

# Does elevated CO<sub>2</sub> affect the biological aspects of *Liriomyza sativae* in melon plants?

## O aumento da concentração de CO<sub>2</sub> interfere em aspectos biológicos de *Liriomyza sativae* em meloeiro?

Jéssica de Oliveira Santos<sup>1</sup>; Francislene Angelotti<sup>2\*</sup>;  
Tiago Cardoso da Costa-Lima<sup>3</sup>

### Highlights

Increase in CO<sub>2</sub> prolongs the duration of the immature stages of *Liriomyza sativae*.  
Elevated CO<sub>2</sub> level does not affect the immature stages viability and longevity.  
CO<sub>2</sub> enriched environment does not influence the survival and fertility of *L. sativae*.

### Abstract

An increase in the carbon dioxide concentration (CO<sub>2</sub>) in the atmosphere has occurred in recent years, influencing the different biological aspects of herbivorous insects. The present study aimed to evaluate the effect of CO<sub>2</sub> increase on the biological aspects of *Liriomyza sativae* Blanchard leafminer in melon plants. For this, two experiments were carried out: (i) to evaluate the effect of melon plants grown in CO<sub>2</sub>-enriched environments on the immature developmental stages of *L. sativae* and *L. sativae* adult longevity, and (ii) to verify the impact of increased CO<sub>2</sub> concentration on *L. sativae* adult survival, feeding punctures, and oviposition. The experiments were carried out in growth chambers maintained in the temperature regime of 20-26-33 °C (simulating the minimum, average, and maximum daily temperature) and under two CO<sub>2</sub> concentrations (400 ppm and 770 ppm). The immature stages and the egg-adult period of *L. sativae* were longer when they developed on plants grown in high CO<sub>2</sub> levels (770 ppm), but no difference in adult longevity was observed. The viability of the immature phases was not different between the two CO<sub>2</sub> concentrations. Furthermore, there was no difference in the number of eggs and feeding punctures between treatments. Thus, the increase in CO<sub>2</sub> concentration prolongs the duration of the immature stages of *L. sativae*; however, it does not affect their viability. Adult survival, fertility, and feeding punctures were also unmodified by the environment enriched with CO<sub>2</sub>.

**Key words:** Agromyzidae. Carbon dioxide. Climate change. *Cucumis melo*. Leafminer.

<sup>1</sup> Master's Course of the Graduate Program in Graduate Program in Environmental Science and Technology, Universidade de Pernambuco, UPE, Petrolina, PE, Brazil. E-mail: jessicadeoliveirasantos01@gmail.com

<sup>2</sup> DSc in Agronomy, Empresa Brasileira de Pesquisa Agropecuária, EMBRAPA Semi-arid, Petrolina, PE, Brazil. E-mail: francislene.angelotti@embrapa.br

<sup>3</sup> DSc in Entomology, EMBRAPA Semi-arid, Petrolina, PE, Brazil. E-mail: tiago.lima@embrapa.br

\* Author for correspondence

## Resumo

O aumento da concentração de dióxido de carbono (CO<sub>2</sub>) na atmosfera tem ocorrido nos últimos anos, influenciando nos diferentes aspectos biológicos de insetos herbívoros. O presente trabalho visa avaliar o impacto do aumento da concentração de CO<sub>2</sub> sobre aspectos biológicos da mosca-minadora, *Liriomyza sativae* Blanchard, em meloeiro. Para isso foram realizados dois experimentos: (i) o primeiro para avaliar o efeito de plantas de meloeiro cultivadas em ambientes enriquecido com CO<sub>2</sub> sobre o desenvolvimento dos estágios imaturos e da longevidade dos adultos de *L. sativae*; e o (ii) segundo para verificar o impacto do aumento da concentração de CO<sub>2</sub> sobre a sobrevivência, puncturas de alimentação e oviposição de *L. sativae*. Os experimentos foram conduzidos em câmaras de crescimento com regime de temperatura de 20-26-33°C (simulando a temperatura mínima, média e máxima diária) e duas concentrações de CO<sub>2</sub>, 400 e 770 ppm. Os estágios imaturos e o período ovo-adulto de *L. sativae* foram maiores quando desenvolvidos em plantas cultivadas em elevado nível de CO<sub>2</sub>, no entanto não foi observado diferença na longevidade dos adultos. A viabilidade das fases imaturas não diferenciou entre as duas concentrações de CO<sub>2</sub>. Não houve diferença no número de ovos e puncturas de alimentação entre os tratamentos. Desta forma, o aumento de CO<sub>2</sub> prolonga a duração dos estágios imaturos de *L. sativae*, porém, não afeta a viabilidade destas. A sobrevivência dos adultos, fecundidade e puncturas de alimentação também não é modificada no ambiente enriquecido com CO<sub>2</sub>.

**Palavras-chave:** Agromyzidae. Dióxido de carbono. Mudança climática. *Cucumis melo*. Mosca-minadora.

## Introduction

The increase in greenhouse gas (GHG) emissions is directly related to global warming (Intergovernmental Panel on Climate Change [IPCC], 2013). Among GHGs, carbon dioxide (CO<sub>2</sub>) represents more than 70% of anthropogenic emissions (Olivier, Schure, & Peters, 2017). Currently, the CO<sub>2</sub> concentration in the atmosphere is approximately 410 ppm, according to data from the Scripps Oceanography Observatory in Mauna Loa in Hawaii, USA (National Oceanic and Atmospheric Administration [NOAA], 2020), and may exceed 700 ppm (RCP8.5) by the year 2100 (IPCC, 2013).

These climate changes may have repercussions in different areas, including agriculture (Paudel & Hatch, 2012). Models formatted for rice, corn, and wheat crops demonstrate that losses caused by insect

pests can increase by 10% to 25% with each 1°C increase in temperature increase (Deutsch et al., 2018). The increase in CO<sub>2</sub> concentration can also influence insects directly or indirectly (Boullis, Francis, & Verheggen, 2018). In the first case, it interferes with biology, development time, survival, fertility, and fecundity (Auaud & Fonseca, 2017). As a consequence of this, its population dynamics are also affected (Marchioro, Krechemer, & Foerster, 2017). The indirect effects are mediated by changes in morphology (Morrison & Morecroft, 2008; Lake & Wade, 2009), biochemistry (Yuan, Himanen, Holopainen, Chen, & Stewart, 2009), and physiology (Gifford, Barrett, Lutze, & Samarakoon, 1996; Yadugiri, 2010) of the host plant.

Herbivorous insects can be divided, according to eating habits, into exophages (e.g., leaf chewing and sap-sucking) and endophages (e.g., gall makers, leafminers,

and stem borers) (Araujo, Vieira, Lewinsohn, & Almeida, 2015). The vast majority of research is concentrated on chewing and sap-sucking insects, while leafmining is the one with the least number of published studies (Sinclair & Hughes, 2010). In the last 10 years, the majority of the studies related to the CO<sub>2</sub> impact on insects have been associated with sap-sucking and chewing insects, and few on leafmining habits (Auad, Fonseca, Resende, & Maddalena, 2012; Srinivasa Rao et al., 2012; Fonseca, Santos, & Auad, 2014; Akbar, Pavani, Nagaraja, & Sharma, 2015).

The larvae of this insect guild, in general, are confined to a single leaf in the vast majority of species (Sinclair & Hughes, 2010); therefore, it is expected that the effects related to CO<sub>2</sub> concentration are differentiated in relation to species that have exogenous habits. Two studies with leafminer flies observed the influence of an enriched CO<sub>2</sub> environment over *Chromatomyia syngenesiae* Hardy (Agromyzidae) (Smith & Johnes, 1998) and *Pegomya nigratarsis* Zetterstedt (Anthomyiidae) (Salt, Brooks, & Whittaker, 1995). The elevated CO<sub>2</sub> levels reduced the development period in the first species while increasing leaf consumption in the second. With lepidopteran leafminers, reduced oviposition of *Tuta absoluta* Meyrick (Sathishchandra, Vaddi, Naik, Chakravarthy, & Atlihan, 2018) and *Phyllonorycter tremuloidiella* Braun were detected with elevated CO<sub>2</sub> levels (Kopper & Lindroth, 2003). Considering the immature stages of lepidopteran leafminers, higher CO<sub>2</sub> concentrations increased *T. absoluta* development time (Sathishchandra et al., 2018), and reduced larval and pupal viability of *Dialectica scariella* (Zeller) (Johns & Hughes, 2002). With leafminer flies from the *Liriomyza* genus, no studies are available

regarding the impact of CO<sub>2</sub> on their biology. Flies from this genus stand out for their agricultural importance, as pests of various vegetables and ornamental plants (Costa-Lima, Silva, & Parra, 2015). In Brazil, it is the main pest in melon crops (Lemos, Costa-Lima, Godoy, Barros, & Barros, 2021).

The present study aimed to evaluate the effect of CO<sub>2</sub> increase on the biological aspects of *Liriomyza sativae* Blanchard leafminer in melon plants.

## Material and Methods

The population of *L. sativae* used in the bioassays was initially obtained from a melon cultivation area in Juazeiro (BA), Brazil. The breeding system was established in a laboratory and greenhouse with cowpea [*Vigna unguiculata* (L.) Walp.], according to Costa-Lima, Geremias, Begiato, Chagas and Parra (2017). The insects used in the bioassay were aged between four and six days, considering the oviposition peak of *L. sativae* (Costa-Lima, Geremias, & Parra, 2010).

Two bioassays were conducted: (i) to evaluate the effect of melon plants grown in CO<sub>2</sub>-enriched environments on the immature stage development and adult longevity of *L. sativae*, and (ii) to verify the impact of increased CO<sub>2</sub> concentration on *L. sativae* adult survival, feeding punctures, and oviposition. Both bioassays were conducted in growth chambers (12 m<sup>2</sup>) with CO<sub>2</sub> concentrations of 400 ppm and 770 ppm. The two concentrations were established according to the current concentration (400 ppm) and the IPCC forecast for 2100 in the scenario RCP8.5 (770 ppm) (Representative Concentration Pathway). The temperature

regime was 20-26-33 °C (20 °C: between 8 pm and 6 am; 26 °C: between 6 am and 10 am; 33 °C: between 10 am and 3 pm; and 26 °C: from 3 pm to 8 pm). The temperatures were determined from the minimum, average and maximum temperatures of the Sub-middle São Francisco Valley, Brazil, where many irrigated crops are hosts for *L. sativae*. The photophase was maintained for 12 h with a relative humidity of  $70 \pm 10\%$ .

For the first bioassay, the yellow type melon seeds, of the Glacial variety, were sown and developed inside growth chambers at the two CO<sub>2</sub> concentrations. Sitrad software was used to monitor the CO<sub>2</sub> concentration inside the chambers. The plants were used in the bioassay only after 15 days of development. One breeding cage (50 × 80 × 62 cm), with all faces composed of a fine polyester mesh, was used in each chamber. Approximately 300 *L. sativae* adults were added to the cages with a 1:1 sex ratio. Eight melon plants were introduced into each cage, which remained exposed to the leafminer adults for 24 h for oviposition. After this period, the plants were removed from the cages and placed in plastic trays (60.5 × 40 × 9.2 cm), while remaining inside the chambers. The melon leaves were observed daily using a stereoscopic microscope (60 X) with light transmission to account for the number of eggs and larvae, and to monitor mortality. Still inside the chambers, when the larvae were four days old, 100 mL plastic cups were attached to the petiole of the plant, accommodating the leaf inside. The larvae that came out of the leaves were collected from the cups. These were individualized for pupation in Petri dishes (8.5 cm diameter) and covered with voile tissue. Daily observations were made to evaluate adult emergence. Upon emergence, adults

were placed in 100 mL plastic containers, with a voile lid and 10% honey solution as a food source, with daily assessments of male and female mortality being performed. The experimental design was completely randomized, with two treatments (CO<sub>2</sub> concentrations of 400 ppm and 770 ppm), and each insect was considered a repetition. The repetition number varied according to the treatment and development stage, with 100 and 102 (eggs), 96 and 98 (larvae), 61 and 68 (pupae), and 46 and 51 (adults), for 400 ppm and 770 ppm, respectively. The following parameters were evaluated: duration and survival of the larva, pupa, and egg-adult, as well as male and female adult longevity.

In the second bioassay, to study the possible CO<sub>2</sub> impact on *L. sativae* adults, the following biological parameters were evaluated: number of eggs, feeding punctures, and survivorship. Melon plants with 15 days of development were used as hosts. Each repetition consisted of a breeding cage (40 × 40 × 55 cm) with five *L. sativae* couples, four to six days old, and a melon plant. The plants were replaced daily with new plants at the same developmental stage. The evaluation was conducted for five consecutive days using a stereoscopic microscope (60 X) with transmission light. The number of eggs and feeding punctures on melon leaves were quantified. During the evaluations, the daily mortality of *L. sativae* males and females was recorded. The bioassay consisted of a completely randomized design, with two treatments (400 ppm and 770 ppm) and 21 repetitions (melon plants).

In addition, 40 melon plants without *L. sativae* exposure were grown in each chamber, adjusted to 400 ppm and 770 ppm CO<sub>2</sub>. For these plants, leaf analysis was

performed for carbon, nitrogen, and carbon and nitrogen ratios (C: N). For the analyses, the muffle method (Goldin, 1987) and the Kjeldahl method (Kjeldahl, 1883) were used.

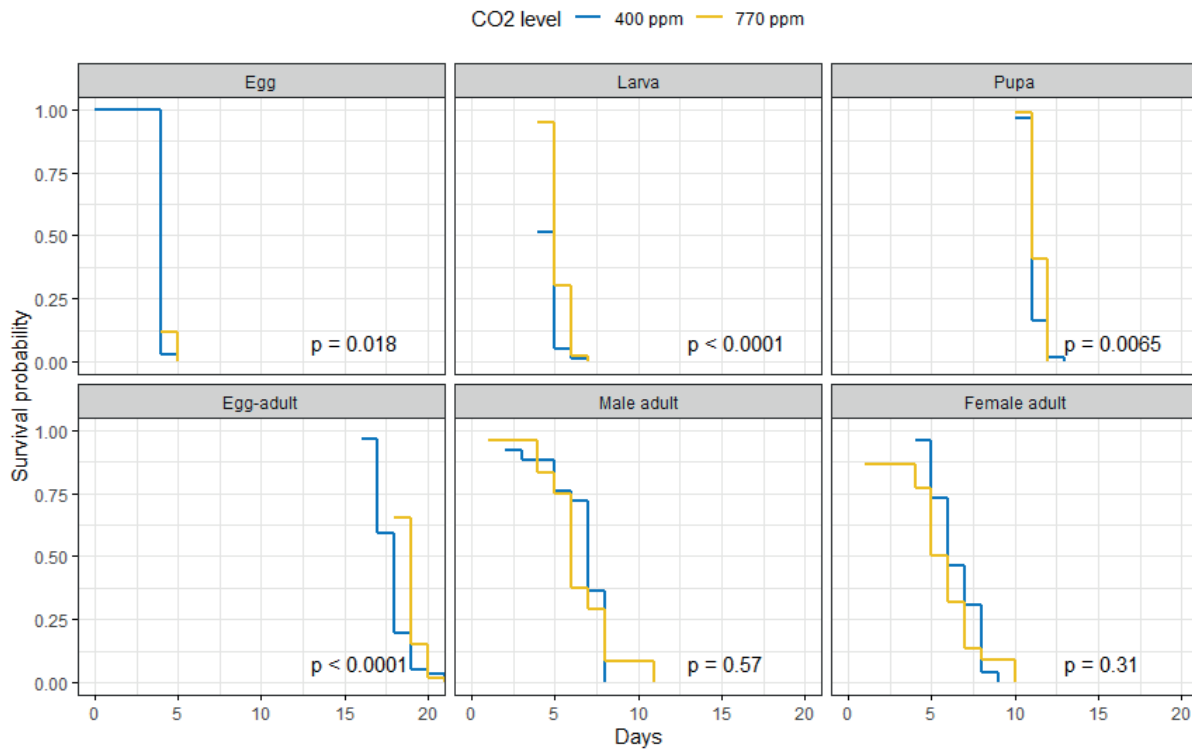
To compare the duration of the immature stages (egg, larva, and pupa), egg-adult period, and adult longevity between treatments, Kaplan Meier survival analysis was performed, and the log-rank test was applied ( $p < 0.05$ ). To verify larval and pupal viability, as well as the number of eggs and feeding punctures, non-generalized linear models with quasi-Poisson distribution (number of punctures and number of eggs) and binomial distributions (larval and pupal viability) were used, followed by an analysis of variance with F and Chi-square test ( $p < 0.05$ ), respectively. All analyzes were performed using the 'R' statistical software, version 3.5.2 (R Core Team [R], 2019).

## Results and Discussion

All immature stages of *L. sativae* presented a development period extension in an environment with high CO<sub>2</sub> levels, while for male ( $p = 0.57$ ) and female ( $p = 0.31$ ) adults, no significant difference was observed (Figure 1). The differences observed in the egg (4.12 and 4.03 days), larva (5.27 and 4.57 days) and pupa (11.21 and 11.18 days) phases for 770 ppm and 400 ppm of CO<sub>2</sub>, respectively, promoted an increase of approximately one day in the *L. sativae* egg-adult period (18.82 and 17.84 days) ( $p < 0.0001$ ) (Figure 1).

The results demonstrate that the increase in the CO<sub>2</sub> level prolonged the period

of the *L. sativae* immature stage; however, it did not interfere with the egg, larvae ( $p = 0.57$ ), and pupa ( $p = 0.14$ ) viability (Table 1). In a study with another Agromyzidae leafminer fly, *C. syngenesiae*, a prolonged development period was also observed in the treatment with 400 ppm compared to 200 ppm of CO<sub>2</sub> (Smith & Johnes, 1998). For the lepidopteran leafminer, *T. absoluta*, the same prolonged period was observed for the larval period in an environment with 580 ppm CO<sub>2</sub> (Satishchandra et al., 2018). The prolonged development period of *L. sativae* may have been caused by the nutritional quality of the leaves available to the larvae. Melons grown in an environment enriched with CO<sub>2</sub> showed lower leaf nitrogen levels; meanwhile, the C: N ratio was higher, with no difference in carbon content (Table 2). Nitrogen is a limiting element in insect development (Kagata & Ohgushi, 2012; Mattson, 1980). This cycle prolongation has already been observed in several insects fed with plants with low N levels grown in an environment enriched with CO<sub>2</sub> (Chown & Nicolson, 2004; Watt et al., 1995). However, it is not possible to generalize this effect, even for insects from the same guild. For example, when working with another leafminer fly in the family Anthomyiidae, CO<sub>2</sub> did not prolong the species cycle, but it did cause an increase in leaf consumption as a form of compensation (Salt et al., 1995). Secondary compounds, based on nitrogen and carbon, can also vary, playing an important role in the feeding behavior of herbivorous insects (Delucia, Nability, Zavala, & Berenbaum, 2012). Consequently, they may have also interfered with the biology of *L. sativae* in the present study.



**Figure 1.** Survival curve of *Liriomyza sativae* in two CO<sub>2</sub> concentrations (400 ppm and 770 ppm). P-values are equivalent to the log-rank test.

**Table 1**

*Liriomyza sativae* viability (%) of egg, larval, and pupal phases (mean ± SE) on melon grown in different CO<sub>2</sub> concentrations

CO <sub>2</sub> Concentration	Viability (%)		
	Egg	Larva	Pupa
400 ppm	100.00	95.9 ± 0.02	65.6 ± 0.05
770 ppm	100.00	94.1 ± 0.02	71.6 ± 0.05

No statistically significant differences were observed between the treatments for the larval and pupal phases ( $p < 0.05$ ). Statistical analysis was not applied to the egg phase because of the absence of data variance.

The mines created by insects transmit the appearance of isolation; however, there is gaseous exchange between the environment and the mine interior (Pincebourde & Casas, 2006). Thus, in a mine with functional stomata and photosynthetically active tissue, CO<sub>2</sub> enters directly through the stomata

(Pincebourde & Casas, 2016). However, the *L. sativae* eggs are found inside the leaf, just beside the tubular opening caused by the female ovipositor (Parrella, 1987), which facilitates gas exchange. In addition to the effects on the insect using the host plant, the environment enriched with CO<sub>2</sub> can also

cause direct effects, such as interference in the respiratory process. In insects exposed to a high level of CO<sub>2</sub>, the effect is similar to that of the low concentration of O<sub>2</sub>, in which both reduce phosphorylative oxidation (Mitcham,

Martin, & Zhou, 2006). Thus, there is a reduction in ATP synthesis and insect metabolism (Zhou, Criddle, & Mitcham, 2000). As a consequence, it can interfere with mortality and development.

**Table 2**

**Carbon and nitrogen concentrations, and the C:N ratio (mean ± EP) in melon grown in two CO<sub>2</sub> concentrations**

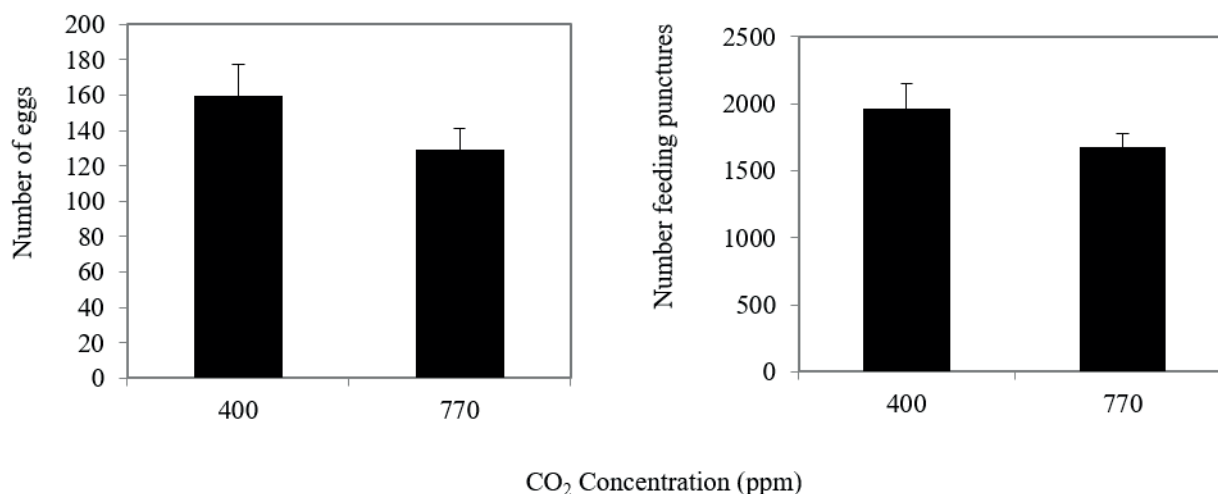
CO <sub>2</sub> Concentration	Carbon (C) content (%)	Nitrogen (N) content (g/Kg)	C:N
400 ppm	41.23 ± 0.80 a	25.20 ± 0,15 a	16.38 ± 0.39 a
770 ppm	41.14 ± 0.15 a	21.14 ± 0.08 b	19.53 ± 0.20 b

Means followed by the same letter indicate no difference between treatments ( $p < 0.05$ ).

The finding of the prolonged *L. sativae* egg-adult period by one day is equivalent to a one-generation reduction of the pest per year. However, we only considered the CO<sub>2</sub> factors assessed in the present study. In the scenario predicted by the IPCC (2013), an increase in CO<sub>2</sub> is associated with a thermal increase. In this way, the cycle prolongation can be neutralized because of the thermal

increase causing a reduction in the *L. sativae* development period (Costa-Lima, Geremias, & Parra, 2009).

No difference was observed between treatments in the total number of *L. sativae* eggs ( $p = 0.147$ ) (Figure 2A), feeding punctures ( $p = 0.159$ ) (Figure 2B), and the survival of adult males ( $p = 0.07$ ) and females ( $p = 0.30$ ).



**Figure 2.** The average number of eggs (A) and feeding punctures (B) of *Liriomyza sativae* in melon, in different CO<sub>2</sub> concentrations. There was no statistical difference between treatments ( $p < 0.05$ ).

This study was the first to evaluate the effect of CO<sub>2</sub> on the reproductive parameters of leafminers, with previous studies focusing only on immature stages (Salt et al., 1995; Smith & Johnes, 1998). In the case of lepidopterans of the same guild, results have demonstrated reduced oviposition in a high CO<sub>2</sub> environment (Kopper & Lindroth, 2003; Satishchandra et al., 2018); whereas another study did not observe any differences (Johns & Hughes, 2002). It is likely that because of the possible damage caused by exposure to high CO<sub>2</sub> levels, such as a reduction in phosphorylative oxidation (Mitcam et al., 2006), it was not possible to observe a biological response within five days of exposure to adults. Another possibility is the use of insect strategies to adapt to situations of hypoxia and hypercarbia, such as discontinuous breathing (Hetz & Bradley, 2005).

The results obtained in the present study help to predict the behavior of an important pest on different agricultural crops. According to our findings, in a climate change situation with an increase in CO<sub>2</sub> levels, it is expected that *L. sativae* would maintain its pest status, considering that no crucial biological aspect was affected. Studies with similar approaches can be conducted with different pest species that allow us to glimpse future scenarios, based on scientific evidence, for the continued growth of agriculture.

## Conclusion

The biology of *L. sativae* in melon plants cultivated under higher CO<sub>2</sub> concentrations (770 ppm) presents an extension of the duration of the immature stages; however, this fact does not affect the viability of these

stages and the longevity of adult males and females. The high CO<sub>2</sub> environment does not affect the survival, fertility, or the number of feeding punctures of *L. sativae* adults.

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