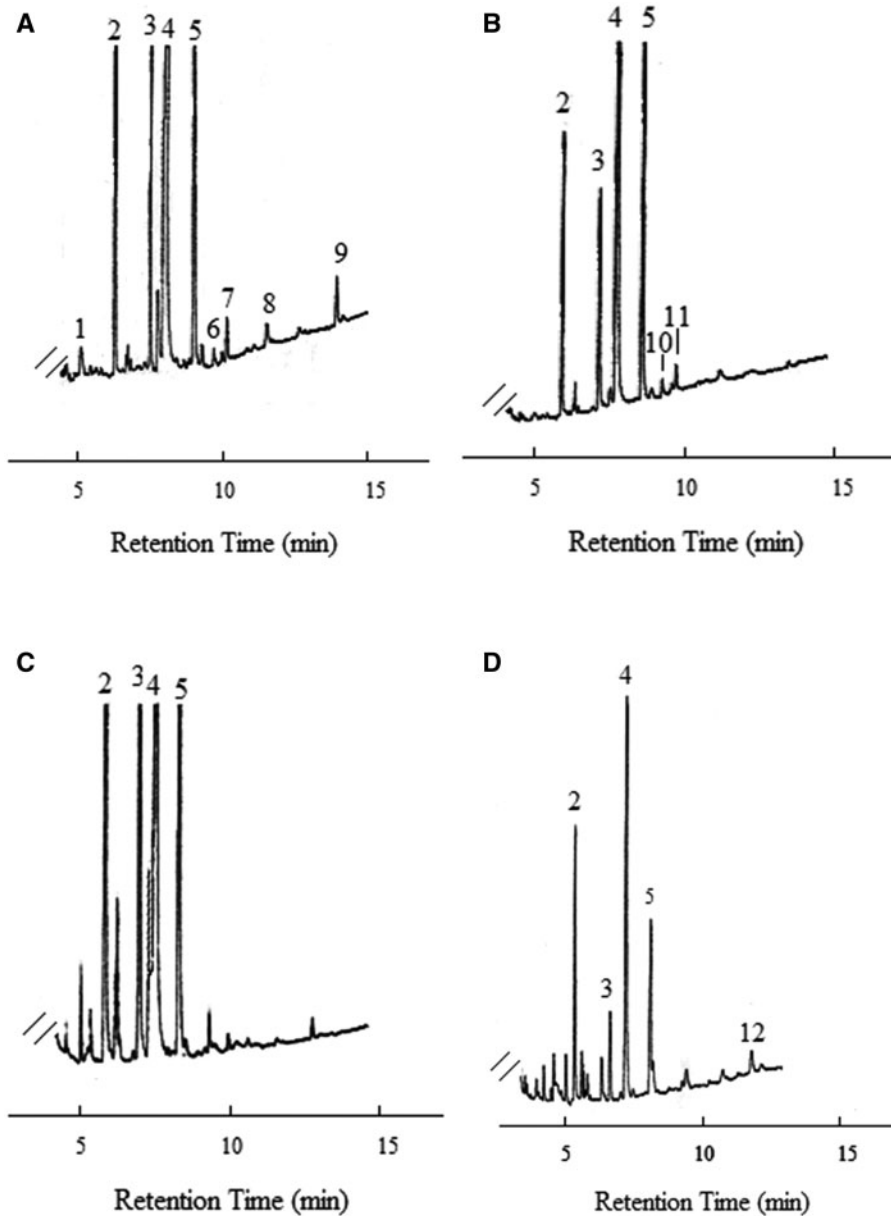
Fig. 5. Gas chromatography of cuticular fatty acids extracted from adults of *Bl. orientalis* (A), *Ba. dubia* (B), *B. germanica* (C), and nymphs of *Bl. orientalis* (D). Myristoleic acid (1), palmitic acid (2), stearic acid (3), oleic acid (4), linoleic acid (5), arachidic acid (6), γ-linoleic acid (7), dihomolinoleic acid (8), behenic acid (9), heneicosylic acid (10), DHA (11), lignoceric acid (12).

$C_{16:1n7}$, $C_{18:1n9}$, $C_{18:2n6}$, $C_{18:3n3}$, $C_{18:3n6}$, $C_{20:2}$, and $C_{22:6\omega3}$ (Table 2). Nymphs of *Bl. orientalis* and adults of *B. germanica* and *Ba. dubia* showed 60% unsaturated fatty acids in samples. Nymphs of *Ba. dubia* and *B. germanica* showed an inverse relationship, with 40% unsaturated fatty acids. In adults of *Bl. orientalis*, 53% of total fatty acids detected in sample were unsaturated. Gołębiowski et al. (2008) described and analyzed presence of fatty acids in *Calliphora vicina*, *Dendrolimus pini*, and *Galleria mellonella* where the dominants fatty acids were 16–18, with 44% concentration in *Calliphora vicina* who was considered the most resistant species to *Conidiobolus coronatus* of the three species studied. Adults of *Bl. orientalis* were not susceptible to the fungus *M. anisopliae*. This cockroach showed a different fatty acid profile of their nymphal stage relative to the other two species tested. Only in adult samples of *Bl. orientalis* were detected fatty acids with $C_{14:1}$, $C_{18:3}$, $C_{20:0}$, $C_{20:2}$, and $C_{22:0}$ atoms of carbon.

The identification of lipid composition in cuticles of thrips *Frankliniella occidentalis* was evaluated by Gołębiowski et al. (2007). They reported some hydrocarbons that are present in larvae and adults, and the highest prevalence were for 3-methylalkanes with 26 and 28 carbons, the saturated fatty acids were ($C_{14:0}$, $C_{16:0}$, and $C_{18:0}$) and unsaturated were ($C_{16:1}$ and $C_{18:1}$). They also reported the inhibitor potential of these compounds against entomopathogenic fungi. In our study, we found that the total fatty acid detected by gas chromatography in cockroaches analyzed were oleic acid ($C_{18:1n9c}$), palmitic acid ($C_{16:0}$), and linoleic acid ($C_{18:2 \, n6}$). The predominant was oleic acid, about 50% in each sample. In vitro tests conducted by Babiarz et al. 2001 in relation to the effect of fatty acids showed that the presence of $C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, and $C_{18:3}$ in the mass culture inhibited growth of fungi *C. coronatus* and reduced the production of conidia. These fatty acids were detected in nymphs and adults of *B. germanica*, *Bl. orientalis*, and *Ba. dubia*. In addition, $C_{18:1n9}$ trans fatty acids was only detected in adult cockroaches.

Saito and Aoki (1983) evaluated the effect of fatty acid compositions from the species *Bombyx mori* and *Hyphantria cunea* with respect to the fungal infection with *B. bassiana* and *P. fumosoroseus*, and they reported that the most prevalent acids found were linoleic acid ($C_{18:3}$) and acids $C_{6:0}$ and $C_{12:0}$ and they presented inhibition of germination and growing of fungi. In our analysis, linoleic acid appears in *Bl. orientalis* and *Ba. dubia*. Cuticular composition of insects, essential to initiate infection, may have affected the adhesion of conidia, resulting in a decrease or increase of insect mortality. From studies reported about cuticular lipids (Jackson 1970), hydrocarbons of *Periplaneta australasiae*, *Periplaneta brunnea*, and *Periplaneta fuliginosa* were identified. In studies reported by Said et al. (2005) about four *Periplaneta* species, 25 hydrocarbons were identified in *Periplaneta fuliginosa*, 23 in *Periplaneta americana*, 21 in *Periplaneta brunnea*, and 19 in *Periplaneta australasiae*. For example, 6.9-heptacosadiene (68.5%) was present in the highest concentrations in cuticular lipids of *P. americana*. This compound was absent in the three other insect species analyzed (Said et al. 2005). There are not any previous references reported on fatty acids of the cuticle from cockroaches. In conclusion, this is the first report of susceptibility of *Ba. dubia* and *Bl. orientalis* to entomopathogenic fungi *M. anisopliae* and the first time that epicuticular fatty acid profiles of *B. germanica*, *Ba. dubia*, and *Bl. orientalis* are reported. These results show that changes in the concentrations of the fatty acids present in nymphs and adults and between species of cockroach can be correlated with the variation in susceptibility related to the action of entomopathogenic fungus. The role of host surface lipids and waxes in fungal pathogenesis of insects is still poorly understood. Determination of the cuticular fatty acid profile is therefore of great importance in understanding the background of insect susceptibility or resistance to fungal infection. However, further research would be necessary to expand the knowledge about the effect of cuticular lipids on germination and infectivity of fungus *M. anisopliae*.

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