



LIFE CYCLE OF IMMATURE STAGES OF *ORYCTES AGAMEMNON ARABICUS* FAIRMAIRE (1896) (COLEOPTERA: SCARABAEIDAE) UNDER SIMILAR NATURAL CONDITIONS OF SOUTHWEST TUNISIA

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ABSTRACT

Few biographies on the biology of *Oryctes agamemnon arabicus* exist in the world. The aim of this work is to study the developmental cycle of immature stages of this pest in breeding trials conducted under natural conditions. Biological cycle of *O. a. arabicus* was composed of egg, three larval, pupal and adult stages. Results showed that incubation of eggs lasted for 12.95 ± 0.16 days and larval periods of 1st, 2nd and 3rd instar were 35.24 ± 4.33 , 60.42 ± 39.2 and 189.15 ± 44.47 days, respectively. Larval development prolonged for 284.94 days. Pupal period lasted for 20.78 ± 2.69 days. Development of one generation, from eggs to adult emergence, lasted for 318.6 ± 17.82 days which indicated that it was univoltine species. Detailed statistical analysis showed that developmental time within the same stage was highly affected by climatic conditions particularly temperature. Thus, we distinguished between adults developed from summer eggs and autumn eggs.

Keywords: Autumn generation, Date palm, Immature stages, Life cycle, *Oryctes agamemnon arabicus*, Summer generation, Tunisia.

INTRODUCTION

Rhinoceros beetles constitute a group of medium to large sized scarabs of the *Dynastinae* whose males are characterized by a large cephalic horn (Rochat *et al.*, 2004). Among rhinoceros beetles, the genus *Oryctes* includes about 40 species (Endrodi, 1985) but only some of them have a real impact on the development of palm trees (Balachowsky, 1962). Species acting as fruit-stalk borers are considered to be among the most serious pests of date palm in the Arabian Gulf area (Al-Sayed and Al-Tamiemi, 1999).

In certain Gulf countries (UAE, Sultanate of Oman and Kingdom of Saudi Arabia), several species develop within oases (Al-Sayed and Al-Tamiemi, 1999). However, *Oryctes* damages to palms cause wounds which are economically secondary and of less importance alone; but, it attracts the xylophage pest *Rhynchophorus ferrugineus* for oviposition which leads to the palm dying.

In some oases of southwest Tunisia, *O. agamemnon arabicus* was accidentally introduced during the last three decades in Djerid region, via offshoots of new varieties imported from United Arab Emirates oases (Endrodi, 1985). It was officially

noted in 1995 (Khoualdia and Rhouma, 1997) in certain oases of Tozeur governorate, mainly focal point from which it is spreading out to other oases, like Rjim Maatoug oases in the extreme west of Kebili governorate. The life cycle of this pest occur on secondary parts of standing alive date palm tree in particularly respiratory roots mass at the base of the whole palm, through the external component of the stem (dry petiole and matrixes of fibrilium) and also in the oldest green palm situated at the base of the crown. These places where eggs were directly laid offered a well protection to the offspring from external aggression either abiotics or biotics (Soltani, 2004; Soltani *et al.*, 2008).

Since this date, no serious work has been done on this subject. An understanding of the life cycle of a pest is a first basic step which can lead to develop better techniques of monitoring and control. Bu the data on the biology, ecology and damages related to this pest are completely lacking. In this study, the principal aspects of *O. agamemnon arabicus* life cycle were outlined under natural conditions of Rjim Maatoug oases in southwest Tunisia.

MATERIALS AND METHODS

Study site

The study was conducted during three years from May 2004 to April 2007 in Rjim Maatoug oases (1610 ha) situated in Tunisian Saharan climate, 120 km from Kebili town. This zone was characterized by an average rainfall lower than 100 mm/year and a mean temperature of 21°C with extremes of 55°C in the shade in summer and 7°C in winter.

Oases of Rjim Maatoug were planted between 1987 and 1995. The soil type was sandy with date palm tree spacing of 9 m × 9 m and a few practices of fodder cultures. The vegetal cover of the Saharan area was limited to few desert species.

Experimental layout for developmental study

Beetles were collected from field as well as obtained from laboratory culture and were confined in pair in plastic boxes of 20 cm × 15 cm × 10 cm filled with decomposed vegetal material collected from natural breeding sites. This breeding medium used in all experiments of present work was originally chosen by Soltani *et al.* (2008) to breed the same species under laboratory conditions. It consisted of fine wood granules of hairy respiratory roots or dry petioles of fronds and fibrillum matrixes, already chewed by larvae during their diets and of less than 3 mm in size. This medium provided an oviposition site for females and food for hatching larvae.

Boxes, with holes in lids for ventilation were kept under natural temperature conditions and 26-34% and 38-69% relative humidity for incubation and hatching, respectively. Fresh laid eggs were collected daily and transferred into small boxes of 100 mm × 50 mm × 50 mm, with the same breeding medium. The incubation period consisted of the number of days elapsing from the removal of eggs until the hatching of the first larval stage. The newly emerged larvae were removed by a group of three individuals within plastic boxes of 20 cm × 15 cm × 10 cm. During development, food was weekly refreshed. At the end of larval development, an intermediate stadium was recorded that started when larvae stopped feeding and became static. Referring to Soltani (2004) who worked on the same species, Hammes and Monsarrat (1974) and Bedford (1976) who worked on *Oryctes rhinoceros* species and Balachowsky (1962) who worked on many coleopteran species mainly *Melolonthinae*, the period comprised between this arrest and pupation was called as pre-nymphal stage. The number of days from pupation to adult emergence called nymphal period was also noted.

Starting with 220 eggs, the require time to the development of each immature stage of *O. a. arabicus* (incubation, first larval stage (L₁), second larval stage (L₂) and third larval stage including pre-nymphal stage (L₃, and nymphose) under Rjim Maatoug conditions was noted. Also, the whole larval development period from L₁ to L₃ was noted. The abbreviations L₁, L₂, and L₃ were used for larval stages throughout the manuscript.

At the beginning of each stage and after each moulting, initial number of individuals (N_i) and newly moulted (N_m) ones were recorded. These data are used to calculate the mortality percentage per stage using following formula:

$$\text{Mortality (\%)} = \frac{N_i - N_m}{N_i} \times 100$$

Statistical analysis

Embryonic and post-embryonic development data were subjected to a statistical analysis using the software SPSS 16. Results were shown as mean (mean ± SE), the mode and the range. The one way ANOVA was applied to verify if there was a difference between registered means.

RESULTS

Embryonic development

Generally, newly laid eggs were ovoid (3.11 x 2.25 mm) and milky white and later became watery & transparent. During development, they increased in size as the embryo grew and absorbed water. Near hatching, their final form was almost spherical.

The mean incubation period measured on a sample of 189 eggs was 12.95±2.16 days. First box plot in Fig. 1 (grouped data) revealed that almost 54 % of incubation or development times were situated between 11 and 13 days with a mode of 12 days and a range of 10 days. The range value was high comparing to some incubation period (superior and equal to certain values) which showed a dispersion values inside their interval.

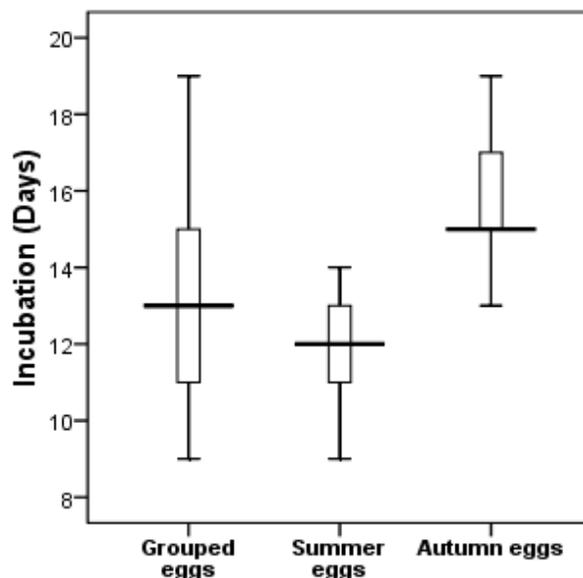


Fig. 1

Incubation period of *O. a. arabicus* under Rjim Maatoug oasis conditions

However, eggs represented the initial stage of development and the period of their oviposition in field affected incubation period and developmental time of future immature stages. Accordingly, revision of relative registered dates of eggs oviposition of *O. a. arabicus* in field revealed that this activity started in late spring (end May), continued mainly in summer and take over in mid-autumn. The differences between the periods of incubation appeared clearly in registered values

mainly expressed by the minimum and maximum relative to each season (Fig. 1). In fact, after season parameter consideration results showed clearly the existence of two intervals of incubation (9-14 days) for eggs laid during summer season which represent 71.43 % of the entire studied cohort with an average of 11.84 ± 1.23 days (Fig. 2), a mode of 12 days and a range of 5 days (Fig. 1) Development period of 13-19 days for eggs during the first half of autumn represented 28.57 % of the entire studied cohort with an average of 15.69 ± 1.42 days (Fig. 2), a mode of 15 days, and a range of 6 days (Fig 1).

Frequencies analysis of collected data relative to each interval revealed that development of 88.9 % of eggs deposited during summer occurred inside the interval of 10-13 days while 83.3 % of eggs in the interval of 14-17 days for those deposited in autumn. Statistical analysis using the one way ANOVA revealed the existence of a significant difference between means (Fig. 2) relative to each period of oviposition ($df=1$, $F=318.04$, $p=0.00$).

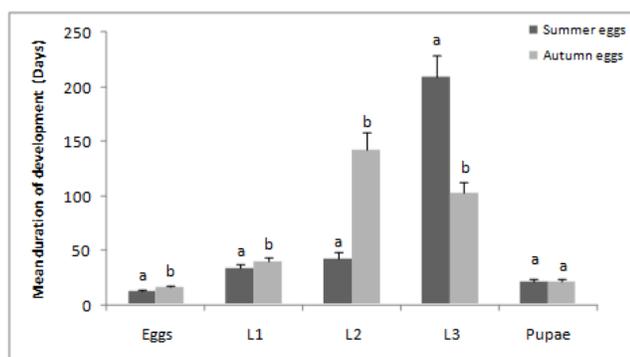


Fig. 2

Means of development of immature stages according to season of oviposition

In conclusion of this part, the results showed the existence of only one continuous period of oviposition activity under natural conditions which started at the end of May and prolonged to the middle of October. In this case, influence of weather conditions on the incubation period appeared clearly on the time of incubation of each cohort. This result leads to distinguish for this species between summer and autumn eggs. First preliminary result and cited data proved the existence of only one generation per year.

Post embryonic development

Larval development

Larval period of *O. a. arabicus* completed through three distinct stages. When hatching, larva of first stage was 7 mm in length, 0.3 g of weight and white in colour. The egg's chorion constituted the first food consumed by larva to liberate itself. After hatching and each moulting process, the entire head capsule of larvae, including mouth parts, was soft and non-functional. Consequently, as first step of its development, larva was still immobilized for 6-12 hours until the end of Chitinisation and sclerotization of the cephalic

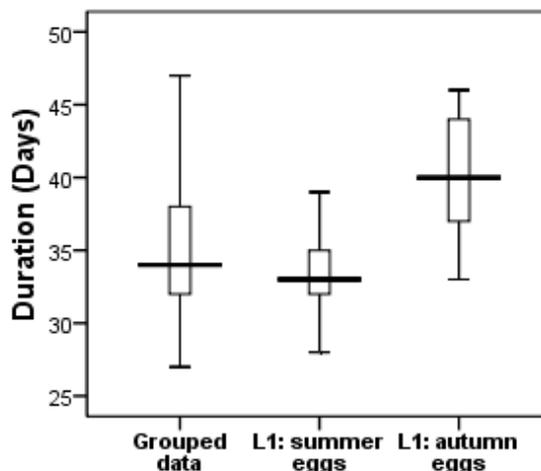
capsule. When ready, they buried inside substrate and start feeding on decomposed wood or in the chipboard prepared by female before oviposition.

General analysis of data under experimental conditions for larval stages, without considering the season of oviposition, showed that the developmental period of L_1 was 35.24 ± 4.33 days with a mode of 33 days and a range of 20 days. After moulting, the L_2 was measured 22.4 ± 4.36 mm in length and 1.7 g of weight; while at the end of this stage it reached to a length of 45 mm. Its development lasted on average 60.42 ± 39.2 days with a mode of 38 days and a range of 133 days. However, duration of L_3 was the longest compared to previous instars and occupied the majority of the developmental cycle of the pest. So, the longest registered duration was 248 days, with a mean of 189.15 ± 44.47 days with a mode of 208 days and a range of 161 days. At full development larva gained a weight of 17 g and a length of 90 mm. The values of standard deviation and range were considered as high (Fig. 3 A, B, C, D).

The larval development relative to the cohort hatching from summer eggs revealed a mean (Fig. 3), a mode, and a range of 33.46 ± 2.45 , 33 and 13 days; 42.66 ± 5.85 , 39 and 23 days; and 208.3 ± 19.13 , 205 and 143 days, for L_1 , L_2 and L_3 , respectively. On the other hand, means (Fig.3), mode and range of developmental duration relative to L_1 , L_2 and L_3 hatching from autumn eggs were 40.06 ± 3.75 , 38, and 14; 142.63 ± 15.11 , 124 and 54; and 102.63 ± 9.87 , 93 and 39 days, respectively.

Statistical analysis using the one way ANOVA at probability level of 5 % revealed the presence of a significant difference on all levels between calculated means of L_1 ($df = 1$, $F = 147.84$, $P = 0.00$), L_2 ($df = 1$, $F = 3,269 E3$, $P = 0.0$) and L_3 ($df = 1$, $F = 1.165 E3$, $P = 0.0$). In fact, this statistical analysis approves the effect of seasonal climatic factors mainly expressed by temperature and humidity on the durations of development of the same stage.

In conclusion of this part, larval developmental period was 284.94 ± 17.94 days without considering the origin of the eggs (Fig.3, D). When origin of the eggs was considered, registered means were 284.85 ± 17.16 and 285.36 ± 20.86 days respectively, for larvae developing from summer and autumn eggs ((Fig.3, D). This result was confirmed using the ANOVA test which showed non significant differences between treatments.



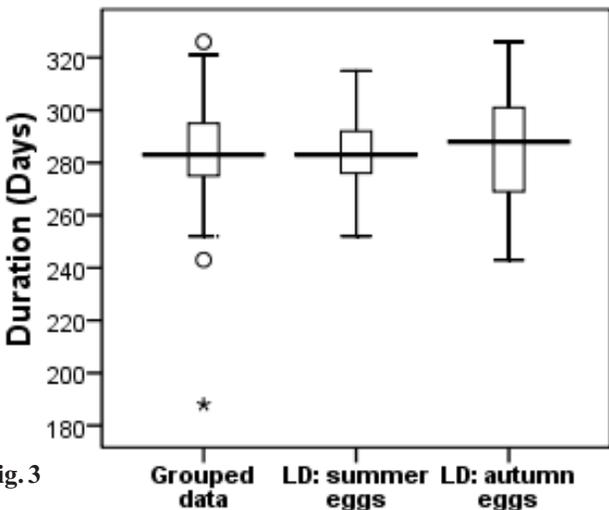
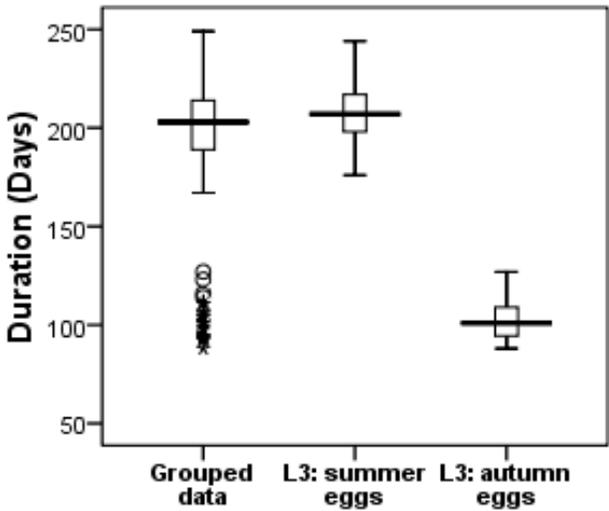
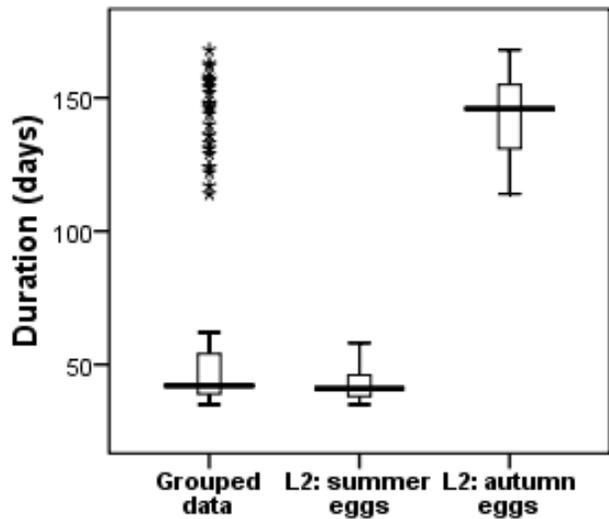


Fig. 3 Larval development of *O. a. arabicus* for summer and autumn eggs: L₁(A), L₂(B), L₃(C) and, all larval development (D)

Pupal development

Pupae occurred at the end of a larval gallery inside a chamber initially created by the L₃ during its feeding activity and then shaped by the repeated movement of the pre-pupa inside until the transformation in pupa. Under breeding conditions, first observation of pupae was noted in early April and last in mid of October. Calculated means, mode and range (Fig.4) relative to nymphs originated from summer and autumn eggs were 20.78±2.69, 21 and 11 days and 21±2.14, 21 and 7 days, respectively. The one way ANOVA revealed no significant difference when used to compare between obtained means which confirmed on one hand the homogenous of values within their interval and on other hand the one continuous period of its development.

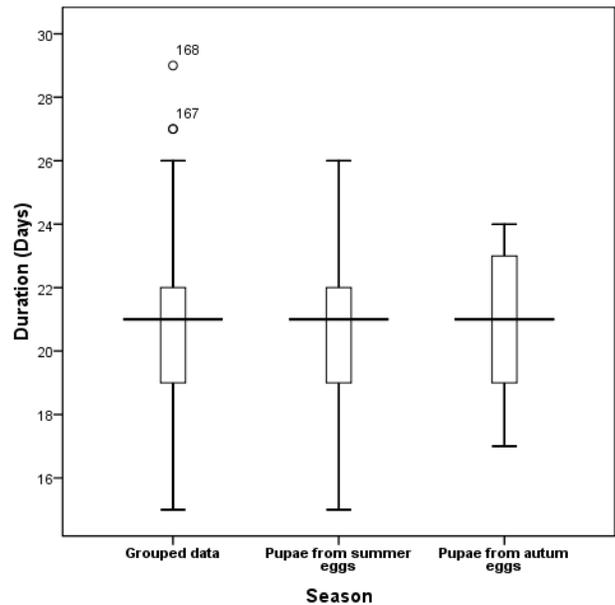


Fig. 4 Pupal period of *O. a. arabicus* under Rjim Maatoug oasis conditions

Chronology of development of *O. agamemnon arabicus* in the nature

After studying the development of different stages of *O. a. arabicus* in boxes; it was important to determine the chronology of its development within oasis. Egg laying in the field started around mid-May by overwintering females and went around mid-June with no observed adult activity out of palm tree in the oases. The flying activity of adults started in the second half of June and oviposition activity peaked between the second decade of July and the third week of August that then decreased progressively until mid October. Larval development occurred through the year without interruption and with a particular larval instar most abundant at a particular time of the year. The L₁ was firstly collected around the end of May. Frequencies analysis of collected data relative to summer eggs revealed that 79 % of larvae developed in 30-35 days, 17.7 % in 36-40 days, and 3.3 % in

30 days. This stage became very abundant within oases between late July and the end of August. However, first larval hatching from autumn eggs appeared in the second half of September, and the last larval hatching was recorded during late October. Frequencies analysis of data revealed that development of 84.9% of larvae occurred in 36-45 days i.e., individuals of this stage hatching from later eggs lasted between middle September and end November to moult to second stage. Thus, this stage didn't constitute a hibernation form of this pest under natural conditions.

First observation of L_2 was recorded in early July when early eggs laid in end spring were hatched and their L_1 instar completed development in June. Using season of oviposition, L_2 originated from summer eggs were divided into three groups: i) larvae in which all development occurred during summer season, ii) larvae in which development occurred between end summer and first half of autumn and iii) larvae in which development occurred during autumn before the onset of unfavourable conditions. Larvae of this cohort hibernated in the L_3 form and never L_2 . Contrary to the cohort of larvae hatched from autumn eggs, they were subject to adverse conditions causing the slackening of their development and then their moulting to L_3 . Larvae hatched from early eggs laid in autumn were more resistant to winter conditions than those hatched from eggs laid in October. Frequencies analysis of L_2 development showed that 82.2% of larvae lasted between 35-60 days, all from summer-laid eggs, and the rest 17.8% developed within the interval of 114-170 days from autumn-laid eggs (Fig.3 B). These results showed that the important fraction of this stage developed in summer and autumn and a little fraction hibernated under this form.

Means of development of L_3 was the longest compared to others. In fact, 81.2% of L_3 which were developed from summer-laid eggs, took 160-260 days while the rest 18.8% coming from autumn-laid eggs molted during spring and their development period ranged between 80 and 140 days (Fig. 3C). Consequently, L_3 stage occupied 73.33% of the total duration of the immature stages originating from summer-laid eggs, even though it was dominated by L_2 stage occupying almost 50% of this duration for the fraction emerged from autumn-laid eggs. So, these two instars constituted the hibernation form of the species within oasis with a marked dominance in number of the L_3 stage.

At the end of L_3 stage, marked by changes in the behaviour and the form of larva, the larva became immobilized as the pre-pupa which lasted between 11-19 days and characterized the favourable period. At the end of feeding tunnel, this later L_3 made a chamber of compact breeding site material to pupate.

Pupae (Nymphae) from summer-laid eggs appeared firstly in oases from late March (spring) through to June, while pupae from autumn-laid eggs appeared in summer due to the development of L_3 stage which occurred during spring and a part of summer.

Number of generations

Oryctes a. arabicus was univoltine species under natural conditions of Tunisian oases. The average period from egg to adult emergence lasted for 318.6 ± 17.82 days with a marked dominance of larval development which occupied almost

73.33% of the life cycle of the pest. According to the origin of the eggs, there was no difference between the duration of immature life cycle of individuals developing from summer eggs and autumn eggs (Fig. 5).

Under natural conditions of oasis, we remarked in few cases that the complete cycle of the pest could be turned off on 5 to 6 months. This was registered for eggs laid and developed in late spring; in this case larval development occurred during summer season and September characterized by favourable conditions of temperatures. The few number of pre-pupae and pupae were collected in late September - October due to high rate of mortality this population i.e., a few eclosion of adults due to bass temperatures which made them dormant & inactive.

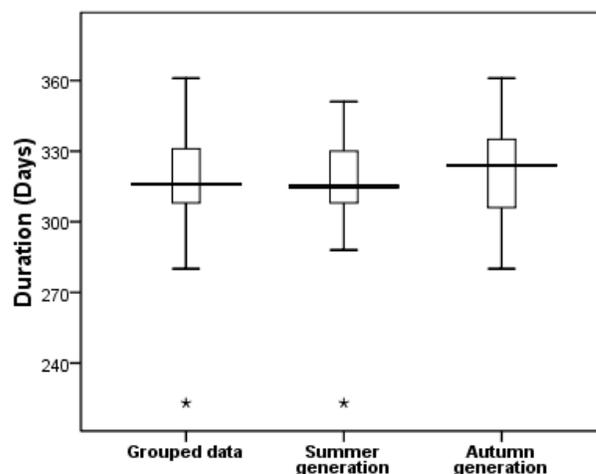


Fig. 5

Development of immature stages of *O. agamemnon arabicus* under Rjim Maatoug oasis conditions

Mortality

Under rearing conditions, 66.36% (146 eggs) of used eggs (220 eggs) reached the pupal stage while 14.09% and 21.16% mortality was observed during egg and larval development, respectively. The highest rates of mortality occurred in eggs (14.09%) and L_1 (10.57%). The lowest rate of mortality was observed in L_3 (3.87%) and pupae (2.01%) (Table 4).

In rearing experiments, eggs suffered mortality under high humidity due to fungal growth, or desiccation under low humidity. Larval mortality had two peaks, initially with L_1 after hatching and later just after molting to L_2 . A part of these results were observed in boxes with fibrous roots which constituted a harder wood to chew. These observations showed that just after hatching newly emerged larvae of different stages needs a decomposed material to be able to feed on and survive. In weekly surveys of palms, signs of mortality of immature stages were low to nil.

DISCUSSION

The differences in incubation periods are the direct result of the influence of breeding conditions affected by abiotic

Table 4Mortality rates of life stages of *O. a. arabicus* under rearing conditions.

	Eggs	1 st instar	2 nd instar	3 rd instar	Pupae	Adult
n	220	189	169	155	149	146
Mortality	31	20	14	6	3	-
%	14.09	10.56	8.28	3.87	2.01	-

Table 5Developmental time in days of many species of the genus *Oryctes*

Species	Time required in days					References
	Eggs	Larval development			Pupa	
		L ₁	L ₂	L ₃		
<i>O. agamemnon</i>	10-16	115-175			20-28	Lepesme, 1947
	11-16	31-38	51-68	98-162	20-29	Soltani, 2004
<i>O. rhinoceros</i>	11	21	21	35-63	21	Bedford, 1976
	8-12	10-21	12-21	60-165	17-28	Bedford, 1980
						Hammes and Monsarrat, 1974
						Waterhouse and Norris, 1987
<i>O. elegans</i>	2-8	270-300			21	Hurpin and Fresneau, 1969

factors acting mainly by temperatures. In fact, late spring and summer season, characterized by favourable temperatures are the most favorable period for adult activities, reproduction and development of immature stages under natural conditions. However, the low temperature of autumn slowed down development of immature stages and increased egg mortality.

Bibliography mentioned that the developmental cycle of *Oryctes* genus beetles was composed of eggs, three larval instars, nymphaea (pupae) and adults but with well diversified life cycle history. However, some species have less than one year life cycle as *O. rhinoceros* (Sivapragasam, 1988; Lokma, 2000; Talhouk, 1982), while others have one year life cycle, example *O. elegans* (Lokma, 2000) and *O. agamemnon* (Lepesme, 1947). A few species, like *O. nasicornis*, have one year in Ivory coast or two to three years in Ukraine (Balachowsky, 1962). Life cycle duration in this case was dependant on existing conditions.

Our data demonstrate that *O. a. arabicus* was univoltine. These results are in agreement with Lepesme (1947) and Soltani (2004) as given in Table 5. Comparison of larval instars duration for each studied cohort shows that summer-laid eggs give immature which have a shorter L₂ and longer L₃ and inversely autumn-laid eggs have a longer L₂ because they run into colder conditions, and shorter L₃. No observed difference on nymphal stage was registered. Compared to other species of the same genus, differences were observed in eggs development which is longer in *O. agamemnon* than *O. elegans* (Lokma, 2000) and *O. rhinoceros* (Talhouk, 1982). Duration of larval life in this study ranged at mean

284.94±17.94 days compared to 270-300 days showed for *O. elegans* (Lokma, 2000). Observed differences with *O. rhinoceros* from which total life cycle lasts for about 4-5 months (Talhouk, 1982; Waterhouse and Norris, 1987) was due probably to climatic conditions more favourable in pacific. Duration of last instar larva was longer compared to previous instars. These results were in general agreed with findings by Bedford (1976,1980) and Lokma (2000) for other species from the same genus.

Low rate of mortality in nature can be explained by the position of the *O. a. arabicus* immature stages being well protected inside attacked parts of date palm tree (Soltani, 2004; Soltani *et al.*, 2008 and the absence of natural enemies owing to its recent introduction in southern Tunisia.

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