

Article

Influence of rainfall regime in the Cerrado biome on the maintenance of traps built by *Myrmeleon brasiliensis* (Navás) (Neuroptera: Myrmeleontidae) larvae and the morphology of adults

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ABSTRACT. Seasonality in the *Cerrado* biome of Brazil is characterized by a dry season and a rainy season, affecting the availability of water and generating nutritional limitations. Thus, plants and animals have developed adaptive mechanisms in order to survive in this environment. Insects known as antlions (Neuroptera: Myrmeleontidae) occur in areas of the *Cerrado* and build traps in dry soil to capture prey items. In the rainy season, these insects are unable to forage due to the waterlogged soil. The aim of this study was to investigate the influence of the rainfall regime in the *Cerrado* on aspects of trap-building behavior, larval development and morphological characteristics of adult antlions. Larvae of the antlion *Myrmeleon brasiliensis* (Navás, 1914) were observed and collected in an area of the *Cerrado* biome in the municipality of Aquidauana (MS), Brazil. Observations were performed in the rainy and dry seasons to determine the abundance of traps built by *M. brasiliensis* larvae. In the laboratory, experiments were performed involving the manipulation of the frequency of simulated rain on the traps. The results revealed that variations in rainfall due to seasonality in the *Cerrado* affect *M. brasiliensis* larvae, with greater foraging observed in the dry season. The laboratory experiments demonstrated that differences in the frequency of rains affect the mortality of the larvae, larval development time and the size of the adults. Thus, variations in rainfall patterns can lead to variations in the characteristics of the population structure of *M. brasiliensis* in areas of the *Cerrado* biome in Brazil.

KEYWORDS. Antlion, seasonality, trap-building behavior, tropical insects.

RESUMO. Influência do regime de chuvas do bioma Cerrado na manutenção das armadilhas de larvas e na morfologia dos adultos de *Myrmeleon brasiliensis* (Navás) (Neuroptera: Myrmeleontidae). No bioma Cerrado a sazonalidade (caracterizada por uma estação seca e outra chuvosa) afeta a disponibilidade de água e produz limitações nutricionais, de maneira que várias plantas e animais desenvolveram mecanismos adaptativos elaborados para sobreviver nesse ambiente. Insetos conhecidos como formiga-leão ocorrem em áreas de Cerrado e constroem suas armadilhas de captura de presas no solo seco. No período chuvoso as larvas ficam impedidas de forragear, dado o encharcamento do solo. Este trabalho tem como objetivo compreender a influência do regime de chuvas do Cerrado em aspectos do comportamento de construção da armadilha, no desenvolvimento das larvas e nas características morfológicas dos adultos. Larvas de formiga-leão *Myrmeleon brasiliensis* (Navás, 1914) foram observadas e coletadas em uma área de Cerrado no município de Aquidauana (MS), Brasil. Para verificar a abundância das armadilhas das larvas de *M. brasiliensis* foram realizadas observações no período chuvoso e seco. No laboratório foram realizados experimentos de manipulação da frequência de chuva nas armadilhas de *M. brasiliensis*. Como resultado foi observado que variações na sazonalidade do Cerrado afetam as larvas de *M. brasiliensis*, as quais são observadas forrageando em maior abundância na estação seca. Os experimentos de laboratório demonstraram que variações na frequência da chuva podem afetar, a mortalidade das larvas, o tempo de desenvolvimento das mesmas, o tamanho das armadilhas e o tamanho dos adultos. Sendo assim, variações no padrão de incidência de chuvas podem levar a variações nas características da estrutura da população de *M. brasiliensis* em áreas de Cerrado.

PALAVRAS-CHAVE. Formiga-leão, sazonalidade, comportamento de construção de armadilhas, insetos tropicais.

The influence of seasonality on tropical insects has been investigated in different regions of the world. Studies describe changes in abundance, the life cycle as well as occurrences of migrations and diapause (WOLDA & FISK, 1981; DUARTE JÚNIOR & SCHLINDWEIN, 2005; SILVA *et al.*, 2011). Macro- and microclimatic changes affect the abundance and diversity of insects both spatially and temporally and can determine the diversity, living habits and morphological characteristics of insects (AMARAL *et al.*, 2013). The rainfall regime is considered one of the

main climatic factors governing the distribution of insect populations (WOLDA, 1988; PINHEIRO *et al.*, 2002).

The *Cerrado* is the second largest biome in Brazil, occupying an area of 1,500,000 km². It is a typical savanna environment formed by a mosaic of different types of vegetation ranging from open grasslands to dense forests and dry forests (COUTINHO, 2006). The pronounced seasonality characterized by a dry season spanning from May to October, which affects water availability and generates nutritional limitations. Therefore, numerous plants and animals have

developed adaptive mechanisms in order to survive in this environment (ASSAD, 1994; SILVA, 2011).

Insects of the family Myrmeleontidae, commonly known as antlions, are holometabolous and have a larval phase divided into three instars. At the end of the third instar, the larva forms a spherical cocoon of sand, within which the pupa undergoes metamorphosis, resulting in the winged adult (MISSIRIAN *et al.*, 2006; SCHARF *et al.*, 2009). Some species of the tribe Mirmeleontini build funnel-shaped traps in dry sandy soil to capture arthropods moving across the surface of the ground (GRIFFITHS, 1986; LUCAS, 1986).

The singularity of this foraging strategy has long drawn attention to these insects (FERTIN & CASAS, 2006; SCHARF *et al.*, 2011). The larva remains motionless buried at the bottom of the pit with its mandibles open, awaiting the prey to fall into the trap. As the trap is basically an inverted cone excavated in soil with low granulometry, its design serves a dual function – first, it channels the prey to the bottom of the pit, where the antlion awaits; second, it slows the escape of the prey, as the unstable grains of sand slip to the bottom of the pit as the prey tries to escape (LUCAS, 1982; FRANKS *et al.*, 2019). Thus, struggle on the part of the prey contributes more to its capture than its escape.

Microclimatological and habitat restrictions determine the building of antlion traps in locations characterized by a specific combination of granulometry, plant density, shade and shelter (GOTELLI, 1993; LIANG *et al.*, 2010). Dry sandy soil facilitates the manipulation of the grains for the building of the funnel trap (GOMES *et al.*, 2004) and is an essential condition to the presence of *Myrmeleon brasiliensis* larvae (Návas, 1914) in areas of the *Cerrado* biome (LIMA & FARIA, 2007). Thus, in the dry season of the *Cerrado*, *M. brasiliensis* larvae build their traps unrestricted by the moisture of the soil. However, the larvae are unable to forage in the rainy season due to the waterlogged soil (FREIRE & LIMA, 2019).

Holometabolous insects accumulate energy in each larval phase to undergo metamorphosis, transforming into adults (MIRTH & RIDDIFORD, 2007). These insects use a good part of this energy during transformation, generating a positive biomass balance in the adult phase (SANTANA *et al.*, 2014; FARIA *et al.* 1994). Thus, body size in the adult is intrinsically related to the accumulation of nutrients during the larval phase (MIRTH & RIDDIFORD, 2007). This variable is of extreme importance and can affect population dynamics, as the size of the female is related to its reproductive potential (SOKOLOVSKA *et al.*, 2000; MILANO *et al.*, 2010). Antlion larvae are unable to forage in situations when the rain is more severe or occurs for a longer period of time, which can exert a negative impact on the size of the adults and, consequently, their fitness.

The purpose of the present study was to investigate the influence of the rainfall regime in the *Cerrado* biome on aspects of trap-building behavior, larval development and morphological characteristics of the adults. The specific objectives were to investigate 1) the correlation between the abundance of traps and the incidence of rain; 2) the

movement of the larvae after rain; 3) the mortality of larvae in response to the frequency of rain; 4) the development time of larvae in response to the frequency of rain; 5) trap size in response to the frequency of rain; and 6) the body size, mass and wing length of adults of *M. brasiliensis* as a function of rain frequency.

MATERIAL AND METHODS

Larvae of the antlion species *Myrmeleon brasiliensis* were observed and collected in a Permanent Protection Area of the *Universidade Estadual do Mato Grosso do Sul* (UEMS) located in the municipality of Aquidauana, state of Mato Grosso do Sul, Brazil. The area has approximately 160 ha. The species was identified by researcher Lionel A. Stange of the Florida State Arthropod Collection (USA).

Vegetation in the region is characterized by different phytophysionomies, including open arboreal savanna with patches of dense arboreal savanna (FINA & MONTEIRO, 2013). The predominant climate is seasonal tropical with a dry winter. The mean annual temperature is around 22-23°C and mean annual precipitation is between 1200 and 1800 mm (Aw in the Köppen classification). Seasonality is marked by two well-defined seasons – a rainy season between November and April and a dry season between May and October (SOUZA *et al.*, 2016). The monthly rainfall index drops considerably between May and September and can reach as low as zero mm (COUTINHO, 2000).

The surface soils in the *Cerrado* result from clay sediments from the alteration of basalts and are formed by sandstone in regions with rocky outcrops (GOMES *et al.*, 2004). The area studied is located in a region composed of sandstone, mainly from the Aquidauana Formation. The presence of sandy soils is favorable to the occurrence of microhabitats for *M. brasiliensis* larvae (LIMA, 2020).

Monthly observations were performed in the rainy season (November 2018 to April 2019) and dry season (May to October 2019) to estimate the abundance of traps by *M. brasiliensis* larvae. In an area of the reserve, a transect measuring 2 km in length by four meters in width was established, within which monthly counts of *M. brasiliensis* larvae were performed through active searches for traps. Rainfall data were obtained from the site of *Centro de Monitoramento do Tempo e do Clima* (CEMTEC, 2019). Pearson's correlation test was used to evaluate the effect of the rain incidence index (mm) on the abundance of traps by *M. brasiliensis* larvae.

Experiment I

In the laboratory, an experiment was conducted to determine the occurrence of movement by *M. brasiliensis* larvae after the simulation of rain. For such, 30 larvae were collected with the aid of a spoon and placed individually in plastic bags with sand from the collection site. In the lab, body length was measured (head-abdomen) and the larvae

were placed individually into plastic pots measuring 21 cm in length by 15 cm in width and 5 cm in depth. Each pot was filled with 200 g of sand from the collection site.

After 48 horas (time allotted for the larvae to build their traps), the diameter of the traps was measured and the occurrence of rain was simulated with the addition of 1 ml of water dispensed directly into the trap with the aid of a pipette. The behavior of the larvae was observed for five days after the destruction of the traps, with the recording of whether the larva moved to a different site rebuild the trap and the distance of the movement. The relation between the distance of the movement after the simulation of rain and body size of the larvae was determined using linear regression analysis.

Experiment II

In the laboratory, 90 second-instar larvae were lodged individually in plastic pots measuring 7 cm in length, 7.8 cm in width and 5 cm in depth. Each pot was filled with 200 g of sand from the collection site. The pots were numbered and separated for use in three treatments (30 larvae per treatment): Treatment I (control) – no simulation of rain; Treatment II – 25 ml of water every 10 days; Treatment III - 25 ml of water every five days.

The larvae in all treatments were equally fed every five days throughout the experiment with ants of the species *Atta sexdens* (Linnaeus, 1758) (Hymenoptera: Formicidae) (mean size: 4.04 ± 0.50 mm). On the day of feeding, three ants were placed into the traps, with an average of two hours between the placement of one ant and another. At the end of each feeding day, which coincided with the 10th day in Treatment II and the 5th day in Treatment III, rain was simulated again.

For the study of the variation in the mortality rate, the number of larvae that died throughout the experiment in the three treatments was recorded. For the study of the variation in trap size, the diameter was measured in millimeters (mm) every five days from the first to the last day of the experiment, totaling 200 days. For the study of the development time of each life stage, the days in which the larvae remained in the larval phase, days of pupation and the day of the emergence of the adults were quantified in each treatment.

The following characteristics of the adults that emerged from all treatments were evaluated: body mass, determined on a high-precision analytical scale with a capacity of 210 g; body length, measured using analytical calipers with a precision of 0.1 mm from the head (not counting the antennae) to the end of the abdomen; and length of the four wings (the wings were removed and mounted on a slide to be photographed under a trinocular microscope; length was measured with the aid of a 2 mm scale).

Repeated-measures analysis of variance (ANOVA) was used to compare mean trap diameter among the treatments. The G test was used to evaluate the effect of rain frequency on mortality and larval development time. The comparison of morphological characteristics (mass,

body length and wing length of the adults) was performed using the Kruskal-Wallis test with Dunn's post hoc test. All analyses were performed with the aid of the Bioestat software (AYRES & AYRES JÚNIOR, 2007).

RESULTS

The mean (\pm standard deviation) number of traps by *M. brasiliensis* larvae per month was 275 ± 80 in the dry season and 20 ± 44 in the rainy season. The largest number of traps ($n = 352$) was found in August. No traps were found in January, February, March or April.

A negative correlation was found between rainfall (mm) and the abundance of *M. brasiliensis* larvae ($r = -0.65$; $p = 0.02$). The months of greater rainfall were February, March and April, when no traps were found. In July, in which no rainfall occurred, 280 traps by *M. brasiliensis* larvae were found (Fig. 1).

In experiment I (conducted to observe the effect of rain frequency on the movement of *M. brasiliensis* larvae), 71.41% of the larvae moved to build a new trap after the simulated rain. The mean distance of the movement in relation to the original site was 51.87 ± 41.71 mm. The high standard deviation demonstrates that some larvae built new traps near the destroyed traps, whereas others sought more distant locations. A positive relation was found between the distance of the movement and size of the larvae ($F = 10.49$; $GL = 1$; $r^2 = 0.24$; $p = 0.0028$); larvae with a larger body size moved a greater distance from the area that received the simulated rain (Fig. 2).

In experiment II (conducted to evaluate the effect of the frequency of rain), a difference in the mortality rate was found among treatments ($\chi^2 = 6.939$; $GL = 2$; $p = 0.03$), as a greater number of dead individuals were found when the frequency of rain increased. In Treatment III (simulated rain every five days), larval mortality was 53%, followed by 33% in Treatment II and 10% in Treatment I.

Besides causing a higher mortality rate, the rain exerted an influence on the development time of the individuals. At the end of the experiment, differences were found in the number of larvae, pupae and adults among the treatments ($G = 11.57$; $p = 0.003$). The larvae in Treatments II and III (with simulated rain) pupated by the 145th day of the experiment. In contrast, 15% of the individuals in Treatment I (without simulated rain) remained in the larval stage after 200 days of experiment. Thus, rain accelerated the larval phase (Fig. 3).

The mean diameter of the traps built by the larvae submitted to rain simulation in Treatment III was 22.02 ± 7.56 mm, which was significantly smaller than that found in the other treatments (30.35 ± 11.28 mm in Treatment I and 30.48 ± 8.48 mm in Treatment II) (ANOVA: $GL = 2$; $F = 8.23$; $p = 0.001$) (Fig. 4).

Among the morphological characteristics, a significant difference was found in body length of the adults ($GL = 2$; $p = 0.00054$). Dunn's post hoc text revealed that the difference occurred between Treatments I and II ($p = 0.05$) as well as between Treatments I and III ($p = 0.05$). The adults that

emerged from Treatment I were the largest (mean length: 21.01 ± 1.71 mm), followed by those that emerged from Treatment II (mean length: 19.74 ± 1.40 mm) and the smallest emerged from Treatment III (mean length: 18.9 ± 1.67 mm)

(Fig. 5). Moreover, Dunn's post hoc test revealed that wing length was significantly smaller in Treatment II compared to Treatment I. No significant differences were found among the treatments regarding mass (Tab. I).

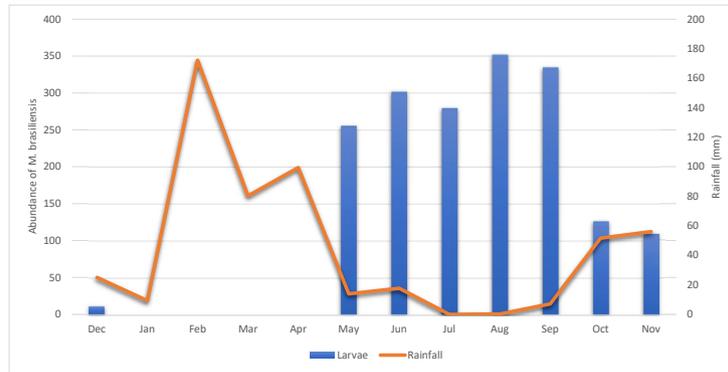


Fig. 1. Abundance of *Myrmeleon brasiliensis* (Návas, 1914) larvae traps and rainfall (mm), recorded between December 2018 and November 2019 in an area of Cerrado, Aquidauana, MS, Brazil.

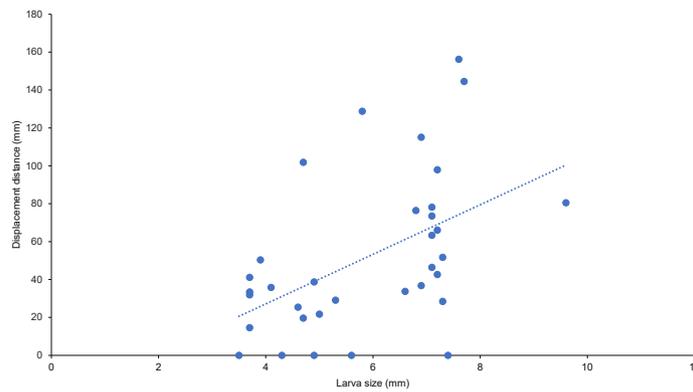


Fig. 2. Displacement of *Myrmeleon brasiliensis* (Návas, 1914) larvae after rain simulation.

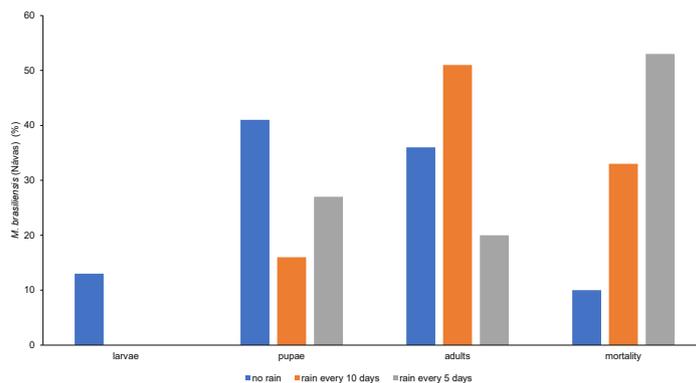


Fig. 3. Percentage of live larvae, pupae, live adults and dead larvae of *Myrmeleon brasiliensis* (Návas, 1914) (Neuroptera: Myrmeleontidae) observed at the end of the experiments (Treatment I: control, without rain; Treatment II: rain every 10 days and Treatment III: rain every 5 days).

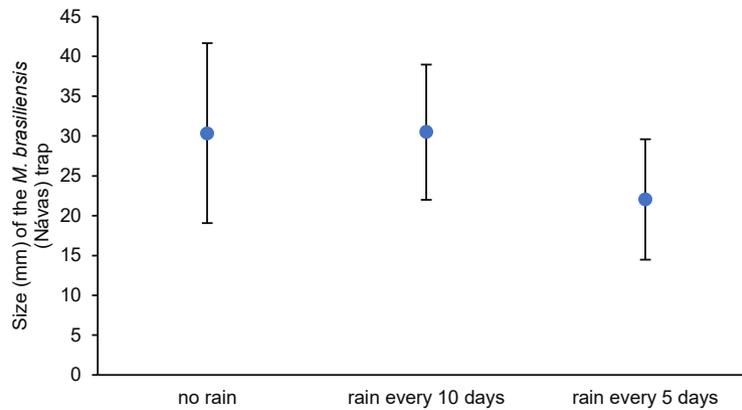


Fig. 4. Size (mean \pm SD) of *Myrmeleon brasiliensis* (Návas) larvae traps submitted to different rain frequencies. (Treatment I: control, no rain; Treatment II: rain every 10 days and Treatment III: rain every 5 days).

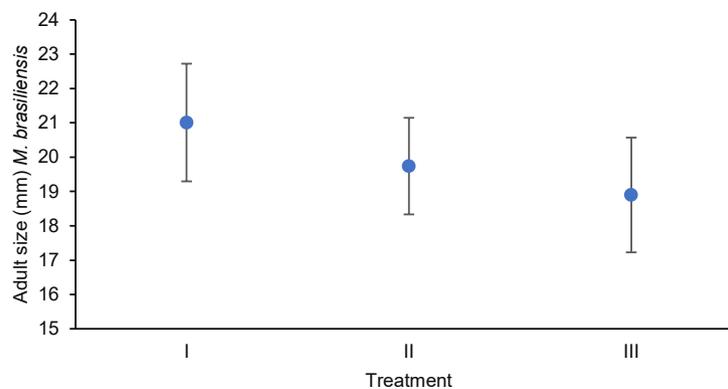


Fig. 5. Mean and standard deviation of the adult body length of *Myrmeleon brasiliensis* (Návas, 1914) from larvae subjected to different rainfall frequencies. (Treatment I: control, no rain; Treatment II: rain every 10 days and Treatment III: rain every 5 days).

Tab. I. Mean (\pm SD) of the measurements of the wings of *Myrmeleon brasiliensis* (Návas, 1914) from larvae submitted to different rain frequencies, and result of the Kruskal-Wallis test and Dunn's posteriori.

	Treatment I	Treatment II	Treatment III	Kruskal-Wallis	
				H	P
Right anterior wing length	25,49 \pm 1,41*	23,58 \pm 1,61*	23,65 \pm 1,82	9,02	p = 0,00
Right posterior wing length	23,85 \pm 1,88*	21,07 \pm 1,70 *	21,79 \pm 1,16	12,00	p = 0,00
Left anterior wing length	25,56 \pm 2,83*	23,21 \pm 1,67*	23,85 \pm 1,26	9,35	p = 0,00
Left rear wing length	23,56 \pm 1,39*	21,15 \pm 1,86*	21,4 \pm 1,60	12,42	p = 0,00

(*) significant difference between Treatments. (Treatment I: control, no rain; Treatment II: rain every 10 days and Treatment III: rain every 5 days)

DISCUSSION

The distribution of living organisms is strongly influenced by both biotic and abiotic factors. The climate is one of the main factors responsible for the regulation of this distribution. In the *Cerrado* biome, which is marked by two well-defined seasons (one dry and one rainy), seasonality

guides this process (WOLDA 1988; PINHEIRO *et al.*, 2002). For antlions of the species *Myrmeleon brasiliensis*, which depend on dry soil for their foraging, the variation in the seasonality of the *Cerrado* (greater or small incidence of rain) exerts an influence on trap-building behavior, larval development and the size of adults.

Antlion larvae generally depend on dry soil for building their traps. However, species of the tribe Myrmeleontini, such as *Myrmeleon formicarius* Linnaeus, 1767 (Neuroptera: Myrmeleontidae) and *Euroleon nostras* (Geoffroy in Fourcroy, 1785) (Neuroptera: Myrmeleontidae), have been observed building traps on the edges of lakes, dams and puddles, where moisture is high (ELIMELECH & PINSHOW, 2008; BOZDOĞAN & SATAR, 2017). For *M. brasiliensis*, trap building only takes place in dry soil and rainfall destroys the traps. Indeed, no larvae were found foraging in the rainy period of the *Cerrado* when the soil was waterlogged (February, March and April). FREIRE & LIMA (2019) also found few larvae foraging in the rainy period, with no larvae observed building traps in the month of January.

In the natural environment, habitat selection by antlion larvae occurs in response to diverse factors, such as the tossing of sand by a neighboring larva, the offer of prey items, protection of the trap from rain and cannibalism among larvae (BARKAE *et al.*, 2014; DEVETAK & ARNETT, 2015; LIMA *et al.*, 2019; DAY & ZALUCKI, 2000). According to GOTELLI (1993), habitat selection by *Myrmeleon crudellis* Walker, 1853 and *Myrmeleon immaculatus* DeGeer, 1773 depends mainly on protection from the rain, which not only destroys the traps, but the saturation of the soil impedes the trap from being immediately rebuilt. As a response, antlion larvae are found building their traps under plantlets and fallen tree trunks, which ensure protection from light rain. LIMA (2020) found *M. brasiliensis* larvae foraging throughout the year in protected locations, such as under the slope of rock formations, where the rain cannot destroy the trap.

In the present laboratory experiments, the larvae moved to build new traps when dry sand was available around the traps. In the natural environment, the soil becomes waterlogged with the occurrence of more severe rain, which would impede this movement. However, such movements may occur in situations of light rain when dry soil can be found in the surrounding area. Moreover, we found that the distance at which the new trap is built is positively associated with the size of the larvae. Indeed, such movements occur with greater frequency among larger larvae, which have a larger energy reserve, enabling the search for more favorable areas (SCHARF *et al.*, 2008; LIMA *et al.*, 2019).

Movement because of rain has also been observed in the species *Myrmeleon carolinus* Banks, 1943, found in clay soil (LUCAS, 1989). In contrast, rain as an isolated factor did not exert an influence on the movement of larvae of the species *Myrmeleon immaculatus* De Geer, and *Myrmeleon crudelis*, but the combination rain and a high temperature caused movement (GOTELLI, 1993). A different strategy was found for other species of *Myrmeleon* Linnaeus, 1767 and *Brachynemurus* Hagen 1888, which also build traps in the soil; the larvae dig centimeters beneath the surface and diminish their metabolism until the soil becomes suitable for rebuilding the trap (SCHARF & OVADIA, 2006).

A greater frequency of rain caused an increase in the mortality of *M. brasiliensis* larvae and those that survived

demonstrated an accelerated development process. Thus, although the increase in the frequency of rain affected mortality, it did not eliminate all individuals in the population, which indicates that *M. brasiliensis* larvae have a seasonal bivoltine life cycle, with slight generation overlap and under the constant pressure of mortality (WOLDA, 1988; KISHIMOTO-YAMADA & ITIOKA, 2015).

The frequency of rain also affected the size of the traps built by *M. brasiliensis* larvae. Despite the positive relation between trap diameter and length of the body and head capsule in *Myrmeleon* Linnaeus, 1767 larvae (YOUTHED & MORAN, 1969; FARIA *et al.*, 1994), the increase in the frequency of rain induces a reduction in trap size. *Myrmeleon* larvae spend more energy rebuilding their traps than maintaining them with the original diameter (GRIFFITHS, 1980; LUCAS, 1986). In the face of constant disturbance, the larva loses biomass every time that it invests in the trap. If it is not able to accumulate sufficient fat during the next foraging, the tendency is for the trap to be rebuilt with a smaller size. Nonetheless, it is fundamental for the larvae to maintain the architecture of the trap in order to catch prey items (LUCAS, 1985; GRIFFITHS, 1986; FARIA *et al.*, 1994; VENNER *et al.*, 2003; ALCALAY *et al.*, 2014).

Regarding the size of the adults, the increase in the frequency of rain to which the *M. brasiliensis* larvae were submitted led to the emergence of smaller adults. It is possible that the energy expenditure with the constant rebuilding of the traps resulted in smaller adults. As all individuals in the experiment were second-instar larvae submitted to the same feeding pattern, these factors can be discarded when seeking to explain the difference in the body size of the adults among the treatments. The opposite was found for *Myrmeleon hyalinus* Olivier, 1811, which is a species occurring in moist soil, as precipitation favors the increase in body size in this species due the increase in the quantity of prey insects, which facilitates foraging (SCHARF *et al.*, 2009).

Body size in insects can vary as a function of temperature, food resources and altitude. Moreover, this characteristic is positively related to the fecundity of females (CHOWN & KLOK, 2003; KANEGAE & LOMÔNACO, 2003). Thus, the variation in body dimensions can affect the size of a population, as it is directly related to the reproductive potential of females (SOKOLOVSKA *et al.*, 2000; HESPENHEIDE, 2006; MILANO *et al.*, 2010). Smaller adults may have their fitness and reproductive success affected over generations.

In seasonal environments, organisms exhibit behavioral responses to variations in the climate and landscape. In the case of the *Cerrado* biome, changes between the dry and rainy seasons affect the larvae of *M. brasiliensis*, which are found foraging in greater abundance in the dry season. Moreover, the laboratory experiments demonstrated that changes in the frequency of rain can affect mortality, larval development, trap size and the size of the adults. Thus, variations in the rainfall pattern can lead to variations in characteristics of the population structure of *M. brasiliensis* in the *Cerrado*.

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