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Original Article

Exposure of insects and host plants to different concentrations of CO_2 affects the performance of *Mahanarva spectabilis* (Hemiptera: Cercopidae) in successive insect generations

A exposição de insetos e plantas hospedeiras às diferentes concentrações de CO_2 afeta o desempenho de *Mahanarva spectabilis* (Hemiptera: Cercopidae) em sucessivas gerações do inseto

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Abstract

The performance of three successive generations of *Mahanarva spectabilis* (Distant) (Hemiptera: Cercopidae) fed on four forages exposed to environments with different CO_2 concentrations was evaluated. In the first bioassay, we utilized the following scenarios: A) plants and insects were kept at high and constant CO_2 (700 ppm) and B) the insects were kept at CO_2 700 ppm and fed on plants from the greenhouse (average of 390 ppm). In the second bioassay, we utilized the following scenarios: C) plants and insects were kept in a greenhouse and D) the insects were kept in the greenhouse and fed on plants kept at CO_2 700 ppm. The survival and duration of the nymphal and adult stages and the number of eggs/female of *M. spectabilis* were evaluated. It was only possible to evaluate the cumulative effects of the increase of CO_2 on three successive generations of *M. spectabilis* kept in a greenhouse, due to the reduced survival of the insects in the first generation in the laboratory. A greater direct than indirect effect of the CO_2 level on the performance of *M. spectabilis* was observed. Furthermore, it should be considered that the effect of CO_2 elevation on the survival, periods of development, and fecundity, when taken together, can significantly impact the population dynamics of *M. spectabilis* in future climate scenarios.

Keywords: spittlebugs, forages, climate change.

Resumo

Avaliou-se o desempenho de três gerações sucessivas de *Mahanarva spectabilis* (Distant) (Hemiptera: Cercopidae) alimentadas com quatro forrageiras expostas à ambientes com diferentes concentrações de CO_2. No primeiro bioensaio, utilizamos os seguintes cenários: A) plantas e insetos foram mantidos em fitotron com CO_2 alto e constante (média de 700 ppm) e, B) os insetos foram mantidos em nível de 700 ppm de CO_2 e alimentados com plantas advindas da casa de vegetação (média de 390 ppm). No segundo bioensaio, utilizamos os seguintes cenários: C) plantas e insetos foram mantidos em casa de vegetação e, D) os insetos foram mantidos em casa de vegetação e alimentados com plantas mantidas em fitotron. A sobrevivência e duração das fases ninfal e adulta e o número de ovos/fêmea de *M. spectabilis* foram avaliados. Só foi possível avaliar os efeitos cumulativos do aumento do CO_2 em três gerações sucessivas de *M. spectabilis* mantidas em casa de vegetação, devido à reduzida sobrevivência dos insetos na primeira geração quando mantidos em fitotron. Observou-se maior efeito direto do que indireto do nível de CO_2 sobre o desempenho de *M. spectabilis*. Além disso, deve-se considerar que o efeito da elevação de CO_2 sobre a sobrevivência, períodos de desenvolvimento e fecundidade, quando tomados em conjunto, podem impactar significativamente a dinâmica populacional de *M. spectabilis* em cenários climáticos futuros.

Palavras-chave: cigarrinhas, forragens, alterações climáticas.

1. Introduction

The atmospheric concentration of carbon dioxide (CO_2) has steadily increased from approximately 280 ppm before the industrial revolution (Qian et al., 2021) to 418 ppm in April 2022 (NOAA, 2022). It is predicted to rise to around 800 ppm by 2100 (IPCC, 2014). This change in climate can significantly alter the relationships between plants and insects in important agricultural production areas (Karthik et al., 2021; Skendžić et al., 2021; Theurillat and

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Guisan, 2001) and influence the geographic distribution of insect as example, the eucalyptus yellow beetle (Vasconcelos et al., 2022).

Brazil has privileged conditions for the cultivation of forage grasses. It's wide territorial extension occupied by the pastures reflects its high productive potential (Rabinovitch et al., 1998). However, the spittlebug, *Mahanarva spectabilis* (Distant) (Hemiptera: Cercopidae), reduces the growth of these grasses and thereby causes a drop in the production of dry mass as well as the quality of the forage, which amounts to a major problem within the cattle industry throughout tropical America (Auad et al., 2007; Souza et al., 2008; Ferreira et al., 2013; Resende et al., 2014; Fonseca et al., 2016; Alvarenga et al., 2017; Silva et al., 2017). Given the impact of this species on grasses, it is vital to understand how global changes may influence its performance and thus assess whether the increase in CO_2 levels may lead to a better adaptation of this pest, triggering the need to search for new control strategies.

Most studies published on the response of insects that feed on plants grown under high concentrations of CO_2 are based on short-term experiments, recording the rates of development in a single generation of an insect (Bezemer and Jones, 1998). Experiments carried out over several generations can reveal differences in responses between generations (Brooks and Whittaker, 1998). Few studies have addressed the impact of high CO_2 on multiple generations of herbivorous insects (Brooks and Whittaker, 1999).

It is worth mentioning that insect-plant interaction can be influenced by the increase in CO_2 concentration due to changes in the nutritional and physiological quality of the host plants (Dáder et al., 2016; Satishchandra et al., 2018) and by the direct effects of elevated CO_2 levels on the fitness performance of the insects (Xie et al., 2015). The increase in CO_2 increases the plants' carbon/nitrogen balance, which affects insect feeding (Coviella and Trumble 1999). The abundance of carbon and the scarcity of nitrogen lead to other changes in a plant that can further affect the insects. A group of carbon-based compounds (such as phenolic compounds and tannins) tend to slow insect growth. Thus, high levels of CO_2 allow many plant species to significantly increase their defenses based on the carbon availability (Trumble and Butler, 2009). Such alterations can modify the pattern of resistant plants against insect pests (Karthik et al., 2021). In this regard, little is known about the intrinsic mechanisms between plant resistance and insect survival strategies under future climate scenarios (Xie et al., 2022). Therefore, considering the use of resistant plants as the main control strategy for spittlebugs, it is essential to know the interference of climate change on the control strategies of this insect pest, to avoid any compromise on the sustainability of the production system. Therefore, based on the future climate scenarios in which CO_2 elevation is expected, the objective was to evaluate the direct and indirect effects of the CO_2 elevation on the fitness performance of multiple generations of *M. spectabilis* fed on four different forages.

2. Material and Methods

2.1. Insects

Adults of the *M. spectabilis* were collected in the experimental field of Embrapa Gado de Leite in Coronel Pacheco-MG. They were then taken to the entomology laboratory in Juiz de Fora-MG, sexed, and kept in acrylic cages (30×30×60 cm). One *Pennisetum purpureum* plant was cultivated in a pot (1L) with a mixture of soil, sand and organic fertilizer (3:1:1), and was placed in the cage. The bottom of the pot was wrapped in gauze and moistened with distilled water, which served as a substrate for oviposition. To remove the eggs retained in the substrate, the gauze was placed on a set of sieves and subjected to running water, where the eggs were retained in the thinnest sieve (400 mesh opening).

The obtained eggs were placed in Petri dishes 5 cm in diameter and lined with filter paper. They were kept in a climatic chamber (25 ± 2 °C, 14 hours of photophase, and relative humidity of 70 ± 10%) until they became close to hatching, i.e., when they showed the stage of advanced embryo (Stage 4- S4), with expected hatching in two days. At that stage, the eggs were used in the experiments.

2.2. Plants and growth conditions

Two species of brachiaria were tested; where one was susceptible to spittlebugs, *Brachiaria ruziziensis*, and the other was resistant to *B. brizantha* (Cardona et al., 2004). In addition to elephant grass (*P. purpureum*), Pioneiro and Roxo de Botucatu cultivars were characterized as resistant and susceptible to *M. spectabilis*, respectively (Auad et al., 2007).

Brachiaria was cultivated from seeds in Styrofoam trays with 140 cells, and on the 15th day after cultivation, it was transplanted in tubes. On the 50th day after sowing, the plants were transplanted in plastic pots with a capacity of 1 kg. Elephant grass was grown by using stakes of 10 cm (with one node) and planted directly in plastic pots. The pots of Brachiaria and elephant grass were kept in environments with different levels of CO_2 as follows: 1) greenhouse (average 390 ppm; minimum of 50 ppm and a maximum of 2499 ppm, i.e., the current level CO_2) and 2) climatized chamber with constant CO_2 level (700 ppm, which is the level expected for the year 2100 by International Panel on Climate Change, IPCC). Each climatized chamber (2.5 x 2.20 x 2.80 m) was maintained at 25 ± 2ºC, 70 ± 10 relative Humidity (RH), and 14:10 (light: dark). In the greenhouse, averages of 27 ± 2ºC and 73 ± 10 RH were recorded. Conditions in the chamber with constant CO_2 were maintained with an automatic control system, COEL HW 4200 (Manaus-Amazonas, Brazil) and an injection system, using a CO_2 cylinder to maintain the desired CO_2 concentration. The CO_2 levels in the floating chamber and greenhouse were recorded with a data logger every 2 minutes, and the data were transferred to the computer using HOBO ware software (Onset Co., Pocasset, Massachusetts, USA).

2.3. Scenarios used in the experiments

The experiments were divided into two bioassays. The first bioassay was carried out in climatized chambers, phytotron type, in the laboratory under two scenarios: A) plants and insects were maintained at high and constant CO_2 (700 ppm) and B) the insects were kept at high and constant CO_2 (700 ppm) and fed on plants from the greenhouse (average of 390 ppm CO_2). The second bioassay was carried out in a greenhouse under two scenarios: C) plants and insects were kept in a greenhouse (average of 390 ppm CO_2), and D) the insects were kept in a greenhouse (average of 390 ppm CO_2) and fed on plants that came from the climatized chamber with high and constant CO_2 (700 ppm).

2.4. Bioassays

In the first generation, 160 plants, at 40 days, were submitted to water jets, aiming to expose the roots for the fixation of spittlebug nymphs. Thirty eggs were placed per pot, which were individually covered by a cage (45 cm diameter × 40 cm long) made of "voile"- type fabric (100% polyester) to prevent the insects from escaping. The assays were carried out using four types of forage (two species of Brachiaria and two cultivars of elephant grass), two scenarios (bioassay 1 and bioassay 2), and three successive generations, with ten replications, totaling 4,800 eggs in state S4 in the first generation. In subsequent generations, the number of repetitions varied as a function of the number of eggs produced by the females.

Due to the low insect survival in the bioassay carried out in the climatic chamber (bioassay 1), in which forages and insects were submitted to Scenarios A and B, it was only possible to analyze the nymphal survival in the first generation in these scenarios (Table 1). In Scenarios C and D, bioassay 2, when insects were always kept in a greenhouse, it was possible to analyze the biological parameters, survival and nymphal duration, longevity of adults, and the number of eggs/female in the first generation of *M. spectabilis*, fed on the four forages. The comparison of the pests' performance between the three generations was possible when they were fed on cultivars Pioneiro and Roxo de Botucatu in Scenario C and Roxo de Botucatu in Scenario D (Table 1). In these treatments, the survival and duration of the nymphal phase, number of eggs/female, and longevity of the adults of *M. spectabilis* in the three generations were evaluated daily.

To evaluate the number of eggs and the longevity of *M. spectabilis* from each treatment, the adults were sexed and separated by pairs. The pairs were individualized, kept in the shoot of the plant of each treatment (scenario), and covered with a cage (45 cm diameter x 40 cm long) made of "voile"- type fabric (100% polyester). The base of the plant was wrapped with gauze moistened with distilled water, which served as a substrate for oviposition. The plants were exchanged when they showed yellowing of the leaves.

All data analysis were performed with the R program (R Core Team, 2022). The obtained data did not meet the normality assumptions related to the residues and the homogeneity of the variance (Shapiro-Wilk p <0.0001). Therefore, nonparametric analysis was used to verify the differences between the treatments. A Kruskal-Wallis (Hollander and Wolfe, 1973) analysis was used, and a comparison among forages was performed by Nemenyi post hoc comparison with a Tukey correction using the 'kwAllPairsNemenyiTest' function in the R package 'PMCMRplus' (Pohlert 2014). The Wilcoxon-Mann-Whitney U-test (Conover 1980) was used for comparison between scenarios, generations, and forages, using the "Wilcox.text" function in the R package 'PMCMRplus' (Pohlert 2014).

3. Results

3.1. Performance of M. spectabilis maintained in a phytotron and subjected to four food sources in two scenarios

Nymphal survival of *M. spectabilis* that were always kept in the future climate scenario (700ppm CO_2) showed

Table 1. Evaluation of the number of individuals sufficient for statistical analysis when *Mahanarva spectabilis* was fed on four forages and its three generations were subjected to four scenarios.

Generation	Scenarios	Forages			
		B. ruziziensis	***B. Brizantha***	**Cv. Pioneiro**	**Cv. Roxo de Botucatu**
G1	A	x	x	x	x
G2		0	0	0	0
G3		0	0	0	0
G1	B	x	x	x	x
G2		0	0	0	0
G3		0	0	0	0
G1	C	x	x	x	x
G2		0	0	x	x
G3		0	0	x	x
G1	D	x	x	x	x
G2		0	0	0	x
G3		0	0	0	x

x = adequate number of individuals for analysis; 0 = Insufficient number of individuals for analysis.

no significant difference when they were fed on four forages regardless of the scenarios in which the plants were kept. (Figure 1). When the nymphal survival is compared among the four foragers within each scenario, it is found that in Scenarios A (x^2=12.95; df=3; *P*=0.004) and B (x^2=16.66; df=3; *P*=0.0008), greater nymphal survival is observed in the Roxo de Botucatu cultivar compared with *B. brizantha* and Pioneiro forages (Scenario B) and *B. ruziziensis* (Scenario A) (Figure 1).

In the laboratory (bioassay 1), where insects were always kept at higher CO_2 level, the evaluation of the cumulative effect of the increase in CO_2 concentration on three successive generations of *M. spectabilis* in the scenarios in which the insects were fed on *B. ruziziensis* and *B. brizantha* was not possible. The low nymphal survival and low fertility of the females of the first generation made it impossible to assess the next generations.

3.2. Performance of M. spectabilis kept in a greenhouse, subjected to two food sources and two scenarios in three generations

No significant difference was observed in the nymphal survival of *M. spectabilis* when the insects were kept in the greenhouse and fed on *B. ruziziensis* (W=69.5, *P*=0.14), *B. brizantha* (W=52, *P*=0.91), and Pioneiro (W=58.5, *P*=0.54), regardless of the scenarios in which the plants were kept. On the other hand, Roxo de Botucatu showed greater survival (W=18, *P*=0.017) of the nymphs in the Scenario D (where the insects were kept in the greenhouse and fed on plants from the climatic chamber with constant CO_2 at 700 ppm) (Figure 2). When the four forages are compared within each scenario, the nymphal survival of those kept and fed in the greenhouse showed no difference (x^2=5.51; df=3; *P*=0.137) among the forages. However, greater nymphal survival (x^2=18.017; df=3; *P*=0.0004) was observed for the insects which were fed on Roxo de Botucatu, maintained at constant and high CO_2 (700ppm), compared with *B. ruziziensis* (Scenario D) (Figure 2).

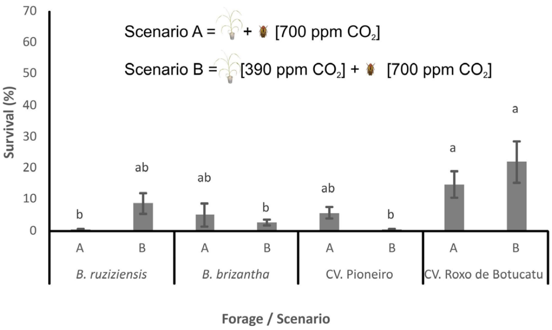

Figure 1. Biological aspects of *M. spectabilis*, first generation, fed on different forages in the following scenarios: (A) plants and insects were maintained at a high and constant CO_2 (700 ppm) and B) the insects were kept at a high and constant CO_2 (700 ppm) and fed on plants from the greenhouse (average of 390 ppm CO_2). Means followed by the same lowercase letter between the forages within each scenario do not differ from each other, by Kruskal-Wallis or Nemenyi post hoc test (*P*<0.01). Means compared between scenarios within each forage did not differ from each other.

The duration of the nymphal phase of *M. spectabilis* showed no difference when fed on cultivars Pioneiro (W=3069, *P*=0.85) and Roxo de Botucatu (W=8765, *P*=0.49), regardless of the scenario. However, shorter durations of the pest insect were found when they were fed on *B. ruziziensis* (W=654, *P*<0.001) and *B. brizantha* (W=3764, *P*=0.003) from climatic chambers with constant CO_2 and kept in the greenhouse (Scenario D) (Figure 2). When the four forages within each scenario are compared, the shortest durations (x^2=25.04; df=3; *P*<0.001) are found for those that were fed on Pioneiro and Roxo de Botucatu kept in the greenhouse (Scenario C), and when fed on *B. ruziziensis,* which was maintained at high CO_2 condition (Scenario D) (x^2=9.26; df=3; *P*=0.026) (Figure 2).

The longevity of *M. spectabilis* adults was observed to be significantly low for the insects which were fed on *B. ruziziensis* (W=527, *P*=0.0010), *B. brizantha* (W=3953, *P*<0.001), Pioneiro (W=3898, *P*=0.001), and Roxo de Botucatu (W=9712, *P*<0.001) from the climatized chambers with constant CO_2 (Scenario D). In Scenario C (x^2=9.90; df=3; *P*<0.019), the longevity of the adults came greater when they were fed on Roxo de Botucatu compared with *B. ruziziensis,* and in Scenario D (x^2=27.74; df=3; *P*<0.001), when fed on *B. brizantha* and Roxo de Botucatu (Figure 2).

No difference was observed in the number of eggs when the females were fed on *B. ruziziensis, B. brizantha,* regardless of the scenario; however, females laid fewer eggs when they were fed on Roxo de Botucatu (W=2357, *P*<0.039*)* and Pioneiro (W=612, *P*<0.0003*)* that was grown at constant CO_2 and kept with the insects in the greenhouse (Scenario D) (Figure 2). Furthermore, females laid a significantly higher number of eggs (*P*<0.01, *F*=3.858) when kept in Roxo de Botucatu compared with *B. ruziziensis* (Scenario C) (x^2=11.65; df=3; *P*=0.042) and Pioneiro (Scenario D) (x^2=15.45; df=3; *P*=0.0014). (Figure 2).

In Scenarios C and D, evaluating the elephant grass cultivars was possible, which provided a sufficient number of individuals for analysis in subsequent generations (Figure 3). No significant difference was found in the nymphal survival of *M. spectabilis* between the three generations when the insects and forages Pioneiro (x^2=1.696; df=2; *P*=0.43) and Roxo de Botucatu (x^2=3.47;df=2; *P*=0.176) were kept indoors in the greenhouse under current CO_2 conditions (Scenario C). The same occurred between the forages in generations 1 and 2. However, lower nymphal survival was found (W=131, *P*<0.0019) in Roxo de Botucatu compared to Pioneiro in generation 3 (Figure 3).

No difference was found in the nymphal duration of the insects fed on forages Pioneiro or Roxo de Botucatu within the first and second generations, whereas it was found longer in the third generation that was fed on Roxo de Botucatu (W=2942, P=0.014). A significantly lower nymphal duration was observed when the insects were fed on both Pioneiro (x^2=24.62; df=2; *P*<0.0001) and Roxo de Botucatu (x^2=44.69; df=2; *P*<0.0001) in generation 1, compared to the other generations (Figure 3). Significantly lower adult longevity was observed for the insects in the first generation that were fed on both Pioneiro (x^2=44.69; df=2; *P*<0.0001) and Roxo de Botucatu (x^2=37.07; df=2; *P*<0.0001) when compared to the subsequent generations.

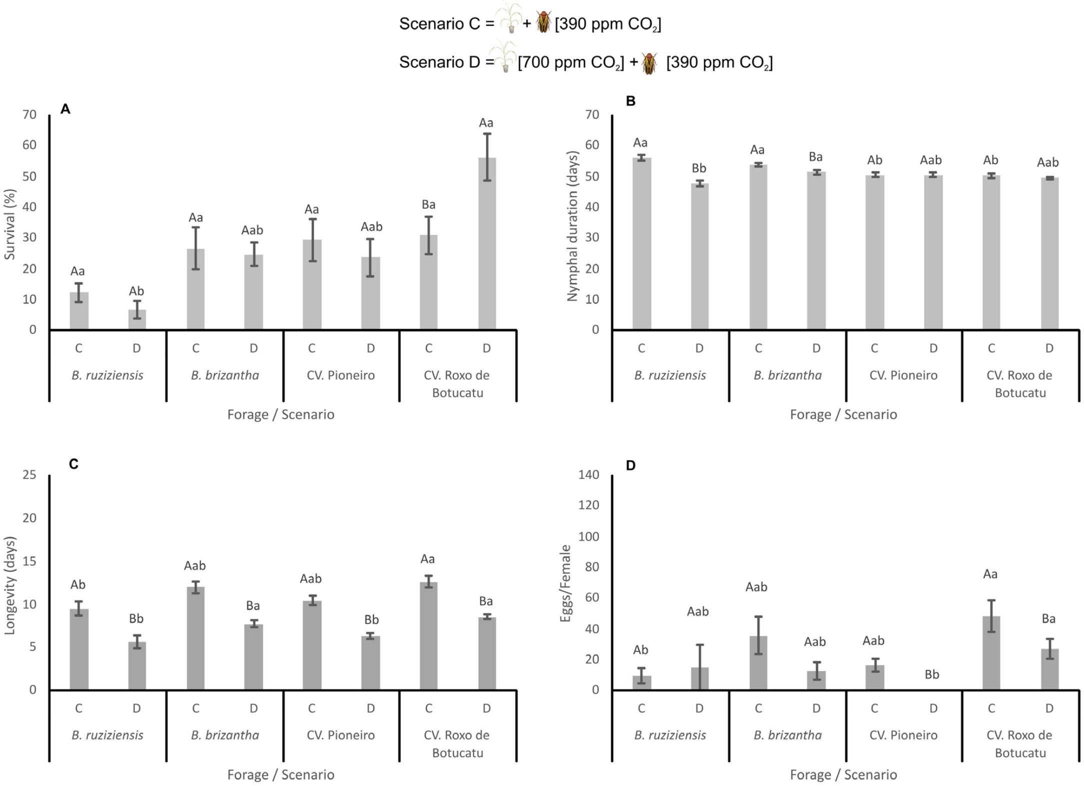

Figure 2. Biological aspects of the first generation of *M. spectabilis* fed on different forages in the following scenarios: (C) plants and insects were kept in a greenhouse (average of 390 ppm CO_2) and (D) the insects were kept in a greenhouse (average of 390 ppm CO_2) and fed on plants that came from the climatized chamber with high and constant CO_2 (700 ppm). Means followed by the same capital letter are compared between scenarios within each forage, and those followed by the same lowercase letter between the forages within each scenario do not differ from each other by Wilcoxon-Mann-Whitney U test ($P<0.01$) or Kruskal-Wallis, Nemenyi post hoc test ($P<0.01$), respectively.

Among the forages, Roxo de Botucatu increased the longevity of the adults of the insect pest in the three generations (Figure 3).

No difference was observed in the nymphal duration ($x^2=5.89$; df=2; $P<0.052$) in the three generations when the insects were fed on Pioneiro; however, it became considerably higher in generation 3, when insects were fed on Roxo de Botucatu ($x^2=15.05$; df=2; $P<0.0001$) compared with the first generation. Within each generation, the number of eggs was observed to be significantly higher when kept in the Roxo de Botucatu cultivar compared to the Pioneiro in generations 1 (W=565, P=0.046) and 3 (W=252, $P<0.0001$) (Figure 3).

Comparing these two factors, generations and scenarios (Figure 4), was only possible when the cultivar Roxo de Botucatu was offered due to the reduced survival of the spittlebugs in the Pioneiro forage in Scenario D. As can be seen in Figure 4, no difference was observed in the nymphal survival in three generations ($x^2=3.52$; df=2; $P<0.172$) when insects were fed on Roxo de Botucatu, that was grown and kept in the greenhouse (Scenario C); however, when fed the same forage but grown at high and constant CO_2 and kept in the greenhouse (Scenario D), significantly higher nymphal survival was obtained ($x^2=12.79$; df=2; $P=0.001662$) in the first generation. In this same generation, greater nymphal survival was observed (W=18, $P=0.017$) in the respective scenario (Figure 4).

The duration of the nymphal phase was observed to be shorter in generation 1 when the insects were fed on Roxo de Botucatu and maintained in the C ($x^2=44.69$; df=2; $P<0.0001$) and D ($x^2=118.83$; df=2; $P<0.0001$) scenarios compared to the other generations. This forage maintained in Scenario D reduced (W=7213, $P=0.049$) the duration of the nymphs of the second generation; however, there was no difference in this parameter in the other generations (Figure 4).

In both scenarios, adult longevity was lower in generation 1 when insects were fed on Roxo de Botucatu. In generations 1 (W=9712, $P<0.0001$) and 2 (W=7504, $P=0.0003$), the longevity of the adults was found to be significantly higher when they were fed on Roxo de Botucatu and kept in Scenario C. This difference was not observed (W=2779, $P=0.059$) in generation 3 (Figure 4).

Significant differences were observed in the number of eggs in generation 2 (W=2091, P=0.0019) and 3 (W=809, P<0.0001) since the females laid more eggs when the insects were fed on Roxo de Botucatu and kept in the greenhouse (Scenario C). Between the generations, only

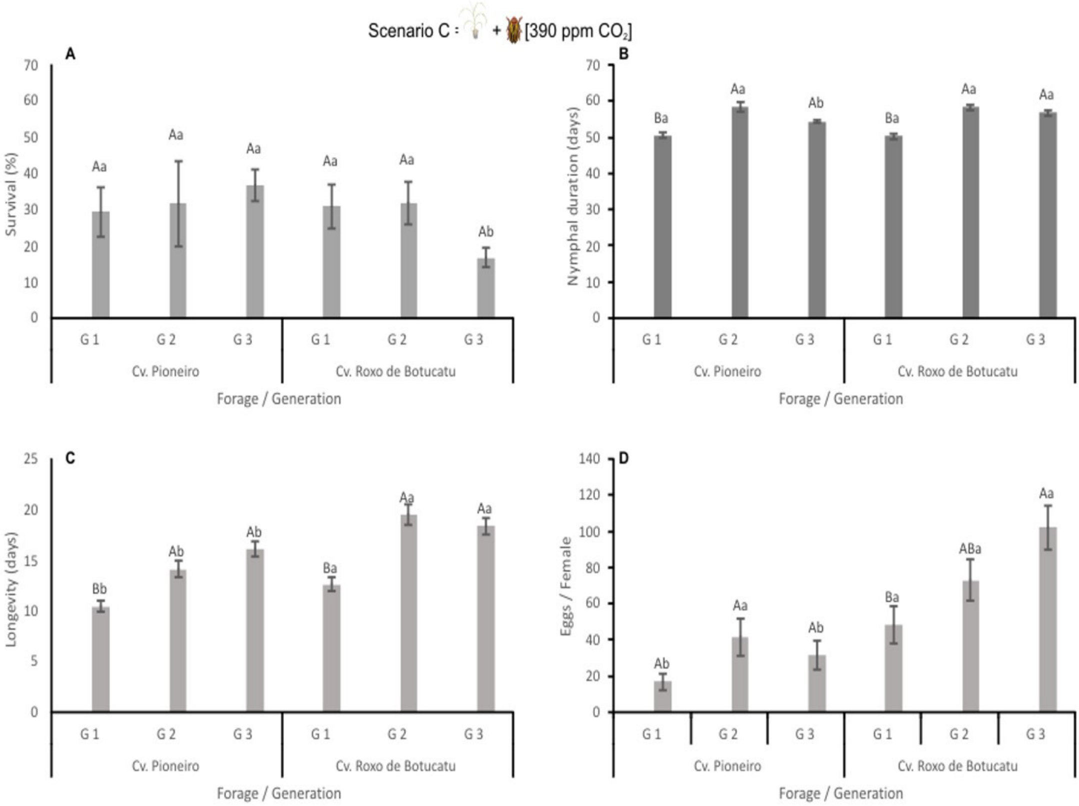

Figure 3. Biological aspects of *M. spectabilis* in three consecutive generations fed on Pioneiro and Roxo de Botucatu cultivars in the following scenario: (C) plants and insects were kept in a greenhouse (average of 390 ppm CO_2). Means followed by the same capital letter are compared between scenarios within each forage, and those by the same lowercase letter between the forages within each scenario do not differ from each other by Wilcoxon-Man-Whitney U test ($P<0.01$) and Kruskal-Wallis, Nemenyi post hoc test ($P<0.01$), respectively.

changes in the number of eggs were observed in Scenario C ($x^2=15.069$; df=2; $P<0.0005$), which was higher in generation 3 (Figure 4).

4. Discussion

Climate change, with rising CO_2 and temperature, can affect the pasture production system through chemical and biological changes in terms of the interaction between the forage and pasture spittlebugs. The defense system of forage plants can influence the survival, fecundity, and development of phytophagous insects and, consequently, their distribution and abundance in the environment (Gould, 1998). The investigation of this process would support control strategies, in which the primary purpose is to find out the best interaction between the organisms and the search for proximity to environmental balance, ensuring low production costs, considering that, according to Dias-Filho (2014), forages are the most practical and economical means of feeding cattle.

Insect survival is an important factor in assessing insect-plant interaction. The low nymphal survival of *M. spectabilis* in Scenarios A and B used in the present study prevented the analysis of some biological parameters of this insect pest (duration, longevity and the average number of eggs/female) by making the number of repetitions insufficient for statistical analysis in the first, second, and third generations (Table 1). Ferreira et al. (2013) also recorded low survival of this insect pest in the first generation, which also varied depending on the scenarios to which the insects and plants were subjected and on the forage species offered, ranging from 2% (*B. brizantha*) to 65% (Roxo de Botucatu).

In the two Scenarios A and B, in which the insects were always kept under controlled conditions, it was possible to verify that the forages provided the same nymphal survival of the insect pest. This result suggests that the quality of forage under high levels of CO_2 did not influence the development of *M. spectabilis*. However, in general, an alteration in the performance of *M. spectabilis* was observed when they were kept in the greenhouse in Scenario D compared to Scenario C (Figure 2). It should be taken into account that the only difference between these scenarios is the place where the four forages were submitted. When they were kept in a scenario with high and constant concentration of CO_2, the nymphal duration, adult longevity, and the number of eggs/female reduced in one or more forages. This proves the indirect effect of plants kept at different CO_2 levels on the performance of the insect pest kept in the current climate scenario (greenhouse with 390 ppm CO_2).

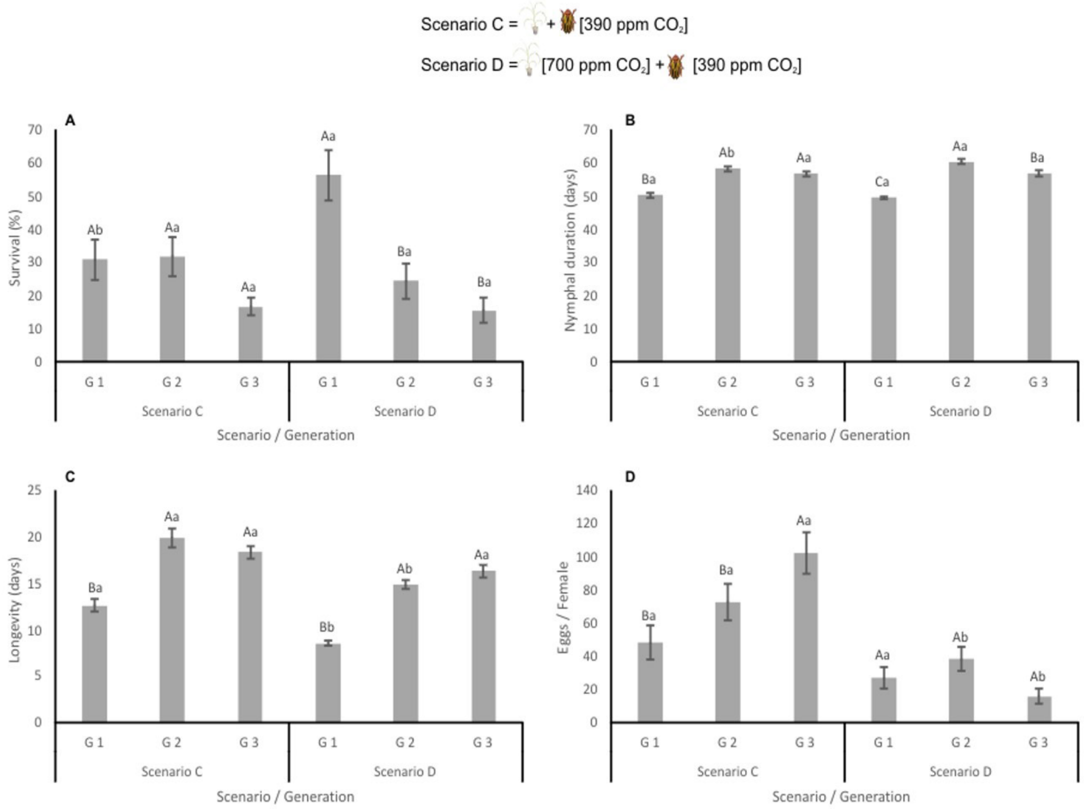

Figure 4. Biological aspects of *M. spectabilis* in three consecutive generations fed on different forages in the following scenarios: (C) the Roxo de Botucatu cultivar and insects were kept in a greenhouse (average of 390 ppm CO_2) and (D) the insects were kept in a greenhouse (average of 390 ppm CO_2) and fed on the Roxo de Botucatu cultivar that came from a climatized chamber with high and constant CO_2 (700 ppm). Means followed by the same capital letter are compared between scenarios within each forage, and those followed by the same lowercase letter between the forages within each scenario do not differ from each other by Wilcoxon-Man Whitney U test ($P<0.01$) and Kruskal-Wallis, Nemenyi post hoc test ($P<0.01$), respectively.

According to Zavala et al. (2013) and Hamann et al. (2021), the effects on herbivorous insects caused by the climate change occur due to changes in the host plants. Yin et al. (2010) emphasized that under high CO_2 concentrations, the lower nutritional quality of plants results in higher insect mortality, reducing the extent of their damage to plants. However, elevated levels of CO_2 also affect insects directly (Xie et al., 2015), which can be evidenced by the low survival of *M. spectabilis* nymphs when they were kept always under high CO_2 level (Figure 1).

It is noteworthy that of the four forages tested, Roxo de Botucatu proved to be the best food for developing *M. spectabilis* in the future (with an expected increase in CO_2). Similar results were reported by Ferreira et al. (2013). They found no significant difference in the nymphal survival of *M. spectabilis* when the insects were fed on *B. decumbens* (susceptible) and *B. brizantha* (resistant) and kept in environments with different concentrations of CO_2. On the other hand, elephant grass cultivars that are susceptible (Roxo de Botucatu) and resistant (Pioneiro) to *M. spectabilis* resulted in a significantly higher percentage of nymphal survival when maintained at 500 ppm than in environments with CO_2 at 250 ppm, stating that the susceptibility of the Roxo de Botucatu increased under high CO_2 levels. This information is vital, since the plant-resistance strategy has been the most indicated strategy for reducing the commitment of the spittlebugs in the productive systems of pastures (Auad et al., 2007; Souza Sobrinho et al., 2010; Resende et al., 2014; Alvarenga et al., 2017).

Still, in pastures, Silva et al. (2019) recorded a direct and indirect effect of the increase in CO_2, which promoted longer duration and lower survival of the *Collaria oleosa* (Distant) (Hemiptera: Miridae) bug, which led to recording the maintenance of the resistance of the forage species *B. brizantha* in the future climate scenario. In addition, Auad et al. (2012) concluded that the population of the aphid *Sipha flava* (Forbes) (Hemiptera: Aphidae), which is also found in forage, would decrease significantly in future climatic conditions, in which the concentration of the CO_2 level and temperature is expected to increase. Thus, despite research suggesting that global climate change may disrupt insect resistance in specific crops, with profound food security implications (Guo et al., 2014, 2017), resistance patterns will remain the same for the primary insect pest species in pastures (pasture spittlebugs, stink bug, and aphid). However, it should be taken into account that the effects of CO_2 elevation on the survival, developmental periods, and fecundity, when taken together, can significantly impact the population dynamics of *M. spectabilis*.

Li et al. (2022) reported that the population of the aphid *Lipaphis erysimi* (Kaltnbach) (Hemiptera: aphidae) decreases under high CO_2 emissions, which indicates that the damage caused by the aphids may be reduced in the future with the increase in CO_2 levels. In contrast, an increase in CO_2 concentration reduced the resistance in wild-type tomato plants infested with *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) (Guo et al., 2012) by lowering Jasmonic acid, an important hormone and polyphenol oxidase in these plants, which could likely exacerbate the problem of this pest in future climate scenarios. A longer duration and no effect on the adult survival and fecundity of *L. sativa* in environments of high CO_2 concentration were found by Santos et al. (2021). It was emphasized that the lengthening of the long developmental duration of phytophagous insects is an important factor that can assist in evaluating the host quality, which suggests poor nutritional quality. Adverse effects on biological parameters also indicate that the damage caused by *L. erysimi* (Li et al., 2022) and *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (De Paulo et al., 2020) will decrease in the future with the increase in the CO_2 level. Hence, there is variation in the responses of different insect species to changes in CO_2 concentration, which corroborates the results of Lindroth et al. (1995), who reported that different species of saturniidae are differently affected by rising CO_2 concentrations.

Much of the research concerning insect responses to future climate scenarios refers to a single generation with plant fragments. However, experiments carried out over more than one generation and on intact plants (Brooks and Whittaker, 1998) reveal differences in responses between the generations and the growth conditions of the host plants, in addition to the insects' freedom of movement in search of feeding places. These characteristics are taken into account in the present research, but it was yet only possible to analyze the performance of the spittlebugs in three successive generations, fed on forages Roxo de Botucatu (in Scenarios C and D) and Pioneiro (Scenario C). Effects on the interaction of generations of *M. spectabilis* in different scenarios are observed in the present research, in which those fed on Roxo de Botucatu plants derived from the phytotron with a high concentration of CO_2 provided a reduction in the nymphal survival (63%) and the number of eggs (80%), in addition to an increase in the nymphal duration (14%) and adult longevity (75%) in the third generation compared to the first generation. Herein, *Neophilaenus lineatus* L (Hemiptera: Aphrophoridae) showed a constant response in an environment of high CO_2 concentration over three generations (Brooks and Whittaker, 1998). In contrast, *Gastrophysa viridula* (De Geer) (Coleoptera: Chrysomelidae) showed reduced performance at high CO_2 emission levels in the second generation of exposure but not in the first generation (Brooks and Whittaker, 1998). Srinivasa Rao et al. (2015) found significant variation in the growth and development of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) as a function of CO_2 concentration and generation. Larval duration increased by 6-8% over four generations under high CO_2, and it was 10% higher in the fourth generation compared to the first generation. Lower survival and fecundity were also reported by Li et al. (2022) for *L. erysimi* kept under 800 µL/L CO_2 compared to 400 µL/L CO_2 in the third generation of this insect pest.

Thus, it is essential to know the effect of climate changes on the control strategies of the spittlebugs in pastures, in a way that does not compromise the sustainability of the production system.

5. Conclusion

Mahanarva spectabilis kept at the current CO_2 level (Scenario C and D) had altered survival and fecundity as a function of the scenarios in which the plants were maintained. On the other hand, when the insects were always kept in the conditions of the future scenario (700 ppm), regardless of the scenarios where the plants were kept, survival was low, and the females of the first generation were not fertile, which made it impossible to assess the next generations, characterized by a greater direct than indirect effect of CO_2 level. Furthermore, it should be considered that the effects of CO_2 elevation on the survival, periods of development, and fecundity, when taken together, can significantly impact the population dynamics of *M. spectabilis* in future climate scenarios.

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