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Bioassay

The double-edged sword effect of warming on the soil predatory mite *Cosmolaelaps brevistilis* (Karg, 1978) (Mesostigmata: Laelapidae)

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Abstract. In this study we used the average annual temperature in Brazil and four warming predictions to propose a maximum thermal threshold for the predatory mite *Cosmolaelaps brevistilis* (Karg, 1978) (Mesostigmata: Laelapidae) as an experimental model. Then, we evaluated the effects of warming on the biological parameters of *C. brevistilis* for conservation biological control. We observed higher rates of oviposition and predation of the prey *Tyrophagus putrescentiae* (Schrank, 1781) (Astigmatina: Acaridae) under warmer conditions at 75 ± 10% RH. The highest rates of prey consumption and oviposition were observed at 28°C compared to the others tested (22, 24, and 26°C). However, the egg viability of *C. brevistilis* was reduced at the same temperature. Therefore, temperature increases both positive and negative effects on the biological parameters of *C. brevistilis* which are important for the ecological interactions that are essential for biological control programs.

Keywords: Conservation biological control, Acari, heatwave.

Predatory mites of the order Mesostigmata are among the most numerous and abundant arthropods in soils (Beaulieu et al. 2011), and are natural enemies of pests in agricultural crops (Castilho et al. 2015). However, the responses of increased temperatures on them and the consequences for biocontrol are poorly explored. Given predatory mites are natural enemies of agricultural pests (Freire et al. 2007; Castilho et al. 2009; Moreira et al. 2015), and that pests can expand their distribution under warming conditions (de Paula & Barreto 2020), understanding their response to warming would aid in the development of better plans for biological control in the face of climate change.

In this study we investigated the biological characteristics of the predatory mite *Cosmolaelaps brevistilis* (Karg, 1978) (Acari: Mesostigmata: Laelapidae) at five temperatures in the range of 20-28°C. Currently, there are records for this predatory mite in grasslands in southern Brazil (Duarte et al. 2017). Knowledge on the biology on *C. brevistilis* will enhance the possibilities for its more effective use as a biological control agent on different crop fields, such as soybean, where our specimens were sampled for this study.

Adults of *C. brevistilis* were collected from the soils of soybean crop sites located at the experimental area of the Department of Entomology and Acarology ESALQ/University of São Paulo, in Piracicaba, SP, Brazil (22°42'47.0"S; 47°37'41.4"W) and reared in the laboratory. The mites were kept in plastic containers according to Abbatiello (1965). Colonies were maintained in the dark, at a room temperature of 22-27°C, a relative humidity of 75 ± 10%, and were fed daily with all stages of the free-living nematodes *Panagrellus redivivus* (Linnaeus, 1767) Goodey, 1945 (Nematoda: Tylenchida: Panagrolaimidae).

The experimental bioassay units consisted of a Petri dish (2.6 cm diameter × 1 cm height) with the bottom covered by a layer of a mixture of activated charcoal and plaster, and it were wetted daily with 0.5 mL of distilled water. The edges of each experimental unit were sealed with plastic film to prevent the mites from escaping. We used one mated adult female of *C. brevistilis* (<72 hours old) in each experimental unit. For each unit, 20 adult females of *Tyrophagus putrescentiae* (Schrank, 1781) (Astigmatina: Acaridae) were offered as food as this is a common biological model (Freire & Moraes 2007; Moreira et al. 2015; Barbosa & Moraes 2016).

Twenty experimental units were subjected to the five different temperatures as follows: a) average annual temperature for the region (20 ± 1°C), b) average annual temperature + Intergovernmental Panel on Climate Change (IPCC) prediction in the best scenario (22 ± 1°C), c) average annual temperature + IPCC prediction in the worst scenario (24 ± 1°C), d) average annual temperature + IPCC prediction in the best scenario + heat wave event (26 ± 1°C), and e) average annual temperature + IPCC prediction in the worst scenario + heatwave event (28 ± 1°C). All temperature treatments were conducted in a climate-controlled chamber with 75 ± 10% of relative humidity and 12 h of photophase.

The experimental units were observed during six consecutive days under a dissecting microscope to determine the number of consumed prey, the eggs laid by the predators, the viability of predator eggs (# of hatched eggs), and the predator survival rates (survival probability over time). For that, we offered 20 new live prey daily. Egg viability was measured daily as the proportion of hatched eggs to the total number of eggs laid. For this purpose, each day, and after counting the eggs, each female predatory mite was transferred to a new experimental unit (containing live prey) under the same temperature conditions, thus leaving the eggs in the original experimental unit, preventing any interferences with regards to egg transfer. The first day of data was discarded to account for any previous feeding event by predators in the rearing colonies.

Normality and homoscedasticity of the data obtained for predation and oviposition rates were separately checked using Shapiro-Wilk's and Bartlett's tests, respectively. The data did not satisfy the normality and variance homogeneity, even after transformations, so Kruskal-Wallis non-parametric Dunn test with Bonferroni correction ($p < 0.05$) was performed in R software 3.6.3 (R Core Team 2020). The egg viability data was analyzed using a generalized linear model with a quasibinomial distribution. The survival of female predatory mites exposed to temperatures overtime was subjected to survival analysis by Kaplan-Meier estimators (Log-Rank), and the survival curves were compared with the Holm-Sidak's method ($p < 0.05$) using the software SigmaPlot 12.3 (Systat Software 2013). To explain the contrast between both prey consumption and egg viability as a function of temperature, the data was analyzed using a Pearson correlation built from a matrix of correlations and their respective coefficients. Subsequently, the data was fit into a Gaussian model to determine whether there was a significant relationship between the two variables.

The number of *T. putrescentiae* consumed by *C. brevistilis* significantly increased with temperature ($\chi^2 = 59.95$; df = 4; $p < 0.001$) (Tab. 1); predators kept at 28°C consumed more prey than mites under all the lower temperatures. The lowest predation rates were recorded for mites kept at 22°C and 20°C. Fecundity (eggs/female/day) and egg viability (% of hatched eggs) were also significantly affected by temperature ($\chi^2 = 61.68$; df = 4; $p < 0.001$; F = 11.84; df = 4; $p < 0.001$, respectively) (Tab. 1). The number of eggs laid by predators increased with warming; females kept at 26°C and 28°C oviposited more eggs per day than females kept at lower temperatures. When the temperature reached 28°C we found a reduction in egg viability by 25% compared to other temperatures.

Table 1. Predation rate, fecundity (eggs/female/day) and egg viability % (mean ± S.E.) of *Cosmolaelaps brevistilis* (Karg, 1978) at different temperatures.

Temperature (°C)	Consumed prey/day⁑	Fecundity⁑ Eggs/ female/day	Egg viability (%)*
28	6.11 (± 0.27)^{a}	2.96 (±0.20)^{a}	76 (±3.97)^{b}
26	4.63 (±0.21)^{b}	2.57 (±0.14)^{a}	92 (±2.76)^{a}
24	3.94 (±0.18)^{b}	1.90 (±0.11)^{b}	95 (±1.51)^{a}
22	3.26 (±0.14)^{c}	1.46 (±0.07)^{c}	96 (±1.40)^{a}
20	2.94 (±0.13)^{c}	1.11 (±0.06)^{c}	93 (±1.91)^{a}

Values followed by different letters within columns are significantly different based on ⁑Dunn test with Bonferroni correction ($p < 0.05$) or *by contrasts from quasibinomial generalized linear model.

Female adult survival was also significantly affected by temperature (Log-Rank = 11.14; df = 4; $p = 0.025$) (Fig. 1A). At 28°C, the survival of predatory mites slowly decreased to 5.50 (± 0.21) days and significantly differed from that at 20°C, for which we found no reduction in the survival probability of *C. brevistilis*. Predation was positively correlated with temperature (Gaussian model corresponded: df= 1; F = 55.51), indicating a tendency to increase as a function of temperature; the values for predation and egg viability rates intercepted by this correlation were $\rho = 0.60$ and -0.07, respectively, albeit non-significant. The values with negative correlation coefficients for egg viability tended to decrease (gaussian model corresponded: df= 1; F = 21.062) as predation rates rose ($\rho = -0.42$) (Fig. 1B).

In this study, we found higher rates of prey consumption by *C. brevistilis* under warming conditions, which was followed by increases in daily oviposition. However, when predators were subjected to the highest temperature (28°C), a trade-off was apparent, and the egg viability and adult survival were significantly lower. The increase in consumption rates is linked to higher metabolic rates of predators at high temperatures (Laws 2017); as metabolic demands increase exponentially, predators compensate for energy expenditure with greater food intake (O'Connor 2009; Lemoine et al. 2013). For example, Ramachandran et al. (2021) observed that the predatory mite *Stratiolaelaps scimitus* (Womersley, 1956) (Acari: Mesostigmata: Laelapidae) consumed more individuals of *Carpoglyphus lactis* (Linnaeus, 1767) (Acari: Carpoglyphidae) when kept at 24°C compared to 16°C.

Notably, high metabolic rates also altered the reproduction of *C. brevistilis* in our study. In this case, the reproductive performance of *C. brevistilis* increased proportionately with increasing temperature. We thus posit that there is a high optimal reproductive performance between 24-26°C, with this temperature range previously suggested to as the ideal limit for the development and reproduction of most arthropods that inhabit the soil in tropical regions (Ikemoto 2005; Dixon et al. 2009; Damos & Savopoulou-Soultani 2011; Skendžić et al. 2021). In addition to prey consumption, high metabolic rates may induce changes in the reproductive parameters of predators (Verberk et al. 2013; King & MacRae 2015). The thermal stress may have caused changes in the reproductive pattern of *C. brevistilis* through the poor formation of oocytes, as observed in *Drosophila melanogaster* Meigen, 1830 (Diptera: Drosophilidae) according in Meiselman et al. (2018). In this case, we observed a reduction in egg viability by 25% at 28°C. We thus suggest that temperatures above 30°C may compromise the fertility of these predators by reducing egg viability.

In summary, changes in the biological parameters of *C. brevistilis* under future climate warming conditions will likely affect their predatory behaviour, which we showed using *T. putrescetiae*, a biological model. Warming increased prey consumption and oviposition but decreased the viability of eggs. These changes can be of high significance when considering that this top predator found in soils can be used as a conservation biocontrol agent to keep the populations of agricultural pests in check in crops, like soybean.

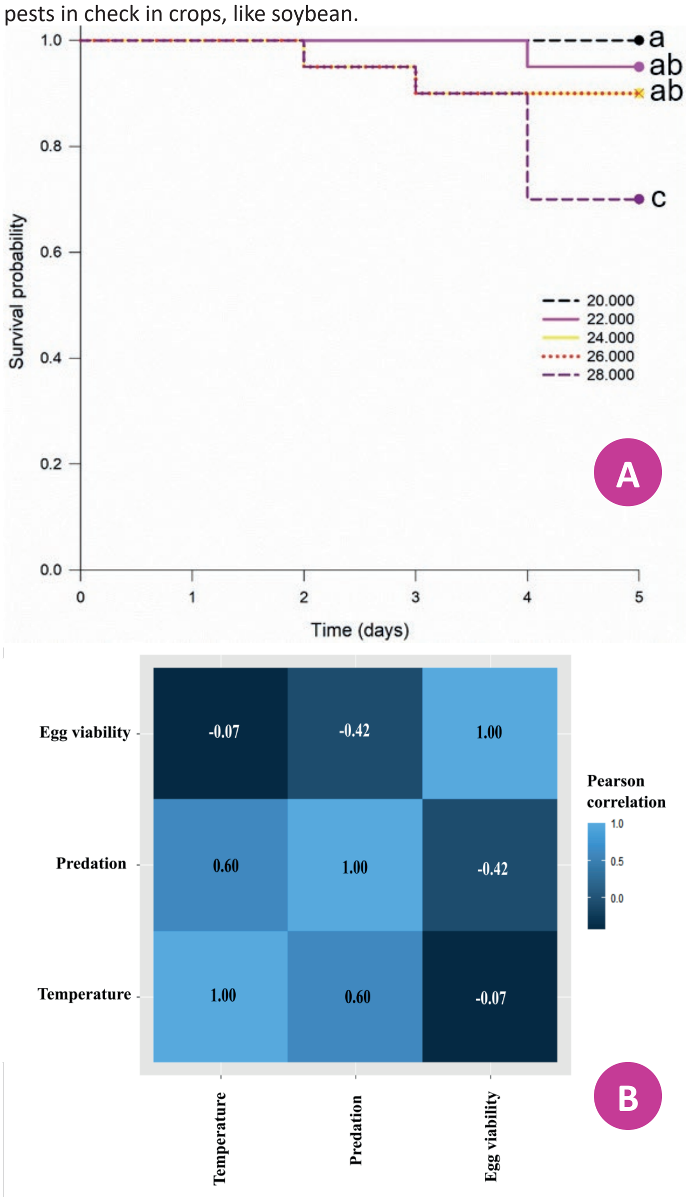

Figure 1. Survival curve of *Cosmolaelaps brevistilis* (Karg, 1978) kept at different temperatures. Curves followed by different letters are significantly different based on the Kaplan-Meier test ($p < 0.05$). Survival proportion varies between 0 to 1, where 1 equals 100% of probability (**A**); Thermal map of the correlation coefficient of predation ($\rho = 0.601$) and egg viability ($\rho = -0.421$) parameters of *C. brevitilis* as a function of the increase in temperature (**B**).

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Authors' Contributions

GB, JCS and PTY conceived and designed the study. GB and LLG conducted experiments and collected the data. ACP and JBP analyzed the data. GB, CB, JCS, ACP, JBP, LLG wrote the manuscript. GB, JCS, ACP, JBP, and PTY contributed to interpretation of results and overall improvement of the manuscript. All authors commented on previous versions of the manuscript and approved the final manuscript.

Conflict of Interest Statement

We declare no conflict of interest.

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