PUBLIC HEALTH

Effect of Constant and Cyclical Temperatures on the Mortality of *Triatoma infestans* (Klug) (Hemiptera: Reduviidae) Treated with *Beauvaria bassiana* (Bals.) Vuill. (Hyphomycetes)

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Neotropical Entomology 34(4):675-679 (2005)

Efecto de Temperaturas Constantes y Cíclicas sobre la Mortalidad de *Triatoma infestans* (Klug) (Hemiptera: Reduviidae) Tratada con *Beauveria bassiana* (Bals.) Vuill. (Hyphomycetes)

RESUMEN - En el presente trabajo se analizó la mortalidad de *Triatoma infestans* (Klug) tratada con *Beauveria bassiana* (Bals.) Vuill. bajo diferentes régimenes de temperatura. Los porcentajes de mortalidad a 26ºC y a 22ºC fueron más altos mientras que los más bajos se registraron a 34ºC constantes. Las combinaciones 26/34ºC o 34/26ºC (12:12h) fueron significativamente diferentes a las de 18/26ºC o 26/18ºC mostrando que es mayor el efecto de altas temperaturas (34ºC) sobre la mortalidad e indicando también que con una temperatura extrema alta unida a una óptima de 26ºC, decrece la susceptibilidad de *T. infestans* a la infección por parte de *B. bassiana*. Se observó que la exposición a temperaturas extremas (18ºC o 34ºC) junto a la óptima de 26ºC en ciclos de 8:8:8h reduce la mortalidad de *T. infestans*. Con alternancia de 6h (6:6:6:6h) se observó que sólo la mortalidad de la combinación 34/30/26/22°C fue similar a las de 22ºC y 26ºC constantes. La ocurrencia de temperaturas extremas durante el primer tramo afectan menos la mortalidad si el período en que ocurren no es tan largo (p.ej. 6h). Sin embargo, si a una temperatura extrema inicial le sigue una caída abrupta (34/22/26/30ºC), la mortalidad se ve significativamente reducida. Los resultados indicarían que es preferible realizar las aplicaciones en el campo al atardecer, para evitar la influencia negativa de cambios bruscos de temperaturas o temperaturas elevadas durante las primeras etapas críticas del ciclo infeccioso de los hongos entomopatógenos.

PALABRAS CLAVE: Regímen fluctuante, germinación, hongo entomopatógeno

ABSTRACT - The mortality of *Triatoma infestans* (Klug) treated with *Beauvaria bassiana* (Bals.) Vuill. under several temperature regimes was analyzed. Mortality rates were highest at 26ºC and 22ºC and lowest at constant 34ºC. The combinations 26/34ºC or 34/26ºC (12:12h cycles) were significantly different from the combinations 18/26ºC and 26/18ºC, showing that high temperatures (34ºC) affect mortality most significantly. The combinations also indicate that when an extreme high temperature is associated with an optimal temperature of 26ºC, the susceptibility of *T. infestans* to *B. bassiana* infection decreases. Exposure to extreme temperature (18ºC or 34ºC) associated with an optimal temperature of 26ºC in 8:8:8h cycles, reduces the mortality of *T. infestans*. In cycles of 6:6:6:6h, only the mortality associated with the 34/30/26/22ºC combination was similar to the combinations at constant 22ºC and 26ºC. Extreme temperatures during the first stage affect mortality less than when this period is not longer than 6h. Mortality decreases significantly when an extreme high initial temperature is followed by an abrupt fall (34/22/26/30ºC). Our results indicate that *Beauvaria bassiana* should be applied to the field in the late afternoon to avoid the negative impact of abrupt changes in temperature, or of high temperatures during the critical first stages of the infectious cycle of this entomopathogenic fungi.

KEY WORDS: Fluctuating regime, germination, entomapathogenic fungus

The blood-sucking bug *Triatoma infestans* (Klug) (Hemiptera: Reduviidae), the most important vector of Chagas disease, is well adapted to domestic and peridomestic habitats (Rabinovich 1972).The entomopathogenic fungus *Beauvaria bassiana* (Bals.) Vuill. (Hyphomycetes) controlled *T. infestans* under laboratory conditions (Lecuona *et al*. 2000).

In insects, development of Hyphomycetes mycosis occurs in 10 stages (Roberts & Humber 1981), the first three (adhesion, germination, and penetration) being highly important for the beginning of the pathogenic cycle. These initial stages develop during the first hours after the contact between host and pathogen. Depending upon the technique of application, approximately 95% of the *B. bassiana* conidia germinate soon after the 24h of inoculation in larvae of *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) (Fernandez *et al*. 2001). Neves & Alves (2004) also showed that conidia germination of *Metarhizium anisopliae* (Metsch.) Sorokin and *B. bassiana* on the tegument of *Cornitermes cumulans* Kollar (Isoptera: Termitidae) occurs between 6h and 12h after inoculation; penetration occurs between 12h and 24h; and colonization of both fungi in the insect, between 24h and 72h.

The disease cycle is influenced by several factors, including environmental conditions such as temperature, relative humidity, and light (Alves 1998). Temperature is one of the most important factors because it affects both the growth of *B. bassiana* (Fargues *et al*. 1997) and its efficiency on *T. infestans* (Lecuona *et al*. 2001). However, despite the fact that most laboratory samples are analyzed under constant conditions, field conditions are variable and unpredictable. Fargues & Luz (2000) have shown the effect of fluctuating humidity and temperature regimes on *B. bassiana* infection in *Rhodnius prolixus* (Stål) (Hemiptera: Reduviidae). Nonetheless, Lecuona *et al*. (2001) concluded that relative humidity does not affect mortality of *T. infestans* with three strains of *B. bassiana*. Based on previous results, we analyzed the mortality of 3rd-instar nymphs of *T. infestans* under constant and fluctuating temperature regimes. No other variables were analyzed in this study.

Material and Methods

Populations of *T. infestans*. Specimens of *T. infestans* were collected in rural areas of Santiago del Estero by employees of the Servicio Nacional de Chagas (Cordoba, Argentina). The insects were reared in the laboratory at 27 ± 1ºC and 80 ± 10% relative humidity (R.H.), and fed on chickens. Third-instar nymphs (N3) that belonged to this colony were taken to the Laboratory of Entomopathogenic Fungi (Laboratorio de Hongos Entomopatógenos - IMYZA-INTA Castelar, Buenos Aires), where the experiments were conducted.

Cultivation of *B. bassiana*. A strain Bb10 that belongs to the fungal culture collection of the IMYZA-INTA Castelar was used. The strain was isolated from *Diatraea saccharalis* (Fabricius) (Lepidoptera: Pyralidae), in Argentina. Before the strain could be used in this study, it was isolated a second time in nymphs of *T. infestans*. The strain was maintained in a petri dish with complete agar medium (CAM) containing (g/l): KH_2PO_4, 0.4; Na_2HPO_4, 1.4; $MgSO_4$, 0.6; KCl, 1; NH_4NO_3, 0.7; glucose, 10; agar, 15; and yeast extract, 5. Conidia were extracted from 14-days old fungal colonies, and were incubated in petri dishes at 26 ± 0.5ºC. Viability was assessed at 18, 22, 26, 30 and 34 ± 0.5ºC by counting the number of conidia that had germinated. The conidia were incubated in CAM with 1 x 10^7 conidia/ml, in 10 microscopic fields 8, 12, 16, 20 and 24h after planting. Conidia are considered germinated when the germination tubule reaches a length that is the same, or greater than the widest part of the conidial body.

Mortality of *T. infestans* under Constant and Fluctuating Temperature Regimes. The activity of the strain Bb10 on *T. infestans* was analyzed by means of a completely random design with 20 N3 nymphs in each repetition. The number of repetitions varied from six to 24, depending on the availability of insects. Nymphs were fed on chickens up to a week before the assays started. Inoculation was carried out by immersion, 6 min after each repetition, in a suspension of 1 x 10^8 conidia/ml in sterile water with 0.01% Tween 80. The insects were placed in a plastic cylindrical sieve (4 x 4.5 cm) for immersion. After immersion in the fungal suspension, each nymph was kept in a separate plastic container (4.5 x 2.5 cm), had their upper bodies covered with a voile fine cloth, and were maintained at different temperatures and in the dark, according to each treatment. The nymphs were not fed for 14 days. Control insects were dipped into sterile water with 0.01% Tween 80, also for six seconds.

The assays were carried out at constant temperatures (18, 22, 26, 30 and 34 ± 0.5ºC), and daily temperature fluctuation cycles (12:12h, 8:8:8h and 6:6:6:6h). All the assays were conducted in the dark, at 80 ± 10% R.H. Nymph mortality was recorded daily, and cadavers were taken to a humid chamber (saturated atmosphere) for fungal sporulation. Mortality was analyzed by ANOVA and SNK, soon after the data arc sine transformation. Percentiles of the survival function were estimated by the Kaplan-Meier method (Kaplan and Meier 1958), by using all insects belonging to each treatment.

Results and Discussion

Mortality of *T. infestans* under Constant and Fluctuating Temperatures. Mortality percentage was higher at 26ºC in the 12:12h and 8:8:8h cycles, and at 26ºC and 22ºC in the 6:6:6:6h cycle (Table 1). The lowest mortality among all cycles analyzed was recorded at 34ºC, and no deaths due to mycosis or other causes were recorded among the control insects. Mortality rates under constant temperature in this study were slightly lower than mortality rates obtained by Lecuona *et al*. (2001). These differences might be due to differences in the geographic sites where the samples were collected. In the above- mentioned study, samples came from a geographic site in Córdoba (Lat. 30-31ºS, Med. Annual Temp. = 17.3ºC, Max. Med. Annual = 24ºC, and Min. Med. Annual = 11.2ºC) where environmental conditions are different from those in Santiago del Estero, the site for our study (Lat. 28-29ºS, Med. Annual Temp. = 20.3ºC, Max. Med. Annual = 27.4ºC, and Min. Med. Annual = 14.1ºC). Lecuona *et al*. (1996), working with specimens of *T. infestans* from Argentina and Brazil, observed differences in virulence for *B. bassiana* and *Metarhizium anisopliae* (Metsch.) Sorokin. Pires *et al*. (1998, 2000) also found morphological and biological differences between Bolivian and Brazilian

Table 1. Mortality (%) of *T. infestans* inoculated with *B. bassiana* (strain Bb10) under different temperature regimes (ºC). H.R. 80%. Castelar, Buenos Aires, Argentina.

T (ºC)	12:12h		T (ºC)	8:8:8h		T (ºC)	6:6:6:6h	
26	73.3 ± 1.91 (18)[1]	a	26	66.3 ± 6.91 (14)	a	26	66.1 ± 4.89 (10)	a
18/26	65.8 ± 1.65 (12)	b	18	55.9 ± 3.61 (18)	b	22	65.9 ± 3.21 (10)	a
26/18	62.2 ± 2.01 (12)	b	18/26/34	49.6 ± 2.03 (24)	bc	34/30/26/22	61.1 ± 2.35 (12)	ab
18	56.3 ± 3.97 (6)	bc	34/26/18	39.0 ± 2.12 (24)	c	22/26/30/34	59.8 ± 2.65 (20)	b
34/26	52.8 ± 2.38 (16)	c	26/34/18	37.5 ± 2.86 (8)	c	30	52.1 ± 4.78 (10)	bc
26/34	50.0 ± 2.89 (16)	c	34	17.9 ± 2.19 (18)	d	26/30/34/22	47.6 ± 2.28 (8)	c
34	21.3 ± 2.31 (12)	d				30/34/22/26	42.3 ± 2.23 (8)	cd
						34/22/26/30	38.1 ± 2.67 (8)	d
						34	14.1 ± 2.81 (10)	e

Mean values ± SEM of mortality percentages in arc sine (repetitions). The same letters in a column do not represent significant differences ($P < 0.05$). Test S-N-K. ANOVA: $F = 121,6$, $df = 7,98$, control n = 14 (12:12h cycle), $F = 54.9$, $df = 6,119$, control n = 20 (8:8:8h cycle), $F = 52.3$, $df = 9,98$, control n =12 (6:6:6:6h cycle).

populations of *T. infestans*.

There were no significant differences in the assay with daily fluctuation cycles (12:12h) and two temperatures: 18/26ºC and 26/18ºC, or 34/26ºC and 26/34ºC (Table 1). These data indicate that mortality is not affected by the order in which extreme temperatures (18ºC or 34ºC) occur, before or after the optimal temperature (26ºC). The associations 26/34ºC or 34/26ºC were significantly different from the 18/26º or 26/18ºC ones, and show that the effect of high temperatures on mortality is most important. These differences also show that an extreme high temperature associated with an optimal temperature (26ºC) decrease the susceptibility of *T. infestans* to *B. bassiana*. Rath *et al*. (1995) studied the virulence of *M. anisopliae* for *Adoryphorus couloni* (Burmeister) (Coleoptera: Scarabaeidae) at constant and fluctuating temperatures (daily cycles of 12:12h at 15/5ºC). These authors found that exposure to a low temperature (5ºC) associated with an optimal temperature (15ºC for *M. anisopliae*) led to a reduction in virulence of about twice as high as that reached at constant 15ºC.

Temperature associations, particularly at 26/34ºC, are common in regions in Argentina with high levels of activity of the species *Triatoma* (Gorla & Schofield 1985). Consequently, temperature associations might affect the effectiveness of the fungal insecticide.

Inglis *et al*. (1996) suggested that high temperatures do not affect conidia germination but impact penetration and proliferation of the fungus within the insect. Our data, however, show that at 34ºC, the maximum germination in vitro was 3% after 24h (Fig. 1). Therefore, we may expect: (1) that a small number of germinated conidia on the tegument of the insect can lead to low mortality (21%), under

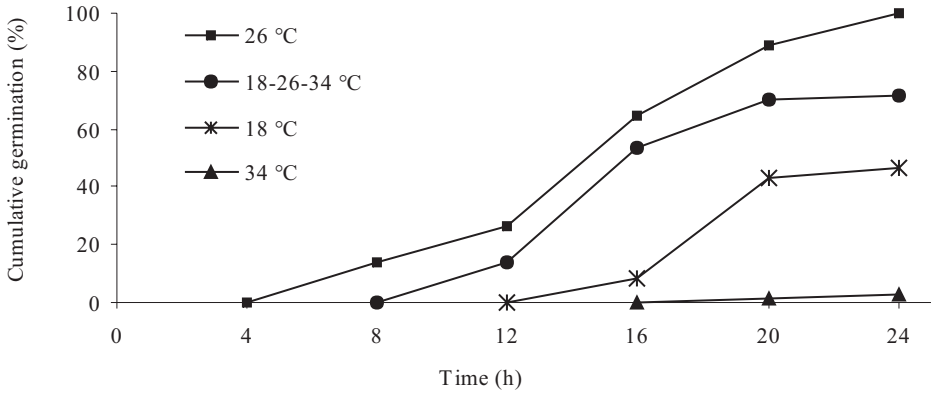

Fig. 1. Germination of *B. bassiana* conidia (strain Bb10) in complete agar medium (CAM) during a 18/26/34ºC cycle (8:8:8h) and constant temperatures. Castelar, Buenos Aires, Argentina.

constant high temperatures (Table 1); and (2) that an increase in mortality (50-52%) may occur when associated with a different temperature (26ºC) within a daily cycle of 12:12h. Hunt *et al*. (1984) suggested that germination and penetration of a very small amount of conidia can cause mycosis in *Dendroctomus ponderosae* (Coleoptera > Scolytidae), under laboratory temperature conditions. Our results support those obtained by Hunt *et al* (1984) in that only a few conidia in our study were able to germinate, penetrate in the blood-sucking bug and cause mycosis at 34ºC. However, exposure to such a high temperature inhibits the normal development of the disease and leads to a low mortality (%) even though conidia germination alone cannot guarantee the penetration of the fungus into the insect haemolymph**,** as mentioned by Lecuona *et al*. (1991) and Fernandez *et al*. (2001).

Results of present investigation support the findings by Fargues *et al*. (1997) that *B. bassiana* can develop within a wide range of temperature (8ºC minimum and 35ºC maximum), the optimal being usually between 25ºC and 28ºC for most strains. Fargues and Luz (2000) did not find any difference in *R. prolixus* under the 25/35ºC (12:12h) temperature associations because mortality rates were the same in all combinations of this cycle. These results indicate that mortality, in this kind of blood-sucking bug does not decrease at 35ºC associated with an optimal of 25ºC.

When the percentage of mortality from assays with three temperature cycles and alternation every 8 h (Table 1) was recorded, the values corresponding to constant temperatures (18 and 34ºC) were significantly different from those at optimal temperature (26ºC). The associations 18/26/34, 34/26/18 and 26/34/18ºC, were statistically similar. Exposure to extreme temperatures (18ºC and 34ºC), associated with the optimal temperature of 26ºC during 24h, reduced mortality of *T. infestans*. At 18ºC, however, as opposed to 34ºC, germination of conidia *in vitro* was delayed (Fig. 1), and caused less impact on the infectious process *in vivo.*

In the assay at constant temperatures and combined with fluctuations every 6h, only the mortality associated with 34/30/26/22ºC was similar to mortality at constant 22ºC and 26ºC. We may infer that extreme temperatures during the first stage affect mortality less if the period of time when they occur is not longer than 6h. However, if an extreme initial temperature is followed by an abrupt fall (34/22/26/30ºC), instead of a slight fall (34/30/26/22ºC), mortality is significantly reduced (38%). Percentages of mortality obtained at 34/30/26/22ºC and 22/26/30/34ºC were different from the associations 26/30/34/22ºC and 30/34/22/26ºC. These differences may be due to the abrupt fall (12ºC) between two consecutive temperatures (34ºC and 22ºC).

Analysis of the mean survival time (Table 2) shows that 50% mortality was reached on the 9th day at constant 26ºC, whereas at constant 34ºC, mortality reached 25% after 14 days. Only four treatments reached 75% mortality on the 13th and 14th days after the beginning of the assay: treatments carried out at constant 22ºC and 26ºC, and the 18/26ºC and 26/18ºC cycles, whereas most combinations reached 50% accumulated mortality between the 13th and 14th days.

Results from our study are important in practical terms because unsatisfactory results can be obtained in the field due to combined temperature effects that occur naturally in the environment, and are seldom taken into account. The results, nonetheless, suggest that it is best to apply the fungus to the field late in the afternoon, to inhibit the negative impacts of abrupt changes in temperature, and of high temperatures during the first stages of the infectious cycle of entomopathogenic fungi.

Table 2. Mean survival time (days) and mortality (%) of *T. infestans* inoculated with strains of *B. bassiana* (strain Bb10) under different temperature regimes (ºC). H.R. 80%. Castelar, Buenos Aires, Argentina.

Temperature regime (ºC)	Accumulated mortality (%)		
	25	50	N[1]
34	-	-	320
34/30/26/22	8	11	300
26/18/34	13	-	600
34/26/18	13	-	600
30/34/22/26	11	-	200
26	7	9	460
18/26	9	11	300
22	11	12	100
30	8	12	100
26/18	9	12	300
22/26/30/34	8	12	500
34/22/26/30	9	12	500
18	10	13	240
26/34	9	13	400
34/26	9	13	400
26/30/34/22	11	13	200
18/26/34	10	14	600

[1] Total number of insects analyzed

Acknowledgments

The authors are thankful to Estela Favret (IMYZA-CICV and A-INTA Castelar) for her valuable help to find relevant bibliography, to Teresa Carpio, Debora Moreyra, and Mariana Turica for the technical support, to Delmi M. Canale (Servicio Nacional de Chagas, Cordoba, Argentina) for providing the triatomines, and to Roberto T. Alves (Embrapa, Brazil) for the critical review of the manuscript. This study was supported by the Secretaria de Ciencia e Tecnica (BID-SEC and T-PICT 08—04526).

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Received 04/VI/04. Accepted 14/XII/04.