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IMPACTO DA TEMPERATURA NA MANUTENÇÃO DOS SIMBIONTES ASSOCIADOS AOS CECOS GÁSTRICOS DE PERCEVEJOS

IMPACT OF TEMPERATURE ON THE MAINTENANCE OF STINK BUGS CAECA-ASSOCIATED SYMBIONTS

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ABSTRACT: Impacts of climate change may affect population performance directly or indirectly through mediated effects on their mutualists. Recently, my colleagues and I showed that the stink bugs species *Nezara viridula*, *Acrosternum hilare*, *Murgantia histrionica* harbor a dominant caecum-associated symbiont. We detected the symbionts presence in the gastric caecum through PCR (Polymerase chain reaction) by using specific primers and monitored the demographic parameters of the insects rearing them at 25 and 30°C. High temperature (30ºC) eliminated the caeca-associated symbionts of *A. hilare* and *M. histrionica.* Mortality of *A. hilare* was substantially higher at 30°C in the first generation, however *M. histrionica* only started to show higher mortality at 30°C during second generation. Here I present the data of Prado et al. (2010) which showed that host fitness was potentially mediated by symbiont loss at 30°C, and that climate changes can have influence on the host's development, ecology, and geographic localization.

KEY WORDS: Bacteria, Climate change, insects.

INTRODUCTION

The effect of climate on organisms, communities, and the environment at large has become a pressing issue for biologists and environmental scientists (PRADO et al., 2010). The ecological impact of the global warming is already apparent (WALTHER et al., 2002) in the effects seen on species fitness (POST et al., 1997), range shifts (PARMESAN; YOHE, 2003), species interactions (HOFSTETTER et al., 2007), and community structure (SAGARIN et al., 1999). Despite the current interest in insect-microbe symbioses, the vast majority of such systems have been poorly studied. The group of insects called stink bugs (Heteroptera: Pentatomidae) has recently received some attention. Recently it was shown that the stink bugs *Acrosternum hilare*, *Chlorochroa ligata*, *Chlorochroa sayi*, *Chlorochroa uhleri*, *Dichelops melacanthus*, *Edessa meditabunda*, *Euschistus heros*, *Loxa deducta*, *Murgantia histrionica*, *Nezara viridula*, *Pellaea stictica*, *Piezodorus guildinii*, *Plautia stali*, *Thyanta pallidovirens* and *Thyanta perditor* are associated with plant-pathogens (*Pantoea* spp.) contained in the gastric caecal region (ventricula 4) of their midguts (PRADO; ALMEIDA, 2009a; PRADO; ZUCCHI, 2011). Although the mechanism of symbiont vertical transmission is poorly understood, females seem to smear the surface of eggs with bacteria while ovipositing (BUCHNER, 1965). Aposymbiotic first instars hatch but remain on the surface of eggs and acquire the symbiont by probing on the egg surface, as evidenced by the fact that surface sterilization of egg masses generates aposymbiotic individuals (PRADO et al., 2006; PRADO; ALMEIDA, 2009b; PRADO et al., 2010). Climate change has already affected stink bug performance and geographic range (MUSOLIN, 2007). Previously it was showed that for the species *N. viridula* high temperature eliminated gut symbionts, without any clear decrease in host fitness (PRADO et al., 2009). Thus, it remained unclear whether temperature change played a role, either directly or indirectly, in these geographic shifts. To better understand the extent to which temperature mediates stink bug ecology and prevalence of their gut bacteria, I present the conducted laboratory studies with two pentatomid species, *A. hilare* and *M. histrionica* (PRADO et al., 2010).

MATERIALS AND METHODS

Experiment: It was tested whether high temperature affects symbiont maintenance in *A. hilare*, using 30 egg masses housed individually in plastic containers with mesh screen covers (PRADO; ALMEIDA, 2009b). Fifteen replicate containers were placed in a controlled temperature chamber at 25°C, while the other 15 were kept in a chamber at 30°C; both had a 16/8 L/D photoperiod. It was censused the insects every 7 days to estimate development and mortality. Censuses continued until all the insects died or reached adulthood. As adults developed, It was transferred them to a separate cage and allowed them to breed under the same conditions. While the insects were in the adult stage, It was evaluated insect mortality and fecundity. To establish the second generation, It was collected 15 egg masses per temperature, laid by first-generation adults. Fifteen egg masses were placed in their respective temperature-controlled chambers (25 and 30°C) with the same measurements taken as for the first generation. It was counted the number of dead insects and the number of egg masses produced during the females' lifetimes at each temperature. A similar experiment was conducted with *M. histrionica*, using 40 egg masses, housed in the same plastic containers. Twenty replicate containers were placed in each of two chambers, set to 25 or 30°C with a 16/8 L/D photoperiod. Second generation was initiated as before, with 45 replicate egg masses at 25°C and 20 egg masses held at 30°C. It was censused all replicate cages every three days for both generations until the insects' death. A suite of demographic parameters were estimated for each of the stink bug species: net reproductive rate (R_0), mean generation time (T), intrinsic rate of increase (*r*), finite rate of increase (λ), doubling time (DT), and gross reproductive rate (GRR). It was also determined adult emergence (day first adult appeared) and oviposition parameters (preoviposition period, oviposition period, number of egg masses, and total number of eggs per egg masses). Effects on development rate were analyzed using the median development time (MDT). Here I will only show the results for mortality and reproduction of the stink bugs and the maintenance of the symbionts.

Symbiont detection: For *A. hilare*, two nymphs of second, third, fourth, and fifth stages and one adult male and female were sampled from each replicate for detection of the symbiont by PCR as described previously (PRADO; ALMEIDA, 2009b). For *M. histrionica*, we sampled one individual of each of the same stages from each replicate (PRADO; ALMEIDA, 2009b).

RESULTS AND DISCUSSION

Mortality:

Mortality in the first generation of *A. hilare* was substantially higher at 30°C than at 25°C (Figure 1A). During the first generation, egg viability was low, and first (26.42±8.8) and fifth nymphal stage (36.67±13.2) mortality rates were higher. The first (40.71±10.5) and second (22.60±5.6) nymphal stages of the second generation also had high mortality rates.

Mortality rates for *M. histrionica* were greater for the first (19.27±4.8), second (20.41±5.6), and fourth (20.24±5.8). nymphal stages at 30°C of the second generation compared with the first generation (5.61± 2.8, 8.92±2.4, and 3.59±2.1, respectively, for first, second, and fourth instars) (Figure 1B).

Reproduction:

Acrosternum hilare reproduction was similarly affected by temperature. The preoviposition period was reduced at the higher temperatures, but so were the number and size of egg masses (Figure 2). *Murgantia histrionica* reproduction showed trends toward reduced oviposition period and number of eggs (Figure 2) at higher temperature.

Maintenance of the symbionts:

The prevalence of symbionts in the two insects varied greatly among temperatures and generations (Figure 3). For *A. hilare* there were significant differences in prevalence between temperatures for all stages. Prevalence was generally high across all stages at 25°C for both

generations. Conversely, at 30°C prevalence dropped off dramatically after the second instar, particularly in the second generation (Figure 3A). For *M. histrionica* there were significant differences in prevalence between temperatures for all but the third nymphal instar. Symbiont prevalence was slightly lower than in *A. hilare* at 25°C, especially in the second generation. Moreover, prevalence in *M. histrionica* showed large differences between generations at 30°C, with no symbionts detected in any of the second-generation insects (Figure 3B).

Our results indicate that high temperature (30°C) has a similar impact on both stink bugs studied, *A. hilare* and *M. histrionica*. First, the prevalence of the gut symbiont was reduced or it was absent in second-generation insects of both species, results similar to those for another pentatomid (PRADO et al., 2009), suggesting that temperature has an important role in the maintenance of gut symbionts in this insect family. In addition, almost all demographic parameters indicated not only lower host fitness at 30°C, but also a population growth rate smaller than one, which is indicative of population decline (see PRADO et al., 2010). At 30°C, *A. hilare* and *M. histrionica* mortality increased and the biological parameters were negatively impacted, despite the fact that nymphal development time remained constant. In addition, results showed a lower percentage of insects hatching and higher cumulative mortality independent of generations. Females of both species had shorter preoviposition periods but laid fewer eggs at 30°C than at 25°C. In previous work performed at 23°C, it was showed that surface sterilization of the egg masses prohibits symbiont colonization of *A. hilare*'s gut, which reduces host fitness and curtails development of a second generation under laboratory conditions (PRADO; ALMEIDA, 2009b). The results obtained in Prado et al. (2010) were similar, suggesting that the impact of temperature on *A. hilare*'s fitness was a consequence of symbiont death rather than a direct impact on the host's biology. The importance of symbionts to *M. histrionica* was not definitively known previously because the surface sterilization technique used was not efficient in eliminating host infections (PRADO; ALMEIDA, 2009b). Assuming that host biology is also not severely impacted by 30°C temperatures, the data presented here suggest that *M. histrionica* is also dependent on its gut symbionts. Based on field observations showing stink bug populations shifting to locations where temperatures have risen in the last century (MUSOLIN et al., 2010, TOUGOU et al., 2009), it is expected that climate is playing a role in altered pentatomid bugs' geographic range via gut symbiont loss. Admittedly, more field and laboratory work is necessary to explicitly evaluate this hypothesis, but temperature has been demonstrated to disrupt mutualistic relationships in a wide range of insect symbioses. Heat shock (CHEN et al., 2000) and constant high temperatures (OHTAKA; ISHIKAWA, 1991) eliminate *Buchnera aphidicola*, resulting in death of its host aphid. Yet the presence of facultative aphid symbionts also increases host/*B. aphidicola* tolerance to temperature, through an unknown mechanism (MONTLLOR et al., 2002), suggesting that complexity exists in these interactions. Such complexity also manifests in the phenotypic variation observed in *Drosophila* interactions with male-killing *Spiroplasma* at different temperatures (ANBUTSU et al., 2008). The consequences of temperature-mediated symbiont loss for host organisms are not well documented, with a few notable exceptions, such as coral bleaching (MCWILLIAMS et al., 2005). Our study has two important limitations, shared with most research on the consequences of climate for micro- and macroorganisms. First, constant temperatures was used, which do not occur in natural conditions. Second, the experiments here done cannot evaluate the extent to which hosts or symbionts may evolve and adapt to new conditions or develop novel associations with other symbionts or hosts. Nonetheless, the work of Prado et al. (2010) in conjunction with studies from other host-symbiont interactions suggests that climate may be an important mediator of the ecology and geographic range of many insect groups through their symbiotic relationships with microbes.

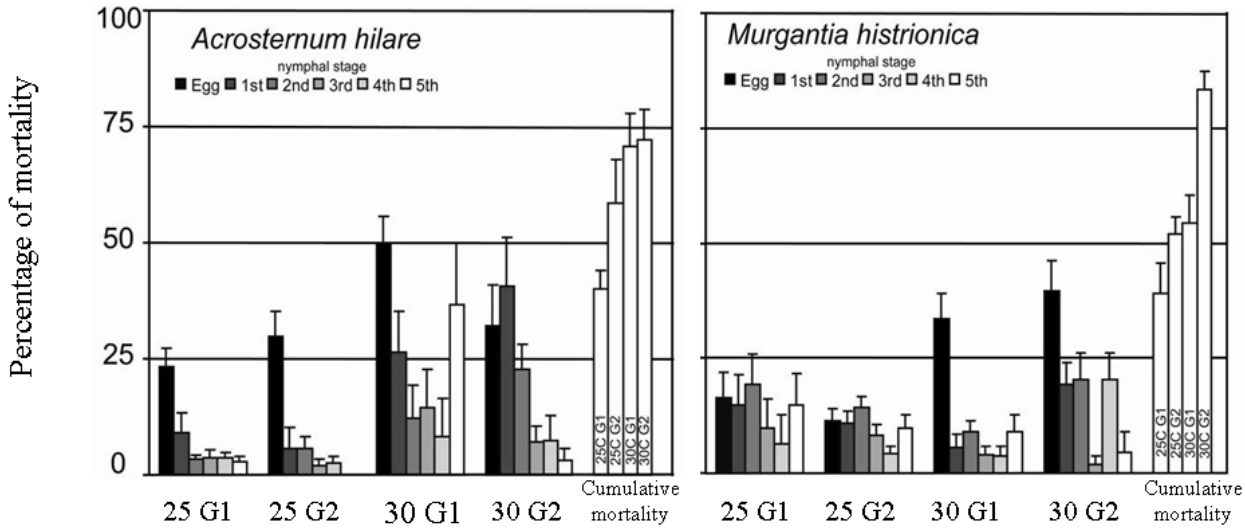

FIGURE 1. Mortality of *Acrosternum hilare* (A) and *Murgantia histrionica* (B) at 25 and 30°C and cumulative mortality during two generations.

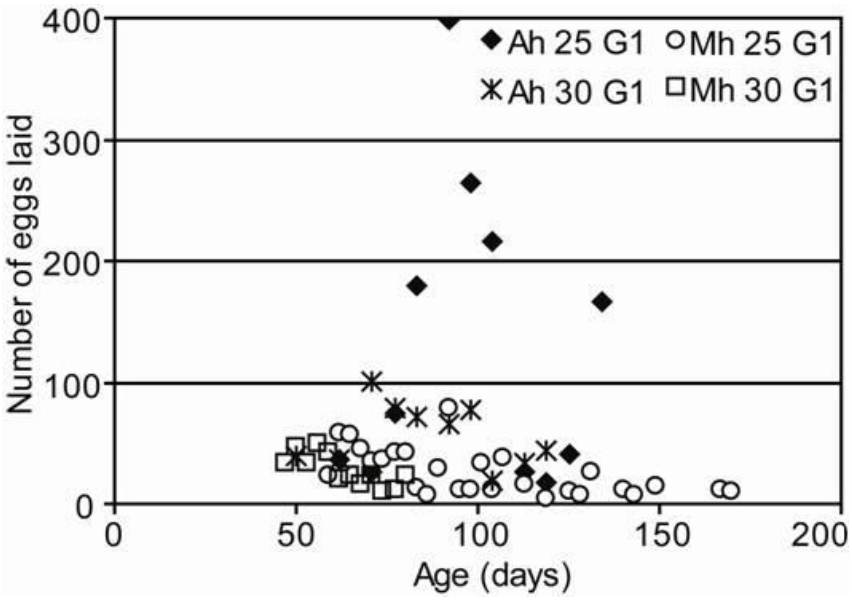

FIGURE 2. Total number of eggs laid by *Acrosternum hilare* (Ah) and *Murgantia histrionica* (Mh) cohorts at 25°C and 30°C during the first generation (G1).

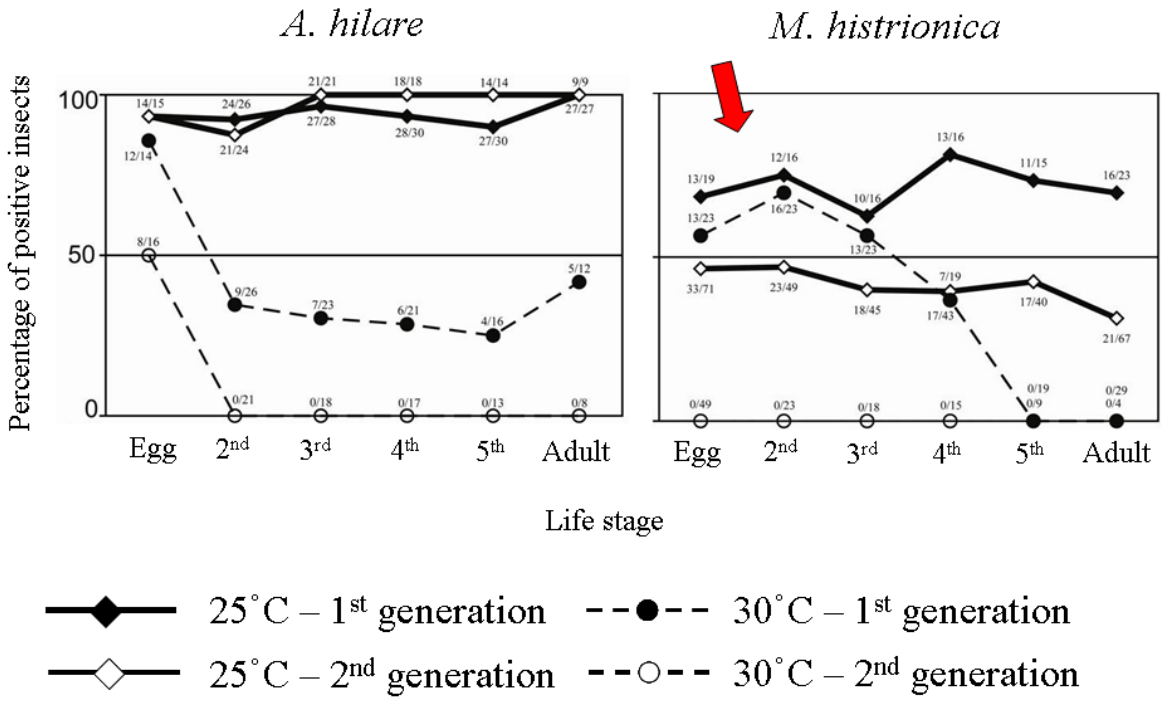

FIGURE 3. Percentage of symbiont-positive *Acrosternum hilare* (A) and *Murgantia histrionica* (B). Solid lines with black and white diamonds are values at 25°C for first and second generations, respectively. Dashed lines with black and white circles are values at 30°C for first and second generations, respectively. Numbers above data points show the number of insects infected and the total number of insects tested.

CONCLUSIONS

Higher temperature affected the maintenance of the symbiosis in both hosts;

Temperature did not significantly alter development;

Degree of mutualism of this association is variable:

A. hilare showed higher mortality at 30ºC and fitness was negatively impacted;

M. histrionica showed higher mortality and no reproduction at 30ºC of 2nd generation.

Global warming changes may also cause negative impacts on the symbiont-host relationship and, consequently, cause interference in insect survivorship and ecology from elevated global temperatures, encouraging more research on these associations (PRADO; ZUCCHI, 2011).

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