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Effect of Density on the Rate of Moulting of Two Locust Species: *Schistocerca gregaria* and *Dociostaurus maroccanus* (Orthoptera: Acrididae)

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ABSTRACT

In this present work, we studied the influence of individual density on the larval moulting rate of three larval stages (third, fourth, fifth instar) in two locust species *Schistocerca gregaria* and *Dociostaurus maroccanus*. The moulting position, their duration and the mortality during the moult were also noted.

The results show that regrouping individuals accelerates their moult rate. Thus the individuals prefer to realise their moults in vertical position considering the short duration and the survival of larvae during moulting is higher. In contrast the moults performed in horizontal position took a longer duration and increased the mortality rate in all instars examined.

Key Words: Moult ; Gregarization; Locusts ; Desert Locust ; Moroccan Locust .

INTRODUCTION

The growth of locusts goes through certain stages of development, where each stage is signified by the detachment of the cuticle, called moult (ecdysis) (Douro et al. 2000). The size of the larvae increases following moulting, but remains latent until the fall of the cuticle (Bouzeraa 2014).The cleavage that separates the cuticle from the locust's body seems to be a result of a secretion due exclusively to hypodermic cells (Dhadialla et al. 2005). Moulting, like in all other insects, plays an important role in the development of Orthoptera, and a better understanding of the process can be useful in pest management (Rackauskas et al. 2006).

The phenomenon of moulting has been analyzed widely, including the physiological and endocrinological aspects (Mondal et al. 2000, Dhadialla et al. 2005, Bouzeraa 2014). However, none of these studies have addressed the influence of the grouping of individuals on the growth and larval development of locusts. Notably, some locust species qualified as formidable pests, such *Schistocerca gregaria* and *Dociostaurus maroccanus*, which are characterized by phase polymorphism reflected by the solitary, transienand gregarious phases. These three phases differ significantly in biological, ecological, ethological and physiological settings (Uvarov 1977, Pener 1991, Pener and Yerushalmi 1998, Simpson et al. 1999). Indeed, in the solitary phase, individual crickets live in low density populations and have less mobility (Ghaout et al. 1991). In comparison, those in the gregarious phase are very active, mobile, and live in high density populations (Ellis 1951, Roffey and Popov 1968).

In the present work, we studied the influence of the density of individuals on the rate of larval development of locust (third, fourth and fifth larvae) in two locust species namely, *Schistocerca gregaria* and *Dociostaurus maroccanus*.

MATERIAL AND METHODS

The larvae used in the study were emerged from two different sources. *Schistocerca gregaria* larvae were stemmed from a massive rearing in the laboratory while, in *Dociostaurus maroccanus*, larvae were collected from the Al Azaghar area (4° 34' W, 33° 38' N). In both species, three larval stages have been studied (third, fourth and fifth instar).

Larval Development

The survey of moulting was conducted using the method described by (Abbassi 2004) and (Kemassi 2008). The larvae of different stages studied were divided into three groups: Group 1 (G1) of 10 individuals, group 2 (G2) of 6 individuals and group 3 (G3) of 3 individuals, with 10 replications for each group. A total of 190 individuals were used for each larval stage.

Individuals were separated into wooden cages that measured 14 (length) x 20 (width) x 7 (height) cm and were fed on fresh grasses. The cages were equipped with special controlled lighting and covered with a net for aeration. The lighting used was 12 h dark and 12 h light cycle. The relative humidity was maintained around 60 per cent and a temperature of 30 °C during the day and 21 °C at night.

Observations of moulting individuals were recorded (number of moults (individuals)performed during 24 hours). Similarly, the positions (horizontal or vertical) and the duration of the moulting were monitored.

RESULTS

The results show that the moulting rates vary from one stage to another and depend on the time (Figure 1). Thus, for larval in stage 3: In group 1 (10 larvae), individuals start moulting during the second day of rearing, and by the 6th day, all individuals are completely moulted. On the other hand, the moulting in groups 2 (6 individuals) and 3 (3 individuals) were completed on the 9th day. In stage 4, for group 1 all moults were performed in 5 days, while in groups 2 and 3, moults were late until the 7th day. In the 5th stage, groups 1 and 2 complete their moults at the same time (6 days), but the group 3 take eighth days). From these results, we conclude that the grouping of individuals (great density) influences larval development in *Dociostaurus maroccanus.*

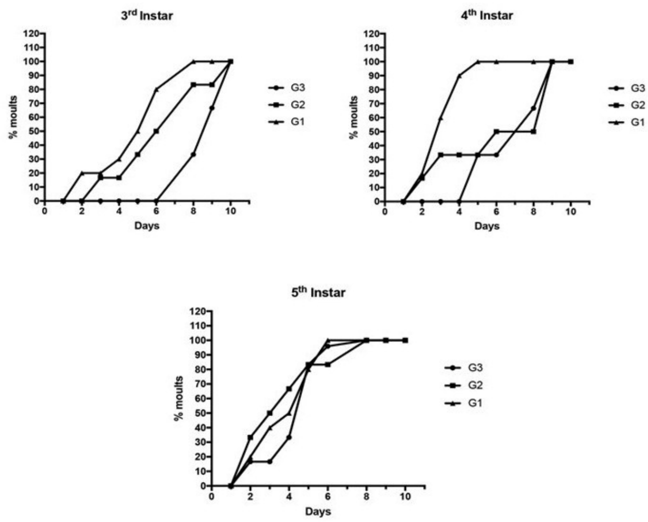

Figure1. Rates of moults as a function of the time of the 3 studied groups and different instars of *Dociostaurus maroccanus*. (G1:Group of 10 larvae, G2:group of 6 larvae, and G3: group of 3 larvae; S3: Third instar; S4: fourth instar and S5: fifth instar).

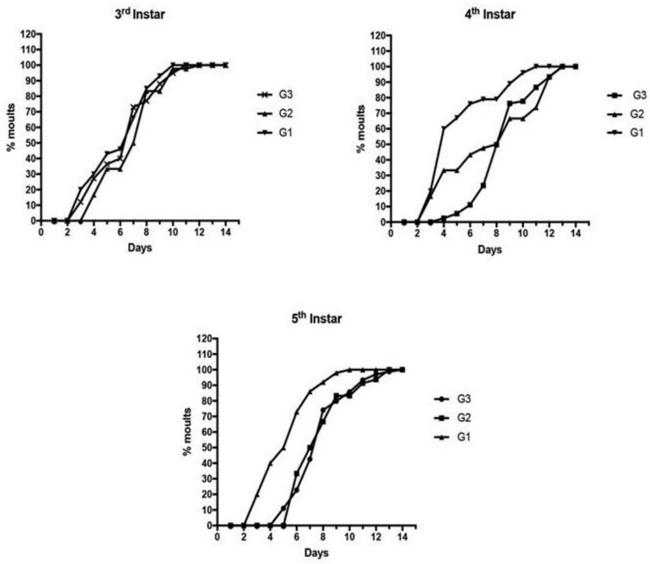

Figure 2. Rates of moults as a function of the time of the 3 studied groups and different instars of *Schistocerca gregaria*.(G1: Group of 10 larvae, G2: group of 6 larvae, and G3: group of 3 larvae; S3: Third instar; S4: fourth instar and S5: fifth instar).

The follow-up of the moultingduring studied stages, depending on the time (Figure 2), shows that the speed of growth in desert locust larvae is faster than that of Moroccan locust larvae. Moulting duration of larvae varies between 12 to 14 days depending on the studied stage. On the other hand, the duration of development in the larvae is influenced by the density of individuals and this for both the species studied. Indeed, in most dense groups, represented by the group of 10 individuals, completed their moults first, before groups of 3 and 6 larvae in all studied stages. During the third stage, the evolution of growth is almost similar between all groups. However,the group of 10 larvae complete its moults first. The maximum moulting interval (4 days) between group of 10 and group of 3 individuals was recorded in the fifth stage.

From these results, it is clear that the grouping of individuals influences larval development in the studied locust species (ANOVA; $F = 3.47$; $df = 2$; $p \leq 0,05$). The higher density of individuals appears to accelerate larval development. However, no statistically significant effect was observed during all stages between the species (ANOVA; $F = 2.28$; $df = 2$; $p \geq 0.05$) and the larval stages (ANOVA; $F = 0.48$; $df = 2$; $p \geq 0,05$).

Realization of Moults

The position of moulting was studied and filmed, in order to define the optimal position of the ecdysis. The larvae were observed moulting in two positions, "vertical" (suspended or "horizontal" grounded.

We noticed that the number of moults performed in vertical position exceeded widely moulting on the ground. The majority of moults were carried out vertically, 83.05% for *Dociostaurus maroccanus* and 86.34% for *Schistocerca gregaria* in comparaison with 16.95% and 13.66 % respectively, on the floor for the two species (Figure 3). Concerning the influence of density, no effect was observed on the ecdysis position.

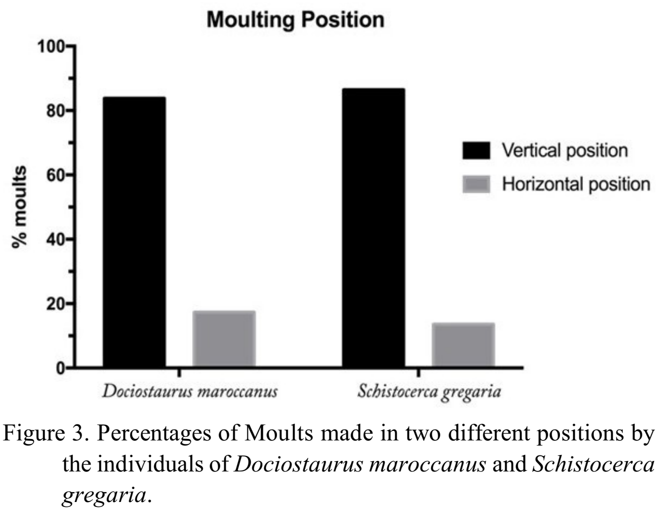

Figure 3. Percentages of Moults made in two different positions by the individuals of *Dociostaurus maroccanus* and *Schistocerca gregaria*.

Mortality During the Moulting

The mortality rate of larvae was recorded in relation to the ecdysis position. The results are shown in Figure 4.

The mortality rate varies according to the larval position during ecdysis. Indeed, mortality during the grounded moulting was 11.0% and 20.0% in *Schistocerca gregaria* and *Dociostaurus maroccanus* respectively, compared with 2.85% and 4.28%, respectively in suspended moulting (Figure 4).

The difference in mortality between the two species may be due to the effects of rearing, since the desert locust that records the lowest rate (13.0% mortality) came from the laboratory, while the Moroccan locust (24.0% mortality) came from field; so it may be more fragile than *Schistocerca gregaria* (Ould el Hadj et al. 2004).

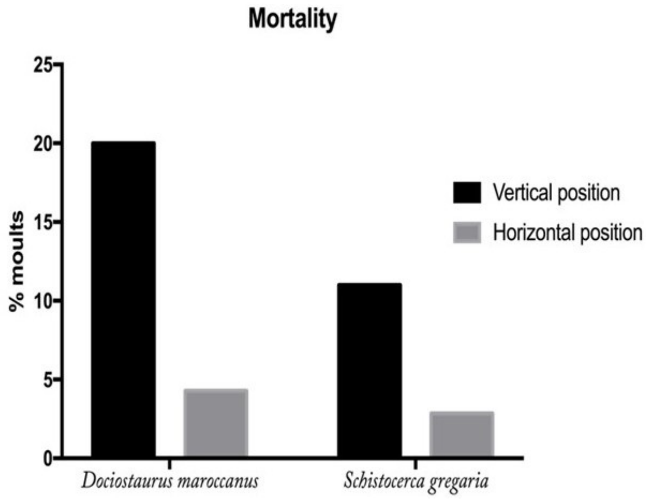

Figure 4. Mortality rate of moulting individuals in suspended and grounded positions

Duration of Moults

To better understand the behavior of the species toward suspended moulting, we analyzed, in the fifth instar, the time required to complete the moulting in 20 individuals, divided equally in both species.

The results show that the duration varies widely depending on the moulting position (Table 1). Indeed, the vertical moulting is very short, with a mean duration of 34 minutes in *Dociostaurus maroccanus* and 41 minutes in *Schistocerca gregaria*. However, moults performed on the floor have a fairly long duration, they

Table 1. Average duration (min) required by each Stage 5 individuals to complete their moult in *Dociostaurus maroccanus* and *Schistocerca gregaria*

	Individuals										
	1	2	3	4	5	6	7	8	9	10	Mean±s.d.
D. maroccanus											
Grounded position	120	1440	2880	1260	1560	180	300	660	360	540	930 ±261
Suspended position	31	42	35	29	35	34	32	38	33	33	34.2 ±1.16
S. gregaria											
Grounded position	240	1440	2880	1620	1500	180	1860	840	360	1200	1212 ±265.8
Suspended position	45	49	35	45	35	42	41	38	41	41	41.2 ±1.40

take more than 17 hours in *Dociostaurus maroccanus* and 8 hours in *Schistocerca gregaria.* On other hand, we noted that locusts on the floor moult hardly (elimination of cuticle) and sometimes lose large parts of their body, including the legs and antennae. This explains the increased mortality of larvae studied in "floor" position compared to that observed in "suspended" position in both species.

DISCUSSION

To ensure its larval development, locust makes a number of moults which vary from one species to another. In this study, we have shown that the phenomenon of moulting is significantly influenced by the density of individuals occupying a defined area.It seems that the density accelerates the development of locusts. These results are consistent with those outlined by many authors (Mcfarlane et al. 1983, Ben Halima et al. 1984, Anstey et al. 2009), who reported that the great density accelerates growth of locust. The importance of wide density can be explained by the competitive behaviour for food during growth. In effect, locusts in the gregarious phase consume large amounts of food (Albrecht 1955, Cassier and Delorme-Joulie 1976, Kaufman et al. 1989), which is likely to accelerate their growth and life cycle (Ernst et al. 2015). Beside diet, hormonal control, via the ecdysone and juvenile hormone, can be also reliable for the growth and the place of realization of the moults in Orthoptera (Nijhout 1994, Riddiford 1996). Indeed, these hormones control the moulting mechanism, the determinism of growth and the development in insects (Gade and Hoffman 2005, Dhadialla et al. 2005, Bouzeraa 2014). In fact, these hormones by transforming locusts to gregarious form accelerate the growth rate of these insects (Rogers et al. 2016).

The locusts that we have studied show a near-total trend (over 80.0%) to assume moulting in suspended position (on the plants, on the edges of the cages, or on the present perches in the cages). It appears that the vertical position provides a favourable support and gravity to the larvae during the moulting phase (Otte and Joern 1977). Actually, the mortality rate and the time required by each position of moulting confirm the advantage of vertical moults. Suspended position reduces the duration of the moulting (<35 min) compared to the surface or floor moulting (over 17 h in *Dociostaurus maroccanus* and 20 h in *Schistocerca gregaria*). This part concerning moulting position is original and it is not analysed elsewhere. Similarly, the survival of the instars during the molting is greater in vertical position, in comparison to the grounded molting, in which mortality reaches more than 20.0% of individuals in *Dociostaurus maroccanus* and 11.0% in *Schistocerca gregaria*. In addition to the gravity provided by the vertical position, growth hormones can also influence the duration of moults in these locusts (Bouzeraa 2014). This relationship between growth hormones, metabolites contained in the haemolymph and growth of locust has been extensively demonstrated in larvae of *Schistocerca gregaria* (Ghoneim and al. 2003 and Tanani et al. 2012). However, these are not the only ones to explain the behavior of the suspended moults in these two species. Other factors, including temperature, humidity, predator avoidance, and the nature of their diet can justify the observed behavior (Simpson et al. 2001, Ernst et al. 2015).

Finally, our study suggests that, in locusts, the grouping of individuals (gregarious) influences the growth of locusts. Most larvae of both *Schistocerca gregaria* and *Dociostaurus maroccanus* species, when they are grouped in a small area, display an accelerated growth rate. This is valid at all three stages (instars 3, 4 and 5) of larval development of these locusts. Concerning the moulting position, the results confirm that these locusts prefer to evolve in suspended position, in order to reduce the mortality of individuals and the time required to complete the moulting.

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