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# *Diaspis echinocacti* (Hemiptera: Diaspididae) on cactus pear cladodes: biological aspects at different temperatures

## *Diaspis echinocacti* (Hemiptera: Diaspididae) em cladódios de palma forrageira: aspectos biológicos em diferentes temperaturas

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**Abstract**

The scale mealybug, *Diaspis echinocacti* (Bouché, 1833) (Hemiptera: Diaspididae), is one of the main pests of the cactus pear in Brazil. The objective was to study biological aspects of *D. echinocacti* at the constant temperatures of 25, 28, 30, 33 and 35 °C with relative humidity of 60 ± 10% and photoperiod of 12 hours in the laboratory on the cactus pear cultivar, "Orelha de Elefante Mexicana", *Opuntia stricta* [Haw.] Haw. The development period (22 to 35 days) and survival in the egg (92 to 100%) and nymph (21.8 to 100%) stages and of the egg-adult cycle (20 to 100%), longevity (34.1 to 59.6 days) and fecundity (33 to 112 eggs) of *D. echinocacti* females with the different temperature and absence of males at the highest temperatures (> 30°C), indicated that the range between 25 °C and 30°C is the most favorable for this scale mealybug. This information may help to improve integrated management programs for *D. echinocacti*, in areas subject to seasonal temperature changes in the Brazilian regions where cactus pear is cultivated.

**Keywords:** development temperature, insect-pest, *Opuntia stricta*.

**Resumo**

A cochonilha-escama, *Diaspis echinocacti* (Bouché, 1833) (Hemiptera: Diaspididae), é uma das principais pragas da palma forrageira no Brasil. O objetivo foi estudar aspectos biológicos de *D. echinocacti* nas temperaturas constantes de 25, 28, 30, 33 e 35 °C, umidade relativa de 60 ± 10% e fotoperíodo de 12 horas em laboratório na cultivar de palma forrageira, "Orelha de Elefante Mexicana", *Opuntia stricta* [Haw.] Haw. O período de desenvolvimento (22 a 35 dias) e a sobrevivência nas fases de ovo (92 a 100%) e ninfa (21,8 a 100%) e o ciclo ovo-adulto (20 a 100%), longevidade (34,1 a 59,6 dias) e fecundidade (33 a 112 ovos) de fêmeas de *D. echinocacti* nas diferentes temperaturas e ausência de machos nas maiores (> 30°C) indicam ser a faixa entre 25°C e 30°C a mais favorável para esta cochonilha de escama. Essas informações podem auxiliar no aprimoramento de programas de manejo integrado de *D. echinocacti* em áreas sujeitas a variações sazonais de temperatura nas regiões brasileiras onde a palma forrageira é cultivada.

**Palavras-chave:** temperatura de desenvolvimento, inseto-praga, *Opuntia stricta*.

## 1. Introduction

Semi-arid regions of Africa, Americas, Asia and Oceania are 37% of the world's land (Huang et al., 2016; Golla, 2021) with 969,589.4 km$^2$ in Argentina, Chile, Colombia, Ecuador and Venezuela (Rossato et al., 2017) and in 1,136 municipalities of eight states in the Northeast region and in the north of the Minas Gerais state in Brazil (Rossato et al., 2017).

The caatinga biome, vegetation native to Brazil with high temperatures, unstable rains, prolonged droughts and unfavorable geological structures to accumulate underground water, characterizes this semi-arid region (Barbosa et al., 2015; Barbosa and Kumar, 2016; Marengo et al., 2017). This reduces the production of forage plants and difficult maintaining and raising cattle, goat and sheep (Moraes et al., 2019).

Plant species adapted to semi-arid regions, such as the forage palms *Opuntia* spp. and *Nopalea* spp. (Cactaceae), can increase the viability of raising livestock (Silva et al., 2015, Gusha et al., 2015). Forage palms are native to the Americas and cultivated in different countries (Miranda-Romero et al., 2018; Perrotta and Arambarri, 2018) in arid and hot environments with reduced cost and food "in natura" or in silage for animals (Gusha et al., 2015). The uniform harvest of cactus pear increases regrowth

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and productivity, reduces costs and the use of labor, and enables the constant production of silage for animals confined during the dry season (Souza et al., 2020).

The scale mealybug, *Diaspis echinocacti* (Bouché, 1833) (Hemiptera: Diaspididae), is one of the main pests of cactus pears in Brazil, infesting the cladodes and sucking the sap of these plants (Oetting, 1984). Yellowing of the cladode racquets followed by wilting, rotting and its death by microorganisms penetrating the lesions caused by mealybug feeding characterize damage to the giant cactus pear (*Opuntia ficus*-indica) as reported in the states of Alagoas, Bahia, Ceará, Paraíba, Pernambuco, Rio Grande do Norte and Sergipe, Brazil (Lima and Gama, 2001; Claps et al., 2001). The effect of the temperature on the life cycle, population dynamics and spatial and seasonal distribution of the scale mealybug *D. echinocacti*, in the main cactus pear producing regions of Brazil, needs to be studied (Bayindir and Birgücü, 2016; Oliveira et al., 2019).

The objective was to study biological aspects of *D. echinocacti* at the constant temperatures of 25, 28, 30, 33 and 35 °C, relative humidity of 60 ± 10% and photoperiod of 12 hours, in the laboratory, on cladodes of the cactus pear cultivar "Orelha de Elefante Mexicana" *Opuntia stricta* [Haw.] Haw.

## 2. Material and Methods

### *2.1. Study location*

This study was carried out at the entomology laboratory (7º 13'32"S latitude and 35º 54'19" W longitude) of Embrapa Algodão in the municipality of Campina Grande, Paraíba state, Brazil in 2021.

### *2.2. Insects and plants*

The females of the scale mealybug *D. echinocacti* were collected on cactus pear cladodes of the cultivar "Orelha de Elefante Mexicana", *Opuntia stricta* [Haw.] Haw. at 90 days after infestation in a plantation at the experimental field of Embrapa Algodão (7º13'50"S latitude and 35º52'52"W longitude) in the municipality of Campina Grande, Paraíba state, Brazil. The cladodes of this plant were collected and packed in Kraft paper bags, placed in a Styrofoam box and taken to the Entomology laboratory at Embrapa Algodão and kept in climate-controlled chambers until the beginning of the bioassay.

### *2.3. Biology at different temperatures*

Freshly eggs (≤ 24 h) laid by *D. echinocacti* females were submitted to constant temperatures of 25, 28, 30, 33 and 35 °C, relative humidity of 60 ± 10% and a photoperiod of 12 hours representing the treatments. Each experimental unit had a cladode disk of the cactus pear cultivar "Orelha de Elefante Mexicana" (9.62 $cm^2$) infested with 10 eggs of *D. echinocacti*, recently laid (≤ 8 hours), with five replications per temperature (treatment) in climatized chamber and observed daily. The total number of nymphs hatched per temperature and egg incubation and development periods for this pest were recorded.

Newly emerged and mobile first-instar *D. echinocacti* nymphs were placed on non-infested cladodes (25 x 15 cm with an ellipse area of 294.4 $cm^2$) in plastic trays (46.5 cm x 29.6 cm x 7.5 cm in length, wide and height, respectively) to evaluate the nymph stage of this scale mealybug per temperature. The epidermis of the cladode, next to the nymph attached to it, was numbered with blue ink to facilitate observing the sessile nymph. The sectioned part of the cladode was wrapped in a cotton swab soaked in distilled water and moistened every two days to reduce water losses. *Diaspis echinocacti* nymphs were divided into five replications with the nearest 10 sessile being each one. These nymphs were observed daily using an EL224 stereomicroscope (BEL Engenharia, Monza, Milan, Italy) with a 20x magnification until the emergence of *D. echinocacti* adults.

The *D. echinocacti* stages were determined according to the external morphology and the period between one molt to the next. *Diaspis echinocacti* eggs, deposited under the armor of the female of this insect, are initially clear and translucent and, near hatching, yellowish (Figure 1A). First-instar nymphs are easily recognized because they are mobile with legs, a pair of red eyes and visible antennae (Figure 1B) and those of the second-instar with marked sexual dimorphism, as female carapaces ou armor increase in diameter (Figure 1C). A posterior extension projecting under it identifies males (Oetting, 1984). Male pupae are white with an elongated oval shape, covered by white mucoprotein filaments (Figure 1D). *D. echinocacti* females are neotenic with white to light yellow color (Figure 1E) and similar to their second-instar nymphs, but differing in size and sexual maturity, while males are winged (Figure 1F). Eggs deposited on cactus pear cladodes were daily quantified at 8:00 A.M. and 04:00 P.M.

The development period, egg viability and nymph and pupae survival, duration of egg-to-adult (immature stage) and longevity of adults and fecundity of *D. echinocacti* females were determinate.

### *2.4. Data analysis*

Survival data per stage and from egg to adult of *D. echinocacti* were transformed into $1/\sqrt{x}$ and those of the development into log (x) (Box and Cox, 1964) and submitted to a two-way analysis of variance (ANOVA) with the averages compared by Tukey's test (P= 0.05) using the Statistical and Genetic Analysis System (Ribeiro Júnior, 2001).

## 3. Results

The interaction of temperature with the survival of eggs, nymphs and in the period of egg-adult of *D. echinocacti* was significant and negative (Table 1).

Survival of *D. echinocacti* females ranged from 52 to 100% at all temperatures and that of males was 100% at 25ºC, 28ºC and 30ºC. These values were higher at the temperature of 28ºC for the former and between 25ºC and 30ºC for the latter (Table 2).

The duration of the egg and nymph stages and egg-adult periods of *D. echinocacti* females, fed on cladodes cultivar "Orelha de Elefante Mexicana", *Opuntia stricta* [Haw.] Haw., decreased, from 2.50 to 4.04, 19.37 to 31.03 and 22.10 to 35.01 days, respectively, between temperatures of 35º C and 25º C. Most individuals emerged between 25º C and 30º C were females and the duration of the period from egg to adult of males ranged from 28.3 to 36.0 days (Table 3).

The periods of pre-oviposition, oviposition and post-oviposition of *D. echinocacti* females, fed on cladodes, were longer and shorter at 25º C and 33 ºC, respectively. (Table 4).

The longevity of *D. echinocacti* females between 25º C and 35º C decreased from 58.2 to 21.3 days, being longer than that of males at the temperatures of 25ºC, 28ºC and 30ºC. (Figure 2).

The total number of eggs and eggs/female/day of *D. echinocacti* was greater and lower at 25º C and 33º C (Table 4). Females of this insect did not lay eggs at 35°C.

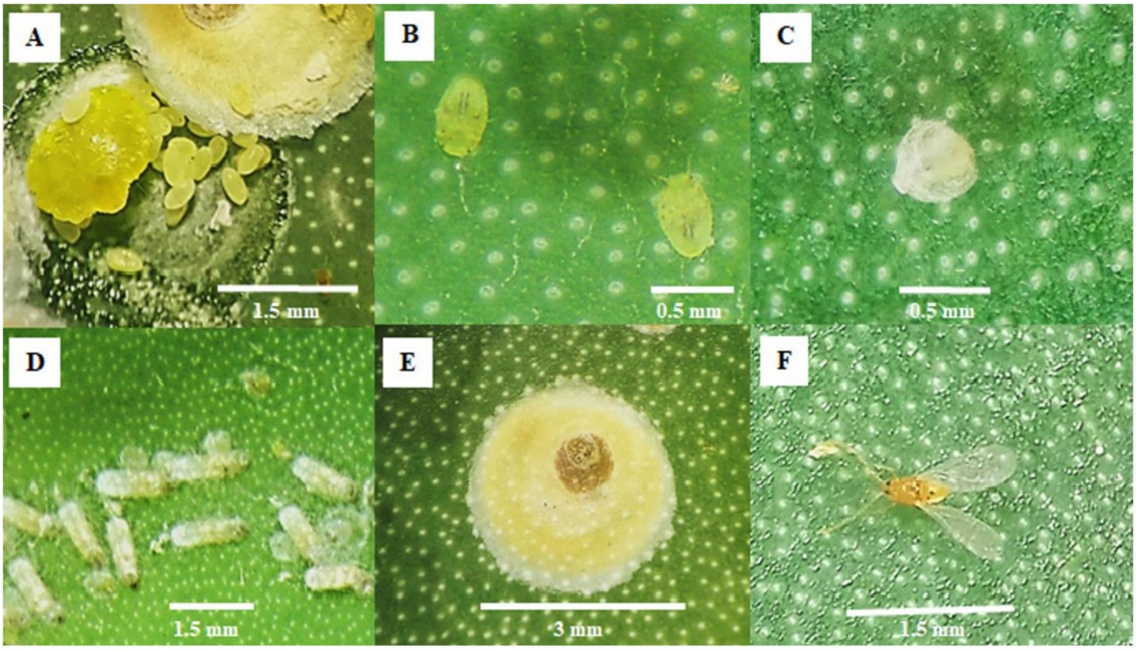


**Figure 1.** Immature stages of *D. echinocacti* (Bouché) (Hemiptera: Diaspididae) on cladodes of the cactus pear “Orelha de Elefante Mexicana” *Opuntia stricta* [Haw.] Haw. with the egg mass next to the female with the carapace removed (A), mobile nymph of the first (B) and sessile of the second (C) instar, male pupa (D), female with carapace (E) and winged male (F).

**Table 1.** Summary models of the effects of temperatures of 25 ºC, 28 ºC, 30 ºC, 33 ºC and 35 ºC on survival[1] and development period[2] of the cochineal *Diaspis echinocacti* (Bouché) (Hemiptera: Diaspididae) using two-way analysis of variance (ANOVA).

| Sexo | Source | Model | DF | *F* | *P* |
|---|---|---|---|---|---|
| Female | Survival | Temperature (T) | 4 | 610.65 | < 0.001 |
| | | Stage (S) | 2 | 377.31 | < 0.001 |
| | | T x S | 8 | 312.83 | < 0.001 |
| | Development | Temperature (T) | 4 | 791.03 | < 0.001 |
| | | Stage (S) | 2 | 50.687.19 | < 0.001 |
| | | T x S | 8 | 5.58 | < 0.001 |
| Male | Survival | Temperatura (T) | 2 | - | n.s |
| | | Estágio (S) | 2 | - | n.s |
| | | T x S | 4 | - | n.s |
| | Development | Temperature (T) | 2 | 45.72 | < 0.001 |
| | | Stage (S) | 3 | 2.158.41 | < 0.001 |
| | | T x S | 6 | 2.51 | = 0.050 |

[1]Survival: data transformed into 1/root(x). [2]Development: data transformed into log(x). DF= Degree of freedom. *F*= Fisher's test. *P*= Probability.

**Table 2.** Survival (Mean ± SE ) during egg, nymph and pupa stages and from egg to adult of *Diaspis echinocacti* (Bouché) (Hemiptera: Diaspididae) on cladodes of the cactus pear "Orelha de Elefante Mexicana", *Opuntia stricta* [Haw.] Haw. at the temperatures (Temp.) of 25°C, 28°C, 30°C, 33°C and 35°C, relative humidity of 60 ± 10% and photophase of 12 hours.

| **Stage/ Life period** | **Temp. (°C)** | **Survival (%)** | | | |
|---|---|---|---|---|---|
| | | **Female** | **N** | **Male** | **N** |
| Egg | 25 | 95.78 ± 2.59 Aa | 41 | 100.00 ± 0.00 Aa | 03 |
| | 28 | 100.00 ± 0.00 Aa | 48 | 100.00 ± 0.00 Aa | 02 |
| | 30 | 100.00 ± 0.00 Aa | 48 | 100.00 ± 0.00 Aa | 03 |
| | 33 | 96.00 ± 2.45 Aa | 48 | - | 00 |
| | 35 | 92.00 ± 2.00 Aa | 46 | - | 00 |
| Nymph | 25 | 91.55 ± 5.18 Abc | 41 | 100.00 ± 0.00 Aa | 03 |
| | 28 | 100.00 ± 0.00 Aa | 48 | 100.00 ± 0.00 Aa | 02 |
| | 30 | 96.00 ± 4.00 Aa | 45 | 100.00 ± 0.00 Aa | 03 |
| | 33 | 54.22 ± 5.10 Bbc | 26 | - | 00 |
| | 35 | 21.78 ± 3.54 Bc | 10 | - | 00 |
| Pupa | 25 | - | 00 | 100.00 ± 0.00 Aa | 03 |
| | 28 | - | 00 | 100.00 ± 0.00 Aa | 02 |
| | 30 | - | 00 | 100.00 ± 0.00 Aa | 03 |
| | 33 | - | 00 | - | 00 |
| | 35 | - | 00 | - | 00 |
| Egg-adult | 25 | 87.33 ± 3.97 Aa | 41 | 100.00 ± 0.00 Aa | 03 |
| | 28 | 100.00 ± 0.00 Aa | 48 | 100.00 ± 0.00 Aa | 02 |
| | 30 | 96.00 ± 4.00 Aa | 45 | 100.00 ± 0.00 Aa | 03 |
| | 33 | 52.00 ± 4.90 Bb | 26 | - | 00 |
| | 35 | 20.00 ± 3.16 Bc | 10 | - | 00 |

Means followed by the same capital letter between stages and temperature or lowercase per stage at different temperatures, respectively, do not differ by Tukey's test at 5% probability. N= number of surviving individuals per stage and temperature.

**Table 3.** Duration (Mean ± SE) of egg, nymph and pupa stages and egg to adult of *Diaspis echinocacti* (Bouché) (Hemiptera: Diaspididae) on cladodes of cactus pear "Orelha de Elefante Mexicana", *Opuntia stricta* [Haw.] Haw. at temperatures (Temp.) of 25°C, 28°C, 30°C, 33°C and 35°C, relative humidity of 60 ± 10% and photophase of 12 hours.

| **Stage/ Life period** | **Temp. (°C)** | **Duration of development (days)** | | | |
|---|---|---|---|---|---|
| | | **Female** | **N** | **Male** | **N** |
| Egg | 25 | 4.04 ± 0.07 Ca | 41 | 3.33 ± 0.33 Ca | 03 |
| | 28 | 3.50 ± 0.04 Cb | 48 | 3.00 ± 0.00 Ca | 02 |
| | 30 | 2.78 ± 0.05 Cc | 48 | 3.00 ± 0.00 Ca | 03 |
| | 33 | 2.69 ± 0.05 Cc | 48 | - | 00 |
| | 35 | 2.50 ± 0.03 Cd | 46 | - | 00 |
| Nymph | 25 | 31.03 ± 0.15 Ba | 41 | 19.67 ± 0.88 Aa | 03 |
| | 28 | 28.35 ± 0.13 Bb | 48 | 17.50 ± 0.35 Ab | 02 |
| | 30 | 24.16 ± 0.25 Bc | 45 | 16.33 ± 0.88 Ab | 03 |
| | 33 | 20.57 ± 0.27 Bd | 26 | - | 00 |
| | 35 | 19.37 ± 0.29 Be | 10 | - | 00 |
| Pupa | 25 | - | 00 | 13.00 ± 0.00 Ba | 03 |
| | 28 | - | 00 | 9.50 ± 0.35 Bb | 02 |
| | 30 | - | 00 | 9.00 ± 0.00 Bb | 03 |
| | 33 | - | 00 | - | 00 |
| | 35 | - | 00 | - | 00 |
| Egg-adult | 25 | 35.01 ± 0.20 Aa | 41 | 36.00 ± 1.00 Aa | 03 |
| | 28 | 31.88 ± 0.16 Ab | 48 | 30.00 ± 0.00 Ab | 02 |
| | 30 | 29.93 ± 0.25 Ac | 45 | 28.33 ± 0.88 Ab | 03 |
| | 33 | 23.25 ± 0.26 Ad | 26 | - | 00 |
| | 35 | 22.10 ± 0.19 Ae | 10 | - | 00 |

Means followed by the same capital letter between stages at the same temperature or lower case per stage at different temperatures do not differ by Tukey's test at 5% probability. N= number of surviving individuals per stage and temperature.

**Table 4.** Reproductive parameters (mean ± standard error) of *Diaspis echinocacti* (Bouché) (Hemiptera: Diaspididae) on cladodes of the cactus pear "Orelha de Elefante Mexicana", *Opuntia stricta* [Haw.] Haw. at temperatures of 25° C, 28º C, 30°C and 33°C, relative humidity of 60 ± 10% and 12-hour photophase.

| Reproductive parameter | Temperature (°C) | | | |
|---|---|---|---|---|
| | 25 | 28 | 30 | 33 |
| Pre-oviposition | 9.40 ± 0.27 bA | 6.70 ± 0.15 cBC | 5.50 ± 0.31 bBC | 5.10 ± 0.23 bC |
| Oviposition | 40.10 ± 0.48 aA | 35.00 ± 0.54 aB | 27.90 ± 0.67 aC | 18.70 ± 0.26 aD |
| Post-oviposition | 10.20 ± 0.33 bA | 8.30 ± 0.21 bB | 6.60 ± 0.27 bC | 4.90 ± 0.23 bD |
| Total eggs | 112.00 ± 2.34 a | 90.00 ± 1.61 B | 71.00 ± 1.15 C | 33.00 ± 0.82 D |
| Number of eggs/day | 3.91 ± 0.06 A | 3.65 ± 0.05 B | 3.63 ± 0.06 B | 2.87 ± 0.05 C |

Means followed by the same lowercase letter in the column or uppercase in the row do not differ by Tukey's test at 5% probability.

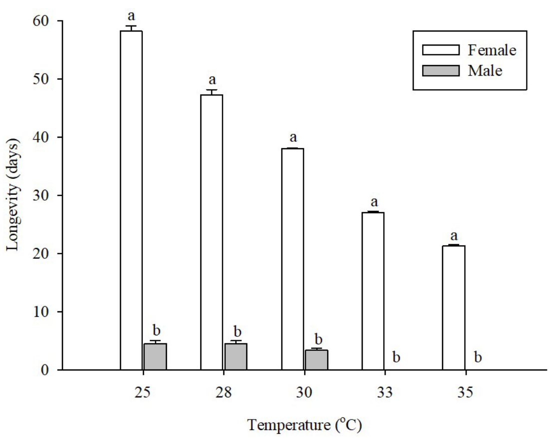


**Figure 2.** Longevity of *D. echinocacti* (Bouché) (Hemiptera: Diaspididae) females and males on cladodes of the cactus pear "Orelha de Elefante Mexicana", *Opuntia stricta* [Haw.] Haw. at the temperatures of 25°C, 28°C, 30°C, 33°C and 35°C, relative humidity of 60 ± 10% and 12-hour photophase. Bars followed by the same lowercase letter per treatment do not differ by Tukey's test, at 5% probability.

## 4. Discussion

The significant and negative interactions between the temperature and survival of *D. echinocacti* in the egg, nymph and egg-adult stages indicate a decrease in this parameter with increasing temperature. This may be related to changes in enzymatic activity denaturizing proteins that regulate process of the development and survival of this scale mealybug at higher temperatures (Sreedevi et al., 2013; Shi et al., 2012). The lack of observation of this effect on male survival may be due to the reduced number of its individuals emerged.

The greater survival of *D. echinocacti* females at 28º C and of males at 25 ºC and 30 ºC indicates that these temperatures are ideal for this insect, as reported for *Aonidiella aurantii* (Maskell, 1879) (Hemiptera: Diaspididae) between 23 ºC and 27 ºC on butternut squash (Mohammed et al., 2020).

The shorter duration of egg and nymph and from egg-adult stage periods of *D. echinocacti* females fed with cladodes of the forage cactus *O. stricta* as the temperature increased is due to the inability of insects to regulate their internal temperature with the environment. The development of most individuals of this insect, composed of females at all temperatures and their males only between 25 ºC and 30 ºC can be attributed to changes in the sex ratio of the progeny, common in Coccidae species (El-Awady et al., 2021). The shorter period of egg-nymph and egg-adult stages of *D. echinocacti* females and males fed with cactus pear cladodes as the temperature increased was expected, as this parameter is inversely correlated for poikilothermic organisms, such as insects (Van Der Meer, 2021). Temperature increase accelerate metabolism and, consequently, movement, fecundity, population size and geographic distribution, but reduces longevity of insects (Hamblin et al., 2017; Just and Frank, 2020). The incubation period of *D. echinocacti* eggs at 25 °C and 28 °C was similar to the 2.9 days and 3.7 days for this mealybug on *Opuntia* sp. in a climate-controlled chamber (27 ºC) and in a greenhouse (26.5º C) (Oetting, 1984). However, the nymph and pupa periods of males of this insect, at 25 °C and 28 °C were longer, respectively, than the 19.8 days and 21.2 days and 7.6 days and 8.1 days for this mealybug with *Opuntia* sp. in acclimatized chamber (27 ºC) and greenhouse (26.5 ºC), respectively (Oetting, 1984). Biological parameters of *D. echinocacti* on cactus pears vary with temperature and the species or cultivar of these plants.

The longer and shorter pre-oviposition, oviposition and post-oviposition periods of *D. echinocacti* females at 25 ºC and 33 ºC reduce the reproduction of this scale mealybug at the first temperature (Beardsley Junior and Gonzalez, 1975). The pre-oviposition and oviposition periods of *D. echinocacti* at 25 ºC were shorter than the 15 and 36 days with cactus pear in a greenhouse (26.5 ºC), but shorter and similar at 28 ºC, respectively, for this insect in a climate-controlled chamber (27 ºC) (Oetting, 1984). This is possibly due to the chemical defenses of the cactus pear, against alkaloids, flavonoids, phenols and saponins, with concentrations varying between cactus species (Pooja and Vidyasagar, 2016; El Aalaoui and Sbaghi, 2022).

The longer longevity of females than males of *D. echinocacti* is typical of species of this cochineal family and is due to the fact that the mouthparts of the latter are not functional, preventing them from feeding and, therefore, reducing their survival to a few days (Beardsley Junior and Gonzalez, 1975).

The highest and lowest total numbers of eggs and eggs/female/day of *D. echinocacti* at 25 ºC and 33 ºC, respectively, indicate that the latter temperature is inadequate for oviposition of this scale mealybug as reported for *Aspidiotus nerii* Bouché (Homoptera: Diaspididae) females fed potato with 99.7 and 55.1 eggs per female at 24 ºC and 28 ºC (Gerson and Hazan, 1979) and *A. aurantii* on butternut squash with 109.7 and 129.4 eggs per female at 23°C and 27°C, respectively (Mohammed et al., 2020).

The inverse correlation between the development periods of *D. echinocacti* females and males with temperature confirms impact of this parameter on males and females of this insect (Li et al., 2017). In addition, the lack of development of males, between 30º C and 35º C, is probably due to sexual dimorphism of this scale mealybug, with these developing in a similar way to holometabolous insects and, therefore, more vulnerable to higher temperatures (Mathenge et al., 2009). The waxy carapace, over the body, increases protection against high temperatures and desiccation, facilitating the development of *D. echinocacti* females (Beardsley Junior and Gonzalez, 1975; Mitov et al., 2018).

The results obtained are important to improve integrated management programs to reduce population outbreaks of *D. echinocacti* in areas subject to seasonal temperature changes in the Brazilian regions where cactus pear is cultivated, especially in the current scenario of global warming.

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