

# Thermal limitations of *Metarhizium anisopliae* efficacy: selection for application on warm-blooded vertebrates

Dana Ment · Naim Iraki · Galina Gindin ·  
Asael Rot · Itamar Glazer · Rula Abu-Jreis ·  
Michael Samish

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**Abstract** Temperature is one of the main obstacles for on-host applications of entomopathogenic fungi for ectoparasite control. The effects of temperatures typical of the body surfaces of warm-blooded animals on the germination, growth and virulence of four strains of *Metarhizium anisopliae* toward engorged *Rhipicephalus (Boophilus) annulatus* females were evaluated. The *M. anisopliae* strains studied can be divided according to their thermal characteristics: (1) strains which germinate (90–100%), grow and infect ticks similarly at 25, 30 and 35°C; and (2) strains

which recover their ability to germinate relatively quickly following a thermal shock (37 or 40°C for 6–48 h) before incubation at a favorable temperature. These latter strains could recover their infectivity after a short thermal shock (6 h at 37–40°C), but not after more prolonged exposure to these temperatures (48–72 h). These two thermal characteristics do not interact, but reflect the efficacy of strains used to control ectoparasites on warm-blooded vertebrates.

**Keywords** *Metarhizium anisopliae* · *Rhipicephalus (Boophilus) annulatus* · High temperature · Thermal characteristics · On-mammal application · Efficacy

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D. Ment · G. Gindin · I. Glazer  
The Volcani Center, ARO, P.O. Box 6,  
Bet-Dagan 50250, Israel

N. Iraki · R. Abu-Jreis  
UNESCO, Biotechnology Centre (BETCEN), Bethlehem  
University, Bethlehem, P.O. Box 9, West Bank,  
Palestinian Authority, Israel

A. Rot · M. Samish  
The Kimron Veterinary Institute, P.O. Box 12,  
Bet-Dagan 50250, Israel

D. Ment (✉)  
The Robert H. Smith Faculty of Agricultural, Food  
and Environmental Quality Sciences, The Hebrew  
University of Jerusalem, P.O. Box 12, Rehovot 76100,  
Israel  
e-mail: danam@agri.gov.il

## Introduction

The entomopathogenic fungus, *Metarhizium anisopliae* var. *anisopliae* (Metschn.) Sorokin (Hypocreales: Clavicipitaceae) is a major candidate for use in tick control (Samish et al. 2008). This fungus very effectively controls several tick stages and also causes a variety of sub-lethal effects, such as reductions in molting and engorging rates, egg production and larval hatching (Gindin et al. 2001; Kaaya and Hassan 2000; Kirkland et al. 2004). However, the application of *M. anisopliae* against parasitic tick stages under field conditions, including on mammalian hosts, has yielded inconsistent results, which may be due to the effects of biotic and abiotic factors on the survival of the fungus and its ability to

cause mycosis (Hall and Papierok 1982; Inglis et al. 2001; Leemon et al. 2008; Polar et al. 2008).

Environmental temperature is known to influence the virulence of many entomopathogenic fungi and can determine their effectiveness as biocontrol agents under natural conditions (Dimbi et al. 2004; Feng et al. 1999). The development of bio-pesticides, including myco-acaricides for the control of ectoparasites on warm-blooded vertebrates, requires the selection of fungal strains which are effective at relatively high temperatures. Such temperatures exceed, in most cases, the optimal temperatures (23–30°C) for germination and growth of entomopathogenic fungi (Jaronski 2009). The skin temperature of mammals like sheep, horses and cattle kept in barns under controlled conditions (20°C and 55% RH) varies across different body parts, from 33 to 36°C (Piccione et al. 2005). This range of temperatures is problematic for the success of a myco-acaricide application. Moreover, mammalian skin temperature fluctuates during the day and can reach 35–41°C on the ears and spine (Piccione et al. 2009; Polar et al. 2005), and even higher temperatures in certain skin areas when the animals are grazing at midday under a clear sky.

*Metarhizium anisopliae* strains differ significantly in various characteristics including their ability to survive and/or grow and infect arthropods at higher temperatures (Brooks and Wall 2005; McCammon and Rath 1994). The thermal death point for *M. anisopliae* conidia has been found to be between 49 and 60°C (Fargues et al. 1992; Walstad et al. 1970; Zimmermann 1982). However, the maximal temperature for conidial germination and mycelial growth is around 35–37°C. Polar et al. (2005) emphasized the importance of the thermal characteristic of *M. anisopliae* strains applied on mammals against vertebrate ectoparasites. Strains which grew better at 34°C in vitro were also more pathogenic towards *Boophilus microplus* (Canestrini 1887) (Acari: Ixodidae) in vivo at 31–35°C. Likewise, *M. anisopliae* isolates that demonstrated optimal growth at higher temperatures in vitro also demonstrated higher rates of infection of the parasitic mite *Psoroptes ovis* (Hering 1838) (Acari: Psoroptidae) at higher temperatures (Brooks and Wall 2005).

Plans for on-mammal applications of entomopathogenic fungi against ectoparasites should take into consideration two possible sources of thermal shock:

first, irregular environmental temperatures and second, the constant high temperature of the mammalian skin. The actual ambient temperature that the fungus encounters on the mammalian host varies from 28 to 41°C depending on the ectoparasites' location on the mammal's body, the conditions under which the mammal is being reared (e.g., open plantation or a ventilated house), solar radiation, environmental temperature and even the age, color, breed and physiological state of the mammal (Monty and Garbareno 1978; Polar et al. 2005).

The effect of higher temperatures on the fungus can be short term, for instance, lasting several hours while animals are under direct sunlight, or long term, lasting 1–3 weeks, during which time the parasitic arthropod feeds on warm parts of a mammal's body (e.g., spine, underarm). These scenarios demand the use of strains that: (1) will survive a thermal shock (up to 40–41°C) lasting several hours and quickly recover to infect arthropods after the ambient temperature has fallen, and (2) will be capable to infect ticks at temperatures of 33–36°C, which are close to the mammalian skin surface temperature (Piccione et al. 2005). The type of thermal resistance which is most critical for successful infection of various tick life stages upon fungal spray application on mammals has yet to be determined. As we search for practical ways to overcome the problem of short-term and/or constant high temperatures, we need to first understand how such conditions temporarily or permanently inhibit fungal development and survival.

The following study aims to develop a selection strategy for the screening of fungal strains for on-mammal applications. Two main temperature regimes, typical temperatures on the mammalian body surface, were applied: (1) constant temperatures (32 or 35°C), which reflect thermal conditions on mammalian skin and fur; and (2) relatively short periods (6, 48 or 72 h) of high temperature resembling the temperatures found on cattle skin (37–40°C). The parameters examined included conidial germination, fungal growth and efficacy against ticks. Heat from the bodies of warm-blooded hosts, together with high ambient temperatures, contributes to the common failures of fungi to control parasitic tick stages. Thus, the selection of heat-tolerant isolates should help to improve the efficacy of this control strategy.

## Materials and methods

### Cultivation and preparation of the fungi

Four strains of *Metarhizium anisopliae* var. *anisopliae* (Metschn.) Sorokin (Hypocreales: Clavicipitaceae) (M.a.) were used. M.a.-Pal-MO2 was obtained from the UNESCO Biotechnology Center (Bethlehem, West Bank, Palestinian Authority) and three strains (M.a.-7, M.a.-PPRC-14, M.a.-PPRC-51) were obtained from the ARO entomopathogenic fungi collection (Department of Entomology, Plant Protection Institute, Volcani Center, Bet Dagan, Israel). Strains M.a.-PPRC-14 and M.a.-PPRC-51 were originally isolated in Ethiopia. All of these strains were re-isolated from ticks (*R. annulatus* females) 1–2 months before the bioassay was performed. The fungi were grown on Sabouraud dextrose agar (SDA, Difco, Becton–Dickinson, MD) for 2–3 weeks at 25°C, and the conidia were harvested by scraping the agar and suspending the scrapings in sterile distilled water containing 0.01% (v/v) Triton X-100, in glass tubes. The material was then filtered through Miracloth (Calbiochem, La Jolla, CA, USA). The suspension was vortexed and sonicated for 5 min with an ultrasonic cleaner (Model D80H, Chemist Co., Taiwan) to break up clumps of conidia. Concentrations of conidia were determined with a haemocytometer. Suspensions were adjusted to the required concentrations in 0.01% (v/v) Triton X-100 and the percentage of viable conidia was determined based on conidial germination tests on SDA 24 h after incubation. Only suspensions with at least 95% germinating conidia were used.

### Tick rearing

*Rhipicephalus (Boophilus) annulatus* (Say) (Acari: Ixodidae) ticks were from a population that has been reared under laboratory conditions since 1984. The ticks were fed on 1- to 3-month-old Friesian calves and their off-host stages were incubated at 28°C and 70–80% RH in the dark.

### Temperature assays

#### *Effect on conidial germination*

A suspension of conidia (0.2 ml,  $1 \times 10^6$  conidia ml<sup>-1</sup>) was spread on SDA medium in Petri

dishes (55 mm ø). The dishes were sealed with Parafilm and incubated at different temperature regimes in the dark: (a) constant temperature—25 (controls), 32 or 35°C, (b) thermal shock—37 or 40°C for 6, 48 or 72 h before being transferred to a constant temperature of 25°C. Germination of the conidia on the SDA medium was observed 18 h post-inoculation (PI) by means of a light microscope at  $\times 200$  magnification. A conidium was considered germinated only if the germ tube was at least the size of half the width of the conidium. The rate of germination was evaluated by counting the germination of 100 conidia from each plate. Four replicate plates were examined for each temperature regime.

#### *Effect on colony growth rate*

x- and y-axes were drawn on the bottom of Petri dishes containing SDA. A filter disk [0.6 cm ø (Cat. # 740-E, Schleicher and Schuell, Keene, NH, USA)] was placed on the culture media at the intersection of the axes. Conidial suspension (10 µl,  $1 \times 10^6$  conidia ml<sup>-1</sup>) was placed on each filter disk and the dishes were sealed with Parafilm-M. Dishes were incubated under the same temperature regimes described above. Every day, the diameters of the colonies along the x- and y-axes of each dish were recorded. If the diameters of the colonies along the two axes were unequal, the average of the two was calculated and considered as the diameter. Four replicate dishes were used for each treatment.

#### *Effect on infection of ticks*

Engorged *R. annulatus* female ticks, 1d post drop-off or within a week of drop-off (kept at 14°C), were dipped in conidial suspension ( $1 \times 10^7$  conidia ml<sup>-1</sup>) for five s, transferred to Petri dishes lined with moist filter paper (five females per dish), and kept at nearly 100% humidity for 14 days. Control ticks were dipped in sterile distilled water containing 0.01% (v/v) Triton X-100 with no conidia. The females were incubated at constant temperature (25, 32 or 35°C), or exposed for 6 or 48 h at 37 or 40°C and then transferred to 25°C for weeks. Tick mortality was checked daily and the weight of eggs laid by five females (in each dish) was recorded on the last day of experiment. Thirty to 40 females were used for each treatment.

## Data analysis

The dead ticks from the bioassay experiments were counted and mortality rates were calculated. Differences in the amounts of germinated conidia, tick mortality or egg mass weight among the different temperature regimes were evaluated using a one- or two-factor analysis of variance (ANOVA) after data was arc-sine transformed (data for tick mortality and conidia germination). If differences among the means of the treatments were found to be significant, Tukey's test was used for comparisons of multiple means.

## Results

### Thermal characteristics of fungal strains

#### *Germination and colony growth at constant temperature (25, 32 or 35°C)*

The germination rate of conidia of all strains incubated at 25 or 32°C was 100% 18 h PI (Table 1). At 35°C, the germination rates of strains differ significantly ( $F = 111.6$ ,  $df$  3, 12;  $P < 0.001$ ); the two strains originally isolated in Ethiopia had high germination rates (86–90% 18 h PI), whereas the strains M.a.-7 and M.a.-Pal-MO2 had very low germination rates (<2% 18 h PI). However, 30 h PI, the germination rates reached  $68 \pm 10$  and  $55 \pm 2\%$  for strains M.a.-7 and M.a.-Pal-MO2, respectively.

The radial growth of the various strains differed significantly ( $F = 16.0$ ;  $df$  3, 36;  $P < 0.001$ ) and was strongly dependent on temperature ( $F = 10.0$ ;  $df$  2, 36;  $P = 0.0003$ ). Incubating the strains M.a.-7 and

M.a.-Pal-MO2 at 32°C resulted in greater than 50% reductions in growth, as compared to incubation at 25°C. Incubating these strains at 35°C resulted in no growth at all (Table 1). The two other strains (M.a.-PPRC-14 and M.a.-PPRC-51) grew as well at 32°C as at 25°C and were even able to grow at 35°C. All of the strains showed heavy sporulation after having been incubated for 7–10 days PI at 25 or 32°C.

#### *Germination and colony growth following thermal shock*

The exposure of conidia to 37 or 40°C for 6 h influenced neither the germination percentage nor the speed of germination (95–100% germination 18 h PI). However, incubating the conidia at 37°C for 48 or 72 h seriously delayed conidial germination in all four strains (Table 2). None of the conidia of the examined strains germinated during the first 24 h after being transferred to 25°C; the conidia began to germinate only after 48 h. The incidence of conidial germination differed significantly between strains ( $F = 20.9$ ;  $df$  3, 12;  $P < 0.001$  for 48 h incubation at 37°C and  $F = 63.8$ ;  $df$  3, 12;  $P < 0.001$  for 72 h incubation at 37°C; Table 2). Incubating the conidia of the tested strains at 40°C for 48 or 72 h dramatically reduced germination; only 0.5–2.0% of these conidia germinated, as compared to the 100% germination observed at 25°C (control).

#### *Effect of temperature on the pathogenicity of the fungi*

Based on the different responses of *M. anisopliae* strains to continuously high incubation temperatures and to temporarily elevated temperatures, two strains

**Table 1** Germination and growth of *M. anisopliae* strains incubated at different temperatures

M. a. strains			Germination at (18 h PI, % $\pm$ SD)			Radial growth (seven days PI, $\phi$ mm $\pm$ SD)		
Strain	Origin	Host	25°C	32°C	35°C	25°C	32°C	35°C
7	Israel	Coleopteran	100 a	100 a	<2c	10.8 $\pm$ 0.4 b	5.3 $\pm$ 0.8 d	0
Pal-MO2	Palestinian Authority	<i>Melolontha hippocastani</i>	100 a	100 a	<2c	12.6 $\pm$ 0.4 a	4.6 $\pm$ 0.9 d	0
PPRC-14	Ethiopia	<i>Pachnoda interrupta</i>	100 a	100 a	86.3 $\pm$ 12.0 b	8.3 $\pm$ 0.1 c	11.4 $\pm$ 0.4 b	1.2 $\pm$ 0.6 e
PPRC-51	Ethiopia	<i>Pachnoda interrupta</i>	100 a	100 a	90.5 $\pm$ 5.0 b	11.1 $\pm$ 0.2 b	11.6 $\pm$ 0.6 b	1.4 $\pm$ 0.4 e

Means followed by the same letter are not significantly different from one another

**Table 2** Germination (%  $\pm$ SD)<sup>a</sup> of *M. anisopliae* var. *anisopliae* strains at 25°C, following 48 and 72 h of thermal shock

Strains	Control (25°C)	Incubated at 37°C for	
	18 h	48 h	72 h
7	100 a	67.4 $\pm$ 15.6 a	21.7 $\pm$ 4.2 a
Pal-MO2	100 a	50.6 $\pm$ 4.5 ab	10.8 $\pm$ 0.8 b
PPRC-14	100 a	38.1 $\pm$ 4.5 c	1.1 $\pm$ 0.5 d
PPRC-51	100 a	6.5 $\pm$ 2.3 d	5.1 $\pm$ 1.0 c

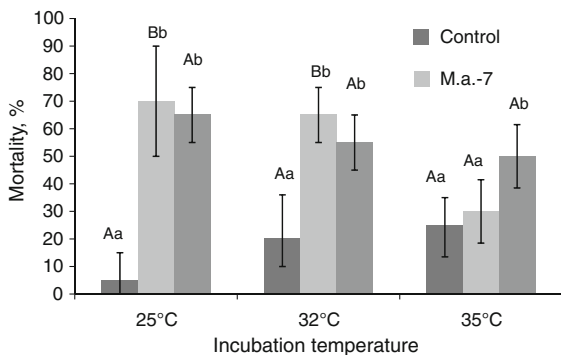
<sup>a</sup> Conidial germination was evaluated in the control dishes following 18 h incubation at 25°C and in the thermal-shocked dishes following 48 h of incubation at 25°C

A thermal shock of 6 h had no significant effect on the conidia. Means followed by the same letter are not significantly different from one another ( $P < 0.001$ )

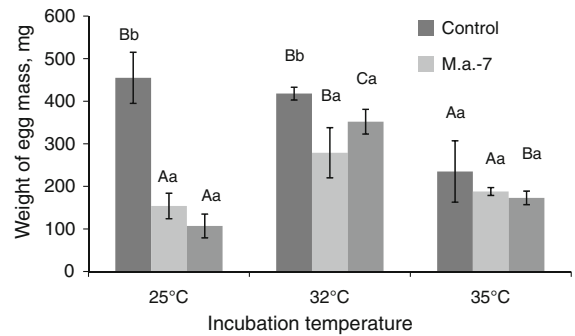
with different thermal characteristics, M.a.-7 and M.a.-PPRC-51, were chosen for further study.

#### Pathogenicity of strains M.a.-7 and M.a.-PPRC-51 to *R. annulatus* kept at a constant temperature of 25, 32 or 35°C

The mortality of ticks infected with M.a.-7 or M.a.-PPRC-51 was significantly higher than that of the control ticks at both 25 and 32°C ( $F = 26.2$ ;  $df$  2, 9;  $P = 0.00018$  and  $F = 14.4$ ;  $df$  2, 9;  $P = 0.0016$ ). At 35°C, only strain M.a.-PPRC-51 caused significantly more mortality than the control ( $F = 5.73$ ;



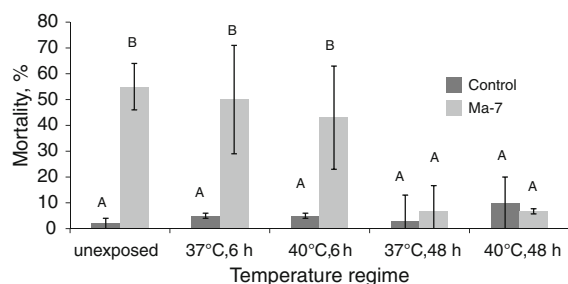
**Fig. 1** Mortality (%  $\pm$ SD, 12 d PI) of engorged *R. annulatus* females following infection with either M.a.-7 or M.a.-PPRC-51 and continuous incubation at the noted temperatures. The control groups were not exposed to fungi. Capital letters denote significant differences among the mortality rates of ticks infected with each of the strains or in the control group at different temperatures. Lower-case letters denote significant differences between strains and the control group at each temperature ( $P < 0.05$ )



**Fig. 2** Average weight of egg masses ( $\pm$  SE, 12 d PI) laid by engorged *R. annulatus* females that had been infected with either M.a.-7 or M.a.-PPRC-51 and incubated continuously at the noted temperatures. The control groups were not exposed to fungi. Capital letters denote significant differences among the weights of egg masses laid by ticks infected with each of the strains or by the control group at different temperatures. Lower-case letters denote significant differences between the weights of the egg masses laid by ticks infected with the different fungal strains and the control group at each temperature ( $P < 0.05$ )

$df$  2, 9;  $P = 0.025$ ; Fig. 1). The average mortality of ticks in the control treatment, as well as the M.a.-PPRC-51-infected groups was not significantly different at any of the tested temperatures ( $F = 2.1$ ;  $df$  2, 9;  $P = 0.17$  and  $F = 2.78$ ;  $df$  2, 9;  $P = 0.11$ , respectively), while the mortality caused by M.a.-7 was significantly lower at 35°C than at 25 or 32°C ( $F = 9.0$ ;  $df$  2, 9;  $P = 0.007$ ). Development of fungi on tick cadavers was observed only on ticks incubated at 25°C and never on ticks incubated at 32 or 35°C.

The weight of egg mass laid by non infected females (control group) incubated at a constant 25°C was similar to that recorded for ticks incubated at 32°C (Fig. 2) and significantly higher than laid by ticks incubated at 35°C ( $F = 12.4$ ;  $df$  2, 6;  $P = 0.0074$ ). The weight of egg masses laid by ticks infected with M.a.-7 or M.a.-PPRC-51 were significantly higher for females kept at 32°C than among those incubated at 25 or 35°C ( $F = 5.46$ ;  $df$  2, 6;  $P = 0.044$  and  $F = 56.3$ ;  $df$  2, 6;  $P = 0.00013$ , respectively). At 25°C, the weight of the eggs laid by infected ticks was 3–4 times less ( $F = 89.9$ ;  $df$  2, 9;  $P < 0.001$ ) than the weight laid by the uninfected (control) ticks (Fig. 2). The weight of the egg masses laid by ticks infected with each of the fungal strains and incubated at 32°C was significantly lower than for the uninfected (control) females ( $F = 13.2$ ;  $df$  2, 9;  $P = 0.002$ ). However, at 35°C, the weight of eggs



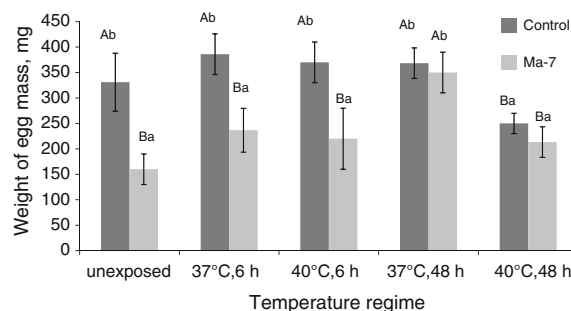
**Fig. 3** Influence of thermal shock on the mortality of ticks infected with M.a.-7. Letters denote significant differences among the mortality rates of ticks infected with M.a.-7 as compared to uninfected ticks under different temperature regimes ( $P < 0.05$ ). Bar: mean  $\pm$  SD

laid by the uninfected (control) females was similar to the weight of eggs laid by females infected by either of the fungal strains ( $F = 2.17$ ;  $df$  2, 9;  $P = 0.17$ ).

#### *Pathogenicity of strains M.a.-7 and M.a.-PPRC-51 to R. annulatus kept temporarily at 37 or 40°C*

After a 6 h incubation at 37 or 40°C, the mortality rate of the ticks infected with M.a.-7 was significantly higher than the mortality rate observed in the control treatment ( $F = 10.69$ ;  $df$  4, 23;  $P < 0.001$ ). Similar mortality was observed among infected ticks that were kept at a constant 25°C ( $F = 0.5$ ;  $df$  2, 17;  $P = 0.62$ ; Fig. 3). However, longer exposure to high temperatures (48 h) reduced fungal efficacy to the same level observed in the control group. The strain M.a.-PPRC-51 lost its pathogenicity after only 6 h of exposure to temperatures of 37 or 40°C. The maximal mortality recorded for this strain following 6 h incubation at 37 or 40°C was 10–15%, the same as in the control group.

A temporal exposure of ticks infected with M.a.-7 and uninfected (control) ticks to higher temperatures had a significant influence on the average weight of the egg masses laid by these ticks ( $F = 16.9$ ;  $df$  14, 79;  $P < 0.001$ ). The weights of the egg masses laid by females infected with the strain M.a.-7 and incubated at 25°C were less than half of the weights of the egg masses laid by the uninfected control ticks (Fig. 4). This strain retained its ability to reduce egg-mass weights even after 6 h of incubation at 37 or 40°C. However, after infected females were exposed



**Fig. 4** Influence of thermal shock on the weight of egg masses laid by females infected with strain M.a.-7. Lower-case letters denote significant differences among the weights of egg masses laid by infected ticks or by the control group at different temperatures. Capital letters denote significant differences between the weights of egg masses laid by infected ticks and those laid by the control group in each temperature treatment ( $P < 0.05$ ). Bar: mean  $\pm$  SD

to temperatures of 37 or 40°C for two days, the weight of the egg masses that they laid did not differ from the weight of the egg masses laid by uninfected ticks (control group; Fig. 4). The effect of the M.a.-PPRC-51 strain on tick oviposition disappeared after the infected females were exposed to only 6 h of these high temperatures.

## Discussion

Environmental conditions, including unfavorable temperatures, are important factors which often determine the success of entomopathogenic fungi in controlling arthropods. The tolerance of fungi to high temperatures is of great importance, not only when the fungi are applied in warm climates or to the warm skin of a vertebrate, but also when they are applied against arthropods exposed to warm conditions for only short periods of time (e.g., arthropods feeding on leaves or walking on bare ground during the day or arthropods exhibiting behavioral fevers) (Arthurs and Thomas 2001; Jaronski 2009; Thomas and Jenkins 1997). Heat tolerance is especially important when choosing fungal strains for controlling ectoparasites while they feed on mammalian hosts.

In practice, even small changes in temperature due to differences in rearing conditions or at different points on the surfaces of the animals can be critical to the development of ectoparasites mycoses. For



on-mammal applications of conidia, higher environmental temperatures, along with the high temperatures found on the surface of the mammal may strongly influence the anti-ectoparasite activity of the fungi (Leemon et al. 2008; Polar et al. 2005). The ventral surface of a feeding tick is often in contact with the animal skin, which, in most cases, is hotter than the environment and less dependent on the ambient temperature. On the other hand, the dorsal surface of a feeding tick can be several millimeters above the host skin and thus the temperature to which it is exposed depends more on the environmental temperature and even irradiation. This suggests that the exact place where the conidia come into contact with the tick may influence the efficacy of the myco-acaricide. Thus, temperature may play an important role in the often inconsistent success of *M. anisopliae* in controlling feeding ticks. The interrelation between air temperature and animal surface temperature depends on many factors, such as wind, animal species and breed, animal rearing conditions, length of hair, coat color, age, physiological state of the animal (e.g., lactation, pregnancy) and activity (Bu and Lephart 2006; Lysyk 2008; Piccione et al. 2005). All of these make animals' surface temperatures sometimes suboptimal or even much higher than the maximal temperature for germination and growth of *M. anisopliae*. Such unfavorable abiotic conditions may last for only a short while or be permanent.

The variability of temperature conditions on animal surfaces makes the selection of a suitable thermo-tolerant strain for on-mammalian host applications even more problematic. Thus, in the development of screening protocols, one needs to choose criteria which reflect the natural situation as much as possible. We have assumed that the efficacy of *M. anisopliae* strains applied on mammals depends, among others, on the following characteristics: (1) the ability of the strain to germinate, grow and infect the target ectoparasite at a relatively high constant temperature near its maximal limit; and/or (2) the ability of the strain to survive a short thermal shock and complete its pathogenic development when transferred to more favorable temperature (i.e., after the ectoparasite discontinues its intimate contact with the mammal and falls to the ground).

In the present study, we observed that the ability of strains to germinate and grow at a constant high temperature (35°C) and their ability to survive a

temporary thermal shock (37–40°C) are not necessarily interdependent factors. For instance, the strain M.a.-PPRC-51 germinated and grew well at a constant temperature of 35°C. However, after having been exposed to a temporary thermal shock (48 or 72 h at 37°C), it germinated poorly. On the other hand, two other strains (M.a.-7 and M.a.-Pal-MO2) germinated relatively well following the same thermal shock, but their germination and growth were suppressed at a constant temperature of 35°C. We also found that the ability of the strains to infect ticks under different temperature regimes is directly related to the strains' thermal characteristics in vitro. While M.a.-PPRC-51 causes similar rates of tick mortality at 25, 32 and 35°C, it completely loses its pathogenicity after a short thermal shock (6 h at 37 or 40°C). The virulence of M.a.-7 was dramatically reduced when it was incubated at a constant 35°C, but there was no change in its virulence following a short thermal shock (6 h at 37 or 40°C). These findings suggest a direct connection between the ability of conidia to germinate, grow or survive in vitro under specific temperature conditions and their virulence toward ticks under these conditions, emphasizing the relevance of in vitro studies for selecting a suitable strain for ectoparasite control. The same correlation was observed between thermal characteristics and a sub-lethal effect of the fungi on the ticks (reduced weight of egg masses).

The influence of continuous high temperature on mortality rates of arthropods infected by fungi has been described in several studies (e.g., Arthurs and Thomas 2001; Bugeme et al. 2009; Lysyk 2008; Polar et al. 2005). Studies have demonstrated that there is no correlation between the fungus's optimal growth temperature and its upper growth-limiting temperature, and its infection of and proliferation within its insect host (Dimbi et al. 2004; De Crecy et al. 2009; Thomas and Jenkins 1997). The optimal temperature for pathogen proliferation within an insect was shown to be higher than the optimal temperature for its development in vitro (Dimbi et al. 2004; Thomas and Jenkins 1997). However, while a selected thermo-tolerant *M. anisopliae* variant was able to grow in vitro at 36–37°C, its virulence at these temperatures was greatly reduced (De Crecy et al. 2009). In our study, strain M.a.-PPRC-51 demonstrated very weak growth at 35°C, while the mortality rates of the infected ticks did not differ significantly

at 25, 32 and 35°C. However, sporulation of fungi on tick cadavers was not observed at 32 or 35°C, even though all of the tested strains show normal sporulation at 32°C in vitro.

The maximal temperatures on animal surfaces under shaded conditions do not normally exceed 40°C (Monty and Garbareno 1978). However, many mammals are not able to keep their body temperature below this limit under extremely hot conditions (Taylor et al. 1981). According to Das et al. (1999), the mean daily surface temperature on the backs of young buffalo calves maintained in an open paddock under direct sun was 42.6°C and often reached 48–50°C for 2–4 h. The tolerance of *M. anisopliae* conidia to extremely high temperatures is highly variable. However, its LT<sub>50</sub> at 48–50°C does not exceed 2.5 h (Li and Feng 2009; Ment D., unpublished results). At a less extreme temperature (40°C), the LT<sub>50</sub> of the fungi varied from 2.7 to 17.6 h (Li and Feng 2009).

Our study demonstrates that exposure of any of the tested strains to temperatures of 37 or 40°C for 6 h followed by incubation at 25°C does not influence later conidial germination. However, exposure to 37°C for 48 h reduced the germination rate to 6.5–67%, depending on the strain. Strain M.a.-PPRC-51, which is less adapted to heat shock, lost its pathogenicity after only 6 h of incubation at a high temperature, although 100% of its conidia survived at this temperature in vitro. In the case of M.a.-7, exposure to a temperature of 37°C for two days made it impossible for the fungus to infect ticks, even after it was transferred and incubated at 25°C.

Obviously the ability of conidia to germinate rapidly and synchronously under temperature regimes found on mammals is a very important characteristic to keep in mind when selecting a strain for on-mammal applications. The ability of fungal strains to develop at elevated temperatures is an essential characteristic, because those strains are more likely to be able to adapt to the temperature of a mammalian coat. At the same time, the ability of a strain to germinate following temporary exposure to high temperatures that exceed its maximal growth limit may also turn out to be a crucial characteristic of strain selection for use on warm-blooded animals (Jaronski 2009). The use of fungi with both types of thermal tolerance may increase the success of microbial control of plant and animal pests. From

the practical point of view, selection of isolates for on-mammal applications may include two series of tests: (1) a test of fungal development at a constant high temperature, and (2) a test of the strain's ability to remain virulent after temporary exposure to temperatures that exist on the surface of mammals (37–41°C).

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