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SEASONAL BIOLOGY OF DACTYLOPIUS OPUNTIAE (HEMIPTERA: DACTYLOPIIDAE) ON OPUNTIA FICUS-INDICA (CARYOPHYLLALES: CACTACEAE) UNDER FIELD AND SEMI FIELD CONDITIONS

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ABSTRACT

Dactylopius opuntiae is the most important pest of cactus (*Opuntia ficus-indica)* around the world. Recently the scale-supping pest have become an increasing threat to the cultivation of prickly pear in many Mediterranean areas. The life cycle of *D. opuntiae* was carried out under field and semi-field in Winter-Spring and Spring-Summer Morocco conditions. The results showed that the duration of life cycle of *D. opuntiae* under field and semi-field conditions was temperature dependent**.** It was long (133.52±3.82 days) in Winter-Spring when temperature was between 2.3°C and 25°C than in Spring-Summer (127.85±2.15 days) when the temperature was between 13.5°C and 47°C. Duration of male life cycle was significantly long under field conditions in Winter-Spring (74.57±4.05 days) and short (37.10±3.0 days) under semi-field conditions in Spring-Summer. For the duration of female life cycle it was significantly long (133.52±3.82 days) under field conditions in Winter-Spring, and no significant difference was observed between field conditions in Spring-Summer, semi-field conditions in Spring-Summer, and semi-field conditions in Winter-Spring. The effect of the availability of food on reproduction and population growth parameters was also evaluated under laboratory conditions at 25 ±2°C and the results indicated that the females pre-oviposition, and post-oviposition periods were significantly long without food than in the presence of food, however oviposition period, females longevity, fecundity, fertility, and growth index were significantly higher in the presence of food than without food. Also the net reproductive rate (R_0), and generation time

(T) were significantly higher for females with food, and no significant difference was observed between females with food and females without food for intrinsic rate of natural increase (r_m), doubling time (DT), and finite rate of increase (λ).

Keywords: *Dactylopius opuntiae,* prickly pear cactus, life history, population growth parameters

1. INTRODUCTION

Since its introduction to the Mediterranean areas where the climate was suitable for cactus development, *Opuntia ficus indica* became a most important species of landscape as natural or cultivated crops (Ochoa & Barbera, 2017).

Recently *Dactylopius opuntiae* (Cockerell) (Hemiptera: Dactylopiidae) have become an increasing threat to the cultivation of prickly pear crops in Morocco and other Mediterranean countries. *D. opuntiae* feeds directly on the plant causing chlorosis and premature dropping of cladodes and fruits (Badii & Flores, 2001). Severe infestations inferior of 75 % of the cladode surface can result in death of the plant (Vanegas-Rico *et al*., 2010, 2015). In Brazil, the damage caused by *D. opuntiae* on *O. ficus indica* used as forage resulted in the loss of 100,000 ha, valued at 25 million US dollars (Lopes *et al*., 2009). In Mexico, damage to fruit and nopalitos (edible young Opuntia cladodes) caused by this scale resulted in lower yields and higher production costs (Badii & Flores, 2001; Portillo & Vigueras, 2006). In Morocco the attack was fast and unpredictable, and consequently, the destruction of large areas planted with cactus knows a fulgurating and dangerous extension in several basins of production (Bouharroud *et al*., 2016; El Aalaoui *et al*., 2019a). The scale pest was also recorded in other Mediterranean countries such as Israel, Lebanon and Jordan (Spodek *et al*., 2014; Moussa *et al*., 2017; Bader *et al*., 2019).

Natural enemies associated to *D. opuntiae* and other Dactylopiidae species include only predators (Baskaran *et al*., 1999; Adalma-Aguilera *et al*., 2005; Vanegas-Rico *et al*., 2010; Castro, 2011; Lima *et al*., 2011; Barbosa *et al*., 2014; Giorgi *et al*., 2017; Bouharroud *et al*., 2019, El Aalaoui *et al*., 2019b) including coleoptera (coccinellidae), dipetra and lipedoptera.

Although *D. opuntiae* is a great devastator of prickly pear crops around the word, the scientific data available about its biology under field conditions is lacking. Many studies found in the literatures were carried out under laboratory conditions (Griffith, 2004; Flores-Hernández *et al*., 2006; Portillo & Vigueras, 2008; Mathenge *et al*., 2009; Silva *et al*., 2013; Luna *et al*., 2018). Generally the fecundity of the genus *Dactylopius* determined on *Opuntia* cladodes (Moran & Cobby, 1979;

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Sullivan, 1990; Luna *et al*., 2018) or by removing the insects from the cladodes to Petri dish (Flores-Hernández *et al*., 2006; Mathenge *et al*., 2009; Luna *et al*., 2018).

The objective of this study was to investigate the seasonal biology of *D. opuntiae* under field and semi-field conditions in Morocco considering the availability of food (presence or absence of cactus cladode)

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in the experimental field of the National Institute of Agronomic Research (INRA- Morocco) in 2017 and 2018 crop seasons. This area is located at 40 km from the Atlantic Ocean (N 32° 21 W8° 22) and 168 m altitude. The site is considered in the semi-arid ecological zone. The rainfall varies from 112.6-607 mm/year and the annual average of last 30 years is 330 mm. Temperature varies from -1°C (Dec-Jan) to 40-45 °C (July-August). The soil has a clay-sand-silty hydromorphic structure with an alkaline PH (Khattabi *et al*., 2004). The climate data were collected using iMetos electronic weather station (Pessl Instruments, GMBH).

2.2 Life history of D. opuntiae under field conditions

To study the biology of *D. opuntiae* under field conditions, 200 plants (1year old) of *Opuntia ficus-indica* susceptible to *D. opuntiae* were planted in a plot of a half hectare which was made available for the study of the biology of the cactus cochineal scale. The plants were infested with one-day-old first instar *D. opuntiae* nymphs (crawlers) (n= 30), which were permitted to settle before being adjusted to the appropriate densities. Daily observations were made on the developing insects, the observations include pre-oviposition, oviposition and post-oviposition periods. For males, their longevity on the cladodes was recorded therefore the plants were sealed at each end with plastic sealing around the base of the cladode to prevent loss of males from the plants (n= 60) (Moran & Cabby, 1979). All experiments were replicated twice under different seasonal conditions in Winter-Spring (December to April) and in Spring-Summer (April to August). The field mean, minima and maxima daily temperature were calculated using iMetos electronic weather station (Pessl Instruments, GMBH).

2.3 Reproduction parameters

At gravid female stage (before oviposition), the females (n= 60) were individually removed from cladodes dewaxed by rolling their wax onto a pin, and transferred into separate Petri dish (diameter

9.5 cm) in the laboratory, or keeping the females (n= 60) attached to the cladodes (Moran & Cobby, 1979; Sullivan, 1990; Luna *et al*., 2018), and transferred all to laboratory conditions (25 ±2°C). In each of two methodologies (females separates in Petri dishes or attached to the cladodes), the females were observed daily, so that we could record their longevity, the length of pre-oviposition, oviposition and post-oviposition periods, the fecundity (number of laid eggs), and the fertility (number of eggs with embryos). To determine growth index and sex ratio male:female, 60 crawlers of one day were placed on uninfested cladode and allowed to develop. The experiment was performed 20 times (n= 1200). The growth index was calculated using Howes (1953) formula:

Growth index= Percent of larva pupated / Mean larval duration (days).

2.4 Population growth parameters

For two methodologies used in this study (females attached to the cladodes or transferred into separate Petri dish) The following population growth parameters were estimated: the net reproductive rate ($R0 = \Sigma\, lx\, mx$) , the mean generation time (T), the intrinsic rate of natural increase $rm = lnR0(T) - 1$, the finite rate of increase ($\lambda =$ exp(r)) and the doubling time ($DT = ln2/rm$). The lx corresponds to the age-specific survival of the females and the mx to the age-specific fertility(= born larvae/female) (Southwood & Henderson, 2000; Vasicek *et al*., 2004; Kontodimas & Stathas, 2005).

2.5 Life history of D. opuntiae under semi-field conditions

D. opuntiae was reared and observed on individually potted plants (1 year old) of *O. ficus-indica* susceptible to *D. opuntiae* in the greenhouse. The plants (n= 200) were infested with one-day-old first instar *D. opuntiae* nymphs (crawlers) (n= 30), which were permitted to settle before being adjusted to the appropriate densities. The design and replication of the study were the same as under field conditions. The biology and life history of the cactus cochineal scale were determined using the same method described in the (***Life history of D. opuntiae under field conditions***). To determine the longevity of males, each pots were sealed at end with plastic sealing tape around the base of the cladode to prevent loss of males (Moran & Cabby,1979). All experiments were replicated twice under different seasonal conditions in Winter-Spring (December to April) and in Spring-Summer (April to August). The semi-field mean, minima and maxima daily temperature were calculated from the thermograms, based on 6 measurements made with intervals of 2 h. The night temperature was determined from the 3 lowest daily values (Vinogradova *et al*., 2013).

3. DATA ANALYSIS

The data of life history, reproduction parameters (pre-oviposition, oviposition, post-oviposition, longevity, fecundity, fertility, and growth index), and population growth parameters (*R_0, rm, T, DT, and λ*) with or without food (*O. ficus-indica* cladodes) were performed using the SPSS general linear model (GLM) by SPSS software (IBM SPSS statistics 23) procedure for ANOVA and Tukey's test at a level of $P< 0.05$ (Carver & Nash, 2011).

4. RESULTS AND DISCUSSION

4.1 Life history of D. opuntiae

The duration of life cycle of *D. opuntiae* under field and semi-field conditions was temperature dependent**.** It was significantly long in Winter-Spring when temperature was ranged from 2.3 to 23.7°C under field conditions and 8 to 25°C under semi-field conditions, than in Spring-Summer when the temperature was relatively higher (13.5°C to 44°C under field conditions and 15-47°C under semi-field conditions) (Table 1; Fig 1; Fig 2) ($F= 55.63$, $df= 3$, $p\leq 0.05$). In both conditions of studies, the mature inseminated female of *D. opuntiae* produced eggs that hatched within a short time (inferior to 1 day). The duration of first moult was significantly lengthy under field conditions in Winter-Spring (5-15 days) and shorty under semi-field conditions in Spring-Summer (1-2 days) ($F= 329.16$, $df= 3$, $p\leq 0.05$), and no significant difference ($F= 329.17$, $df = 3$, $p\leq 0.05$) was observed between field conditions in Spring-Summer and under semi-field conditions in Winter-Spring (Table 1). For the duration of second moult, it was significantly long under field conditions in Winter-Spring (53-64 days) and short under semi-field condition in Spring-Summer (13-17 days) ($F= 6915.12$, $df= 3$, $p\leq 0.05$). The duration time from pupation to emergence was significantly long under field conditions in Winter-Spring (16-19 days) and short under field (5-12 days) and semi-field (6-8 days) conditions in Spring-Summer ($F= 1055.47$, $df= 3$, $p\leq 0.05$). Adult male lifespan was not significantly affected by seasons under both conditions ($F= 2.73$, $df= 3$, $p= 0.092$). The mean physiological time from first instar to (i) adult male was significantly long under field conditions in Winter-Spring (59-73 days) and short under semi-field condition in Spring-Summer (19-25 days) ($F= 3623.36$, $df= 3$, $p\leq 0.05$), to (ii) young female was significantly long under field conditions in Winter-Spring (60-72 days), no significant difference was between field conditions in Spring-Summer, and semi-field conditions in Winter-Spring and Spring-Summer ($F= 4161.42$, $df= 3$, $p\leq 0.05$), to (iii) gravid female was significantly long under field conditions in Winter-Spring (113-117 days) and short under semi-field conditions in Spring-Summer (36-51 days) ($F= 6547.63$, $df= 3$, $p\leq 0.05$), no significant difference was observed between field conditions in Spring-Summer, and semi-field conditions in Spring-Summer, and to (iv) first egg production (mature female) was significantly long under field conditions in winter-Spring (115-122 days) and short under semi-field condition in Spring-Summer (47-53 days) (F= 13767.80, df = 3, p≤ 0.05). Male life cycle was significantly long under field conditions in Winter-Spring (65-85 days) and short under semi-field conditions in Spring-Summer (32-43 days) (F= 1758.77, df = 3, p≤ 0.05). For female life cycle it was significantly long under field conditions in Winter-Spring (126-136 days) (F= 55.63, df= 3, p≤ 0.05), and no significant difference was observed between field conditions in Spring-Summer, semi-field conditions in Spring-Summer, and semi-field conditions in Winter-Spring. No production of eggs was observed between December and February under field conditions due probably to a significant temperature decline in those two months. To the best of our knowledge, there is insufficient information available on the biology of *D. opuntiae* under field, and semi-field conditions in the Mediterranean area. In Mexico, Luna *et al*. (2018) reported that the incubation period of *D. opuntiae* was 61.78±24 minutes at 25±1 °C and 40±10 % relative humidity. In order to compare to other *Dactylopius* species, Guerra & Kosztarab (1992) reported that the eggs of *Dactylopius coccus* hatched within 30 min of laying at 25–26°C. *Dactylopius austrinus* eggs hatching took place within 67 min at 26°C (Moran & Cobby, 1979). For *Dactylopius tomentosus,* Mathenge *et al*. (2009) reported an incubation period of 17 days at 26°C. Other *Dactylopius* spp. eggs hatching was occurred within 3–5 h at 26°C accordnig to Karny, (1972) and Sullivan, (1990). The duration of first moult of *D. opuntiae* was listed as 8.59±1.54 days at 25±1°C (Luna *et al*., 2018) compared to 15, 18 and 35 days for *D. ceylonicus*, *D. austrinus* and *D. tomentosus*, respectively, at 26°C (Moran & Cobby, 1979; Sullivan, 1990; Mathenge *et al*., 2009). It is not possible to distinguish male and female first-instar nymphs, but after fixation to cladode it can be easy to make distinction. Like other *Dactylopius* spp: the male develops long glassy filaments on the abdomen posteriorly only, while the female developed longer conspicuous filaments on the dorsal surface of the body anteriorly and posteriorly (Gunn, 1978; Mathenge *et al*., 2009) and the sexual dimorphism became apparent during the second instar stage. The mean duration of female life cycle from egg to the commencement of oviposition was reported as 40–50 days for *D. austrinus* at 25 and 26°C (Moran & Cobby, 1979; Hosking, 1984), *D. opuntiae* at 26°C (Githure *et al*., 1999; Volchansky *et al*., 1999; Flores-Hernandez *et al*., 2006) and *D.coccus* at 25-26°C (Guerra & Kosztarab, 1992). The current work showed that the duration of female life cycle from eggs to first eggs production was ranged from 51.58±1.86 to 119.70±2.92 days in both study conditions. The fluctuation of temperature under field and semi-field conditions lead to extend duration of female production life cycle (under semi-field conditions: from

51.58±1.86 to 77.55±1.85 days ; Under field conditions: from 54.78±1.33 to 119.70±2.92 days). Generally, the life-history of the male cactus cochineal scale, as other holometabolous insects, has been a source of confusion in the literature. Morocco biotype *D. opuntiae* male have 5 biological stages egg, nymph (1st,2nd,3rd,4rd and5th), pre-pupa, pupa and adult. The last 3 stages and 3rd,4rd and5th nymph develop in the cocoon. The male was short lived and mean male developmental time from egg to death was between 37.10±3.01 and 74.57±4.05 days in both studied conditions (field and semi-field conditions). These results intersect with that found by Flores-Hernandez *et al*. (2006) under controlled conditions between 19-23 °C. The duration of male life cycle was found as 57 days for *D. tomentosus* at 26°C and 60% RH (Mathenge *et al*., 2009), 37.1 days for *D. austrinus* at 25 °C (Hosking, 1984) and 31.3 days for *D. ceylonicus* at 26°C (Sullivan,1990).

Table 1. Development time in days (± standard deviation) of *D. opuntiae* on *O. ficus-indica* under field and semi-field conditions.

	Field		Semi-Field	
Life Stage	Winter-Spring	Spring-Summer	Winter-Spring	Spring-Summer
Egg incubation period	<1day	<1day	<1day	<1day
First moult	10.60±2.96a	3.17±1.70b	3.47±0.50b	1.33±0.48c
Second moult	58.03±3.03a	16.83±0.94c	23.43±1.06b	15.70±1.63d
Pupation–emergence	17.73±1.22a	7.52±1.96c	16.20±0.99b	7.50±0.75c
1st instar–adult male	66.53±3.51a	34.03±1.37c	41.17±1.54b	23.05±2.46d
Adult male lifespan	8.02±1.93a	8.80±1.82a	8.10±1.94a	8.65±1.63a
1st instar –Young female	66.25±4.44a	26.60±0.98b	27.17±0.81b	26.43±1.11b
1st instar–gravid female	117±2.57a	48.20±1.90bc	48.47±1.84b	46.70±5.51c
1st instar–mature female (first egg production)	119.70±2.92a	54.78±1.33c	77.55±1.85b	51.58±1.86d
Male life cycle	74.57±4.05a	42.68±2.25c	49.97±2.48b	37.10±3.01d
Female life cycle	133.52±3.82a	128.85±2.24b	128.90±1.90b	127.85±2.15b

Lines with different letters are significantly different according to the Tukey’s test ($p \leq 0.05$).

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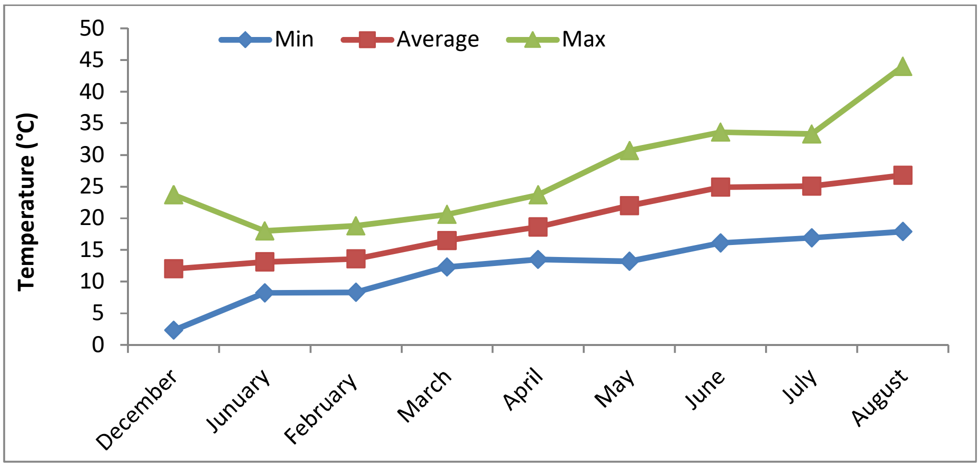

Fig 1. Field average, minimum and maximum values of air temperatures during the study period (December to August).

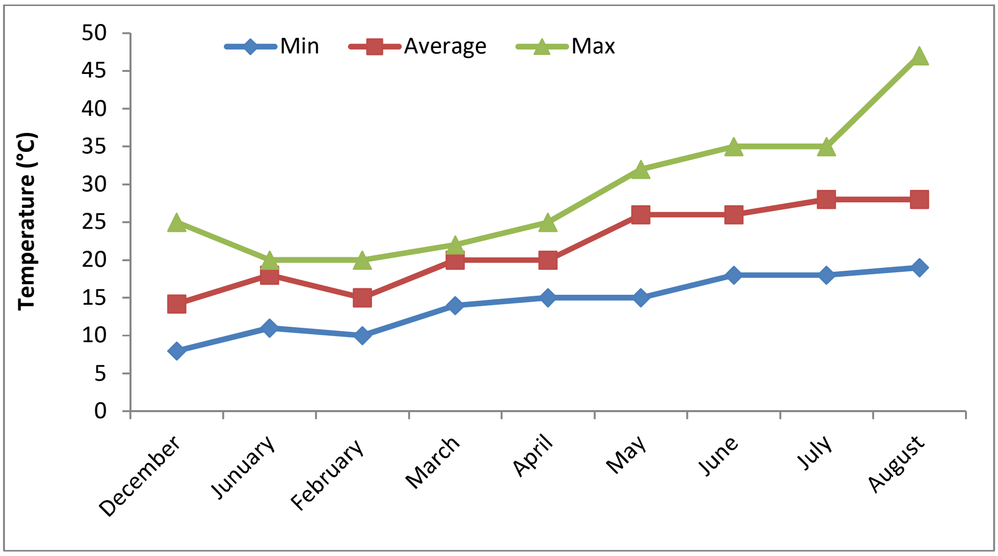

Fig 2. Semi-Field average, minimum and maximum values of temperatures during the study period (December to August).

4.2 Reproduction parameters

D. opuntiae reproduction parameters had a response related to the presence or absence of food (*O. ficus-indica* cladodes) (Table 2). Pre-oviposition, and post-oviposition periods were significantly long without food than in the presence of food (Pre-oviposition, $F= 93072.83$, $df= 1$, $p= 0.02$; Post-oviposition, $F= 985.88$, $df= 1$, $p\leq 0.05$). However, oviposition period, females longevity, fecundity, fertility, and growth index were significantly higher in the presence of food than without food (Table 2) (Oviposition period $F= 5109.08$, $df= 1$, $p= 0.018$; Females longevity, $F= 16.13$, $df= 1$, $p\leq 0.05$; Fecundity, $F= 274.10$, $df = 1$, $p\leq 0.05$; Fertility, $F= 295.50$, $df = 1$, $p\leq 0.05$; Growth index, $F= 9.51$, df= 1, p= 0.004). The oviposition period (28.33±1.20 days with food, and 15.28±1.68 days without food) was not similar to that reported by Luna *et al*. (2018) as the oviposition lasted six or nine weeks depending on the availability of food at 25±1°C. The fecundity was also different for what Flores-Hernández *et al*. (2006) (131 individuals per female without food), and Luna *et al*. (2018) (567.58±164.67 with food, and 351.25±131.98 without food) reported. This difference may be due to host plant used, or other factors such as quality nutrition and stress of the cladodes before withdrawing the females (Luna *et al*., 2018). Indeed, a higher fecundity was reported for *D. austrinus* females (1,145 individuals for female) (Moran & Cobby, 1979), and *D. ceylonicus* females (1,200 individuals for female) (Sullivan, 1990), which were evaluated with females attached to the cladodes. The sex ratio (females: males) of *D. opuntiae* obtained in the current study was similar to that reported by Flores-Hernández *et al*. (2006) for the same species (1:1) (Table 2), and was different (3.7:1) from that reported by Luna *et al*. (2018). In this case Nur *et al*. (1987) indicated that the physiological factors of the egg and environmental conditions can be modified the sex of scale pest. The same authors have observed that in the field, the sex ratio of this species is different during specific seasons of the year.

Table 2. Reproduction parameters of *D. opuntiae* with or without food (*O. ficus-indica* cladodes) at 25 ± 2°C.

Reproduction parameters	Females with food	Females without food	Significance
Pre oviposition	6.98±0.91	11.05±2.31	**
Oviposition	28.33±1.20	15.28±1.68	**
Post oviposition	2.55±0.79	8.52±1.24	**
Longevity	37.87±1.56	35.85±3.56	**
Fecundity	451.82±12.09	425.33±2.71	**
Fertility	96.92±1.08	93.32±1.21	**
Growth index	6.30±0.66	5.68±0.60	**
Sex ratio	1 :1	1:1	ns

** significant ns non significant by Tukey's test ($p \leq 0.05$).

4.3 Population growth parameters

The net reproductive rate (R_0), and generation time (T) were significantly higher for females with food than for females without food (The net reproductive rate: F= 642.55, df = 1, p≤ 0.05; Generation time: F = 1625.68, df= 1, p≤ 0.05). For intrinsic rate of natural increase (r_m), doubling time (DT), and finite rate of increase (λ), no significant difference was observed between females with food and females without food (Table 3), similar results were found by Luna *et al*. (2018) for Mexican *D. opuntiae* biotype. Also this results can be explained because the females without food laid the totality of the eggs and nymphs in a shorter time than died, therefore the availability of food favored the net reproductive rate and increased the generation time for females with food (Luna *et al*., 2018).

Table 3. Population growth parameters of *D. opuntiae* with or without food (*O. ficus-indica* cladodes) at 25 ±2°C.

	Females with food	Females without food	Significance
R_0 ($\Sigma\ lx\ mx$) *	437.88±11.57	396.90±4.79	**
r_m larvae/female/day*	0.050±0.001	0.052±0.001	ns
T(days) *	128.90±1.90	114.90±1.90	**
DT(days) *	3.75±0.02	3.65±0.02	ns
λ (days^{-1}) *	1.05±0.001	1.05±0.001	ns

*Based on 60 females

** significant; ns= no significant by Tukey's test (p ≤ 0.05).

5. CONCLUSION

The results of this paper showed that the duration of life cycle of *D. opuntiae* under field and semi-field conditions was temperature dependent**.** The cycle was long in Winter-Spring and short in Spring-Summer. The results indicated also that the reproduction and population growth parameters of *D. opuntiae* is influenced by the availability of food. The fecundity of *D. opuntiae* was higher when the females were attached to the cladodes. Our study generated plenty of information on the biological parameters and life cycle of *D. opuntiae*, which should facilitate the development of management options for the control of this very devastating cactus mealybug in Morocco and many other Mediterranean countries.

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