

How will *Mahanarva spectabilis* (Hemiptera: Cercopidae) Respond to Global Warming?

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Subject Editor: Evan L. Preisser

Received 1 October 2015; Accepted 13 January 2016

Abstract

The aim of this study was to determine the favorable constant temperature range for *Mahanarva spectabilis* (Distant) (Hemiptera: Cercopidae) development as well as to generate geographic distribution maps of this insect pest for future climate scenarios. *M. spectabilis* eggs were reared on two host plants (*Brachiaria ruziziensis* (Germain and Edvard) and *Pennisetum purpureum* (Schumacher)), with individual plants kept at temperatures of 16, 20, 24, 28, and 32°C. Nymphal stage duration, nymphal survival, adult longevity, and egg production were recorded for each temperature*host plant combination. Using the favorable temperature ranges for *M. spectabilis* development, it was possible to generate geographic distribution. Nymphal survival was highest at 24.4°C, with estimates of 44 and 8% on *Pennisetum* and *Brachiaria*, respectively. Nymphal stage duration was greater on *Brachiaria* than on *Pennisetum* at 20 and 24°C but equal at 28°C. Egg production was higher on *Pennisetum* at 24 and 28°C than at 20°C, and adult longevity on *Pennisetum* was higher at 28°C than at 20°C, whereas adult longevity at 24°C did not differ from that at 20 and 28°C. With these results, it was possible to predict a reduction in *M. spectabilis* densities in most regions of Brazil in future climate scenarios.

Key words: forage, spittlebug, climate change, biological aspects

Insects are among the organism groups of organisms most likely to be affected by climate change because climate has a strong direct influence on their development, reproduction, and survival (Bale et al. 2002). The short generation time and high reproductive rates of many insect species means that they can respond faster to climate change than longer-lived organisms (Menéndez 2007). Temperature is often the primary abiotic determinant of insect development and survival (Bale et al. 2002). Although insects can survive and reproduce within a range of temperatures, their performance is often worse near their minimum and maximum temperature limits (Haddad et al. 1999).

In the last 30 yr, there has been a mean global temperature increase of 0.2°C (IPCC 2007). In February 2007, the IPCC released the results of its Fourth Assessment Report of the Intergovernmental Panel on Climate Change, called IPCC-AR4 (Alley et al. 2007). These results suggest a global mean temperature rise of 1.8–4.0°C by 2100. Although this increase may be even greater (6.4°C) if human populations continue to grow rapidly and intensive fossil fuel consumption continues, the most reliable estimates project a mean increase of 3°C over this period. This estimate also indicates, with 90% reliability, that most of the temperature increase observed in the last 50 yr was caused by human activities.

Brazil has favorable conditions for forage grass cultivation and large areas of highly productive pasture land. The production of cattle meat and is directly linked to cattle forage quality (Souza Sobrinho et al. 2011), and grasses in the genera *Brachiaria* and *Pennisetum* are widely used as cattle forage throughout Brazil. *Mahanarva spectabilis* spittlebugs that feed on both plants have caused significant losses for forage producers and the Brazilian economy (Auad et al. 2010). The nymphs and adults of this insect harm the host plant by sucking its sap and injecting a toxin that causes plants to yellow and wilt (Resende et al. 2013, Aguiar et al. 2014).

Global warming is expected to force many species to alter their distribution by expanding into novel regions and disappearing from formerly suitable areas (Hughes 2000). Such range shifts are why climate change is one of the main factors involved in species extinction (Thomas et al. 2004, Franco et al. 2006). It is important to determine how temperature changes will affect *M. spectabilis* development and to identify geographic areas where global warming may cause this species to become a problem.

This study was conducted to determine the favorable constant temperature range for *M. spectabilis* development and to generate geographic distribution maps of this insect pest for future climate scenarios.

Materials and Methods

Plants and Insects

The studied host plants were *Pennisetum purpureum* (elephant grass) and *Brachiaria ruziziensis*; both plants are widely used throughout Brazil to feed cattle. *Brachiaria* cultivation was initially performed in trays of 140 cells, and after 15 d, the seedlings were transplanted into plastic tubes. Fifty days after sowing, the plants were transplanted into plastic pots (300 ml). *Pennisetum* cuttings were planted directly in plastic pots (300 ml). Plants were kept in the greenhouse (Florida Estufas Agrícolas, Jaguariúna, São Paulo state, Brazil) until they reached a mean of 55.0 cm in height and could be used in experiments.

Insects were collected in pastures located at the Embrapa Dairy Cattle Research Station, Brazil. This site has a rainy season that usually spans October through March and has an average temperature of 23°C, during which *M. spectabilis* were collected. Insects were then taken to the Entomology Laboratory, sexed, and maintained in acrylic cages (30 by 30 by 60 cm). One *Pennisetum* plant was placed in each cage. The base of each plant was wrapped with gauze moistened with distilled water, which served as an oviposition substrate. To remove the eggs that had been deposited on the substrate, the gauze was placed on a set of sieves and subjected to water jets, such that the eggs remained on the thinnest sieve (400-mesh opening). Eggs were then placed in Petri dishes (2 cm in height by 5 cm in diameter) lined with moistened filter paper and kept in a climate chamber (Eletrolab, São Paulo, São Paulo state, Brazil). ($25 \pm 2^\circ\text{C}$, 14 h of photoperiod and $70 \pm 10\%$ relative humidity) until the S4 stage (near hatching) before experimental use. Because this species has diapausing periods during its embryonic stage, S4 eggs were used because they were not in diapause. This developmental stage of the egg is characterized by two red spots on each side of the operculum; the operculum corresponds to the eyes, and the red spots represent the nymph's abdominal pigments (Peck 2002).

Viability and Duration of the *M. spectabilis* Immature Stage

S4 stage eggs were placed on filter paper (1 by 1 cm) strips and transferred to plastic pots containing *Pennisetum* or *Brachiaria*. The plants were subjected to water jets in order to expose the nymphs' feeding sites (roots). To prevent the nymphs from escaping, the pots were sealed with a plastic cover and gauze and kept in trays in climatic chambers ($70 \pm 10\%$ RH and 12 h of photoperiod). The study was conducted as a factorial experiment with two host plants (*Brachiaria* and *Pennisetum*) and five temperature levels (16, 20, 24, 28, and 32°C) based on completely randomized design. Each treatment was replicated 20 times using 10 eggs per replicate.

Reproductive Aspects and Longevity of *M. spectabilis* Adult Stage

M. spectabilis adults maintained at each temperature were separated in males and females. A single male–female pair was placed on the plant shoot and covered with a cage made of “voile” fabric. The plant base was wrapped with gauze moistened with distilled water, which served as an oviposition substrate.

Male–female pairs maintained in *Pennisetum* were placed in trays and kept in a climatic chamber ($70 \pm 10\%$ RH and 12 h of photophase) at 20°C ($n = 17$), 24°C ($n = 34$), and 28°C ($n = 26$). Plants were exchanged when their leaves showed yellowing. Data on oviposition and adult longevity were evaluated at each temperature.

Nymphal survival was so low on *Brachiaria* that we were not able to study adult longevity or reproduction on this host plant.

Statistical Analyses

Regression and ANCOVA-type analysis were conducted for all response variables, aiming to investigate the form of relationship between each of them and the temperature and also to assess if there exists an interaction effect between host plant and temperature. The initial full models for nymphal stage viability and duration included the main effect of host plant, linear and quadratic effects of temperature, and the interaction terms plant*temperature and plant*temperature², whereas the models for egg production and adult longevity only considered the polynomial terms involving temperature. The terms involving a quadratic effect of temperature were included in order to approximate a possible nonlinear relationship.

Nymphal stage viability data were analyzed using a logistic regression model. Model goodness-of-fit was assessed using the Hosmer–Lemeshow test; type III Wald chi-square tests were used to evaluate significance of model parameters. Nymphal survival on different host plants and at different temperatures was compared using odds ratios (ORs) estimated from the logistic model. Results were reported as ORs with 95% confidence intervals (Wald-type CIs). The LOGISTIC procedure of SAS System version 9.2 (SAS Institute Inc. 2009) was used for this analysis.

Nymphal stage duration was analyzed using ANCOVA, with significance of effects assessed by type III *F*-tests and goodness-of-fit assessed by the adjusted coefficient of determination (R_{adj}^2). Adult longevity data for *Pennisetum* were examined by a simple normal linear model, considering temperature first as a covariate and then as a factor, in order to compare means among its levels using the Tukey–Kramer test. SAS' GLM procedure was used for fitting both models.

Oviposition data were analyzed by a generalized linear model with negative binomial distribution and square root link function. The NB distribution was employed to accommodate the overdispersion, relative to the Poisson distribution, exhibited by the counts, and the square root link function was chosen over the identity or logarithmic ones because it provided the best fit for the data. Goodness-of-fit was assessed by the Pearson chi-squared test and significance of the polynomial terms for temperature was evaluated using type III likelihood-ratio tests. The model was then refitted, now considering temperature as a factor, aiming to compare means among its levels by the Tukey–Kramer test. SAS' GENMOD procedure was used.

Graphs were created in R software version 3.1.3 (R Core Team 2015) and, for all tests, statistical significance was considered when $P \leq 0.05$.

Generation of Geographic Distribution Maps of *M. spectabilis*

For map generation, IPCC data conversion was used in a half-degree spatial resolution (0.5° by 0.5°) with interpolated meshes of geographic coordinates in the raster model for the Fourth Assessment Report (AR4) of IPCC (2007) using a Geographic Information System (GIS). These data were generated through global circulation or climate models (GCM), such as BCCR-BCM 2.0, CNRM-CM3, CFCM3.1.T47, GFDL-CM2.0, and CCSM3 (Hamada et al. 2009).

IPCC defines scenarios (Special Report on Emission Scenarios) that comprise different greenhouse gas (GHG) emission projections and relate socio-economic and technological development aspects. IPCC A2 scenarios from the Fourth Report to spittlebug were used (high GHG future emissions) for GIS layout composition and future

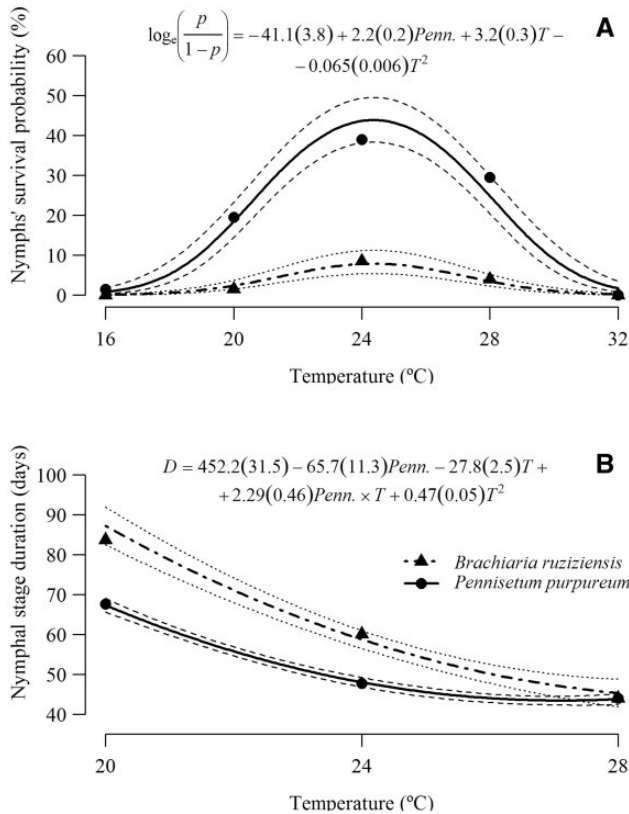


Fig. 1. Fitted models for viability (%) (A) and duration (d) (B) of the nymphal stage (egg–adult) of *M. spectabilis* fed on *Brachiaria ruziziensis* and *P. purpureum* at different temperatures. Points marked with triangle and circle symbols represent observed mean values and curves represent fitted values with respective 95% CIs. Values in parenthesis in the fitted model equations are the standard errors for the parameters estimates. *P*, survival probability; *D*, duration; *Penn.*, differential effect of *Pennisetum* compared to *Brachiaria*; *T*, temperature.

monthly scenario map generation. These scenarios focused on the effect of the average temperature on the insect during the reference period (1961–1990) of the Climate Research Unit (Ghini 2011) in order to predict conditions in 2020, 2050, and 2080: temperatures of 24–28°C (favorable), 20°C (less favorable), and extreme values of 16 and 32°C (not favorable).

The A2 GHG emission scenario was used, which simulates climate, agriculture, and future emission heterogeneities, current fertilization and tradition standard maintenance, high population growth and fragmented income distribution, and economic growth with slow technological advancement. Temperature data were converted to rasters in the GIS in GRID format, and temperature-specific data on spittlebug performance were used in monthly matrix reclassifications (*rasters*) for future scenarios, which arranged them in each layout with map element adjustment performed later.

Results

For the nymphal stage viability logistic model, none of the interaction terms plant*temperature² ($\chi^2 = 1.21$; *df* = 1; *P* = 0.27) and plant*temperature ($\chi^2 = 0.51$; *df* = 1; *P* = 0.48) were significant, yielding parallel negative quadratic curves as a function of temperature for the host plants, with no lack-of-fit according to the Hosmer–Lemeshow test ($\chi^2 = 10.46$; *df* = 8; *P* = 0.23). The maximum survival probability occurred at 24.4°C, with maximum

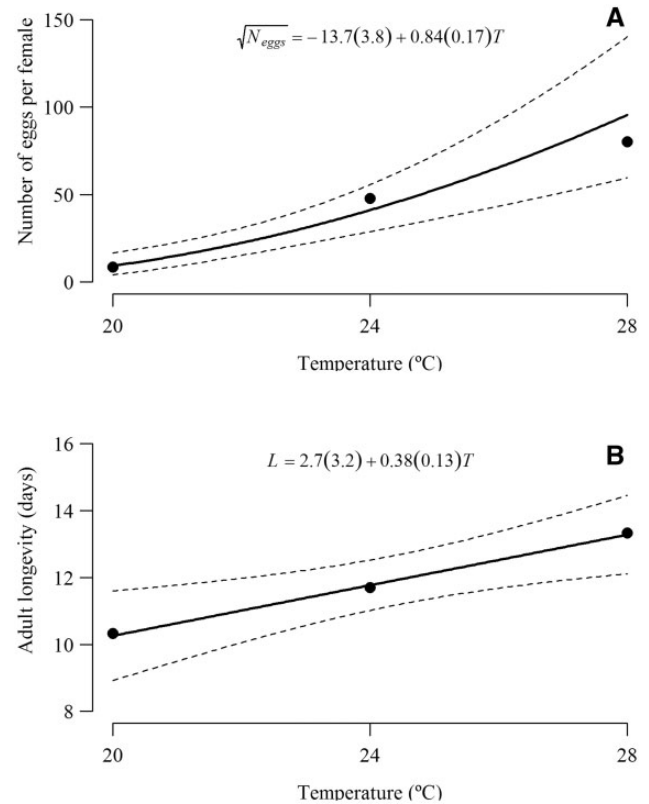


Fig. 2. Fitted models for egg production (A) and longevity of adults (B) fed on *P. purpureum* and maintained at 20, 24, and 28°C. Marked points represent observed mean values and curves represent fitted values with 95% CIs. Values in parenthesis in the fitted model equations are the standard errors for the parameters estimates. *N_{eggs}*, number of eggs per female; *L*, longevity.

estimates of 44% (95% CI: 38–50) and 8% (95% CI: 5–11), for *Pennisetum* and *Brachiaria*, respectively. Extreme temperatures were lethal to *M. spectabilis*: nymphal viability was zero at 16°C for *Brachiaria* and at 32°C for both host plants (Fig. 1A).

For any temperature value in the investigated range, nymphal survival was higher on *Pennisetum* (OR = 9; 95% CI: 6–14) than on *Brachiaria*. For both host plants, nymphal survival at 24°C was approximately three times (OR = 3.4; 95% CI: 2.5–4.6) higher than at 20°C and two times (OR = 2.3; 95% CI: 1.8–3.0) higher than at 28°C. Nymphal survival at 28 and 20°C did not differ significantly (OR = 1.5; 95% CI: 0.98–2.17; Fig. 1A).

The viability of insects was zero when maintained at 16°C and fed with *Brachiaria* as well as when they were maintained at 32°C on either host plant. Nymphs survived in only two *Pennisetum*-fed at 16°C replicates; the large number of zero-survival replicates prevented us from analyzing between host-plant differences in nymphal duration at these temperatures.

Nymphal stage duration decreased nonlinearly with increasing temperature, a relationship approximated ($R_{adj}^2 = 0.76$) by a quadratic function for both host plants, with significant effect of the interaction plant*temperature ($F_{1,201} = 25.4$; *P* < 0.0001) and the squared temperature ($F_{1,201} = 90.5$; *P* < 0.0001) terms. When host-plant performance was compared at similar temperatures, nymphal stage duration was longer on *Brachiaria* than on *Pennisetum* at 20°C ($t = 8.2$; *P* < 0.0001) and 24°C ($t = 9.0$; *P* < 0.0001); there was, however, no difference at 28°C ($t = 0.82$; *P* = 0.41; Fig. 1B).

The square root of the expected number of eggs per *Pennisetum*-reared female increased linearly with temperature with no lack-of-fit

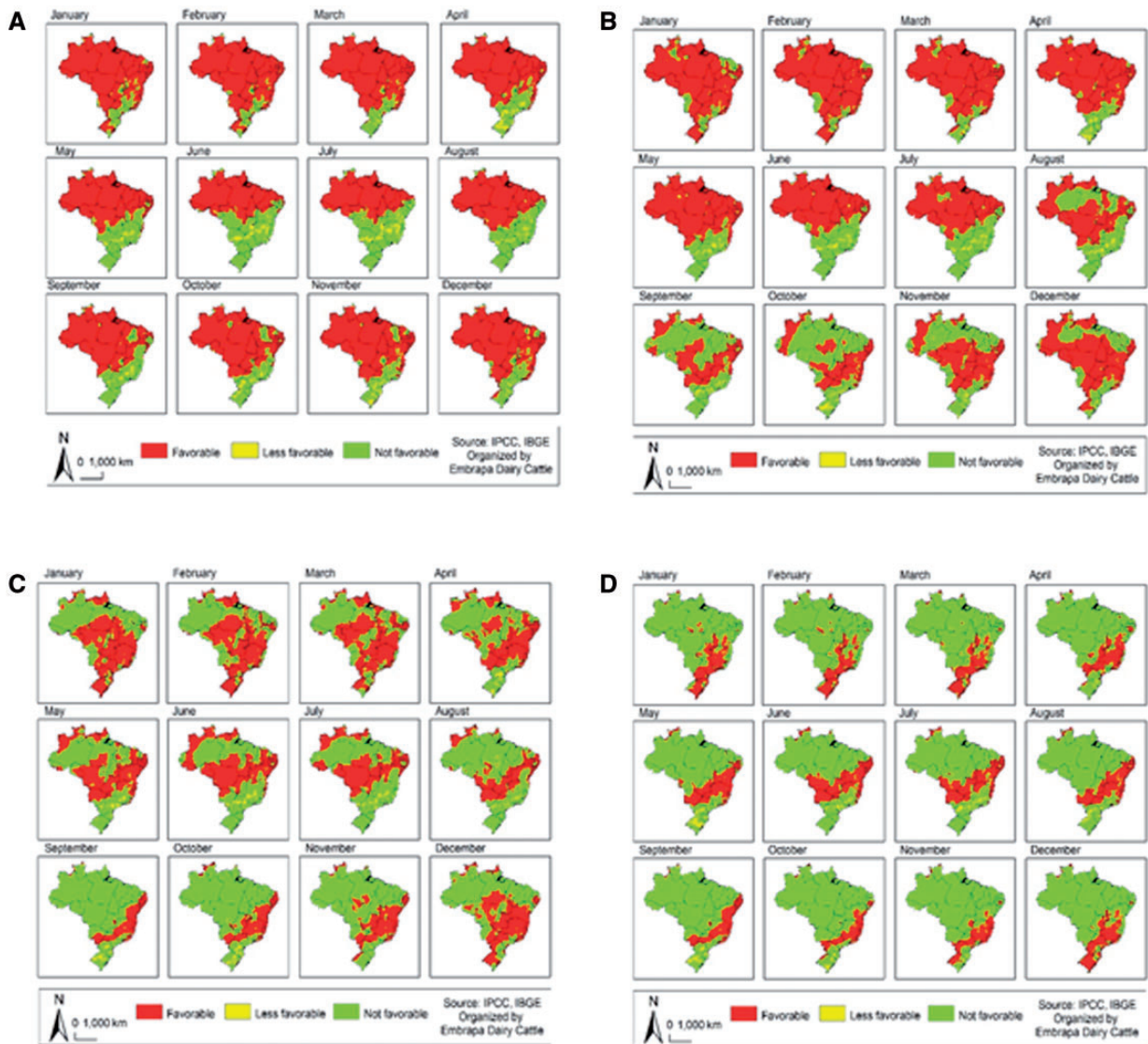


Fig. 3. Favorability for occurrence of *M. spectabilis* as projected by global climatic models from the fourth report of the IPCC for the reference period (1961–1991) (A) and by the years 2020 (B), 2050 (C), and 2080 (D) under the “A2” scenario for Brazil, considering temperatures of 24–28°C (favorable), 20°C (less favorable), and extreme values (16 and 32°C) as not favorable to the insect.

($\chi^2=45.6$; $df=75$; $P=0.99$). The dispersion parameter for the negative binomial distribution was estimated as 2.00 with standard error of 0.34, which shows that the model was successful in capturing the overdispersion exhibited by the counts. Although egg production at 24°C ($z=4.05$; $P=0.0002$) and 28°C ($z=4.49$; $P<0.0001$) was significantly higher than at 20°C, there was no difference in egg production at 24 and 28°C ($z=-1.36$; $P=0.36$) (Fig. 2A).

Longevity of *Pennisetum*-reared adults increased linearly with temperature and was higher at 28°C than at 20°C ($t=2.89$; $P=0.012$), whereas no difference was found when comparing the mean longevity for 24°C with that for 20°C ($t=1.38$; $P=0.35$) or 28°C ($t=-1.87$; $P=0.15$) (Fig. 2B).

The above-mentioned data on *M. spectabilis* biological and reproductive parameters demonstrate that temperatures of 24–28°C are favorable, 20°C is slightly favorable, and extreme temperatures (16 and 32°C) are unfavorable for insect development. According to the analysis of the temperature ranges of insect survival over the reference period (1961–1991) obtained from the IPCC AR4, the

occurrence of *M. spectabilis* is favored in all regions, except in the Southern region from March to December (Fig. 3A). This favorability continues with the same tendency for the A2 scenario in 2020 (Fig. 3B), except mainly in the north part of Brazil from August to December, will become unfavorable. In the 2050 scenario, there will be an overall reduction in the insect favorable areas, and the South will continue to be unfavorable (Fig. 3C). By 2080, there will be increase in the occurrence of insects in favorable areas in the South, especially in the months of January to March. In that same scenario, the North and Northeast regions, which were considered highly favorable in previous scenarios (2020 and 2050), will become unfavorable places for pest insect occurrence due to an increase in the global temperature. The Southeast and Midwest regions will remain favorable for *M. spectabilis* attack (Fig. 3D).

Discussion

Although nymphal stage viability averaged less than 40% across all of the tested temperatures, it was high enough to determine their

optimum temperature range on the two host plants. Nymphal viability was highest at 24 and secondly at 28°C on both *Pennisetum* and *Brachiaria*. Auad et al. (2009) observed that *M. spectabilis* population peaked in the field at 25°C, and Garcia et al. (2006) observed that the average survival of *Mahanarva fimbriolata* (Stal) nymphs at 25°C in the laboratory was 94%.

In contrast, nymphal viability was essentially zero below 16–20°C and above 32°C, showing the narrow thermal requirements of *M. spectabilis* with respect to survival and development. This result is consistent with those from other species; a small deviation from the optimum conditions for *Philaenus spumarius* (L.) resulted in high mortality (Karban and Strauss 2004). According to Parra (2001), food quality affects insect development time, viability, fertility, and longevity. In this study, nymphs kept at 20, 24 and 28 °C were, respectively, about 16, seven and 10 times more likely to survive, when kept on *Pennisetum* than on *Brachiaria*. Ferreira et al. (2013) also observed higher nymphal viability for *Pennisetum*-reared *M. spectabilis* than on *Brachiaria*.

Amarasekare and Sifuentes (2012) suggested that temperature has similar effects on all insect life stages in terms of accelerating development. Although the impact of temperature on development can vary among species, development time is generally higher at lower temperatures. Nymphal duration of *M. spectabilis* decreased with increasing temperature on both host plants up to 28°C, although no nymphs survived at 32°C. The impact of food type and nutritional quality, in addition to temperature, must be considered in insect development (Gilbert and Raworth 1998, Bertin et al. 2013).

M. spectabilis fertility was significantly influenced by temperature. Egg production at 28°C was approximately 1.7 and 9 times higher than at 24 and 20°C, respectively. The optimum temperature range of *M. spectabilis* is 24–28°C on both host plants but with the highest survival and fecundity on *Pennisetum*. These results allow us to predict *M. spectabilis* distribution in future climate scenarios in Brazil to understand how the specie will respond to global warming.

According to the analysis of the temperature ranges of insect survival over the reference period (1961–1991) obtained from the IPCC AR4, the occurrence of *M. spectabilis* is favored in all regions, except in the Southern region. Paula-Moraes et al. (2008) sampled *M. spectabilis* in the North, Northeast, Midwest, and Southeast regions of Brazil. The predicted increases in global temperature by 2050 and 2080 will change *M. spectabilis* distribution in Brazil and decrease the total area in Brazil where conditions are favorable to this insect. In 2080, *M. spectabilis* is expected to decrease in northern Brazil and to become more abundant in southern Brazil, an area where climatic conditions are unfavorable currently and in the 2020 future scenario. The fact that this insect diapauses during its embryonic stage, which usually lasts from March to August (Auad et al. 2011), suggests that this insect will be less damaging to pastures in the future even if these months become more favorable to *M. spectabilis* occurrence. It is noteworthy that adaptations of spittlebug populations to new climatic configurations over time were not considered, which could alter such obtained responses. In summary, the IPCC Fourth Report scenarios predict reduced *M. spectabilis* densities in most regions of Brazil by the end of the century.

Acknowledgments

This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, Brazil).

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