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# LABORATORY OBSERVATIONS OF *RAPHITELUS MACULATUS* WALKER 1834 (HYMENOPTERA: CHALCIDOIDEA) A PARASITOID OF THE ALMOND BARK BEETLE *SCOLYTUS AMYGDALI* GUERIN-MENEVILLE 1847 (COLEOPTERA: CURCULIONIDAE) IN TUNISIA

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**A B S T R A C T**

The almond bark beetle *Scolytus amygdali* is an economic insect pest of many cultivated species of stone fruit trees, especially peach, plum, apricot and almond, grown in the Mediterranean region. Although the potential use of parasitoids as biological control agent against economically insect pests only few studies have been carried out against *S. amygdali*. The present study focused on the biology and the life cycle of *S. amygdali* in Tunisia during the past three years. Infested branches of almond trees were reared under laboratory conditions in rearing cages. Adults of *S. amygdali* and their natural enemies including all life stages of *Raphitelus maculatus* were collected and identified. *S. amygdali* females deposited around 303.8 eggs throughout 10.8 maternal galleries. The average density of attack was 0.07 galleries per $cm^2$ of bark whereas the rate of parasitism with *R. maculatus* was 2.1. The mortality percentage of *S. amygdali* was 39.6%. The mortality of the population is statistically linked to the presence of *R. maculatus*. Moreover, the parasitism rate was increased by increasing the number of *S. amygdali* adults and the multiplication rate.

**Keywords**: Parasitoids, *Raphitelus maculatus*, *Scolytus amygdali*, Tunisia.

## INTRODUCTION

Bark beetles are the most destructive insect pests in coniferous forests and also important in fruit trees in both temperate and tropical climate (Mendel, 1986). The almond bark beetle *Scolytus amygdali* Guerin-Meneville 1847 (Coleoptera: Curculionidae) is a native insect pest to the Mediterranean region; it feeds and develops mainly on stone fruits (Amygdaloideae). It is reported in all countries around the Mediterranean (Russo, 1931; Cherif and Trigui, 1990; Zeiri *et al*., 2010), the Middle East (Kinawy *et al*., 1991; Youssef *et al*., 2006), the Caucasus and Central Asia (Janjua and Samuel, 1941; Choate, 1999). This insect is one of the principal pests of almond, peach and other fruit trees in Tunisia (Cherif and Trigui, 1990; Zeiri *et al*., 2010; Zeiri *et al*., 2011). Plantations of plum, apricot and peach in Israel (Mendel *et al*., 1997), cherry in Spain (Teresa Garcia Becedas, pers. commu.), and almond in Morocco (Mahhou and Dennis, 1992) were severely affected. Adults feed during their maturation on the buds and the nearby twigs for reproduction, the females rely on weakened trees and excavate egg laying galleries under the bark. Hymenopterous parasitoids are the most important natural enemies of bark beetles, and have been largely studied on Scolytines in many countries (Noyes, 2012). According to Balachowsky (1963), their role is much stronger than other natural enemies. On *Ruguloscolytus rugulosus* (Schvester, 1957), *Ruguloscolytus mediterraneus* (Gurevitz, 1975) and *Phloeotribus*

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*scarabaeoides* (Russo, 1938) authors indicate that the rate of parasitism by Hymenoptera is generally not negligible, but it can be considered as primordial limiting factor. Hymenopteran family Pteromalidae is an economically important family regulating the abundance of many insects including xylophagous ones. Several Pteromalidae were reported on *S. amygdali* (Mendel, 1986; Benazoun, 1983; Benazoun, 1999). *Raphitelus maculatus* Walker and other Pteromalid wasps were also reared from other Scolytinae like *Leperisinus varius* (Lozano and Campos, 1991) on Olive, *Hylesinus fraxini* (Nakladal and Turcani, 2007), *S. nitidus* (Buhroo *et al*., 2002; Buhroo *et al*., 2006), and elm bark beetles in Serbia (Stojanovic and Markovic, 2007). On *P. scarabaeoides* these wasps were reared by Russso (1938), Gonzalez and Campos (1990), and Benazoun (1999).

The identification of parasitoid and behavior study of their hosts is an essential demand in order to utilize in any biological control program. In Tunisia, there is little information, particularly in the systematics of parasitoids attacking bark beetles. For this reason, the present study aims to explore more information on the almond bark beetle parasites as important natural enemies for bark beetles.

**MATERIALS AND METHODS**

The almond trees infested with *S. amygdali* were chosen in an orchard under the supervision of the Professional Training Center of Agricultural Delegation Djemmal with GPS value (35°37'60" N; 10°46'0" E) Governorat of Monastir (Tunisia).

The rearing of *S. amygdali* on infested branches was carried out in the laboratory. The infestation of almond beetles was induced by cutting branches from the field and putting them in the rearing cabinets. Adult beetles and parasitoids in the boxes were collected daily and counted until the emergence stopped. The parasitoids were identified and listed. Moreover, parasitoid number and the proportion of a particular species out of the total (i.e., dominance coefficient) were determined. Collected parasitoids were stored in 80% alcohol and examined under microscope for morphological analysis. Photos were taken with a Canon Power Shot S50 camera attached to a Stereomicroscope LEICA (DMLB2), a MZ 12.5 Binocular and a SEM (Quanta 200 FEI).

After emergence, the length and diameter of each log was measured. The penetration holes (with or without secretion of gum) of adult bark beetles and natural enemies were observed to estimate the intensity of the attack. All life stages of beetles as well as natural enemies were counted separately. On the infested area of the branch pupal impressions whether superficial, deep, fully open, semi-obliterated or defaced were counted. This work gives idea about the population of the beetle and the parasitism. The attack density (AD) was calculated by counting the number of galleries per $cm^2$ area. The attack number (AN) represents the number of maternal galleries. The oviposition and the multiplication rate (MR) i. e., number of emerged adults divided by the number of maternal galleries were also calculated. The parasitism rate (PR) is the number of collected parasites divided by the number of maternal galleries. These parameters were determined according to Benazoun (1983). Statistical analyses were performed by SPSS version 17.0 using descriptive analysis and Pearson Correlation.

**RESULTS**

In Tunisia, there are many recorded parasitoids of *S. amygdali* collected from the Center of Tunisia at Djemmal in 2010 (Table 1).

Table 1. Dominance coefficient of *S. amygdali* parasitoids collected from the Center of Tunisia in 2010.

| Species | Dominance coefficient % |
|---|---|
| *Cerocephala eccoptogastri* (Zeiri *et al*. 2010) | 53,26 |
| *Cephalonomia hypobori* (Zeiri *et al*. 2011) | 19,06 |
| *Pteromalidae* | 14,88 |
| *Raphitelus maculatus* | 10,44 |
| *Eurytoma striolata* | 1,3 |
| *Eusandalum merceti* | 0,26 |
| Braconidae | 0,78 |

*R. maculatus* was first seen as a parasite on larva of *S. amygdali* during December 2009 and then it was reared in June 2010. The dominance coefficient for *R. maculatus* was 10.44% among the parasitoid complex attacking *S. amygdali*. However, *Cerocephala eccoptogastri* was more abundant parasitoid throughout the almond orchards having 53.26% coefficient of dominance. During bark dissection of rearing logs, larval, pupal and adult stages of *R. maculatus* were collected and the morphological studies carried out (Figures 1-3) according to Hedqvist (1963).

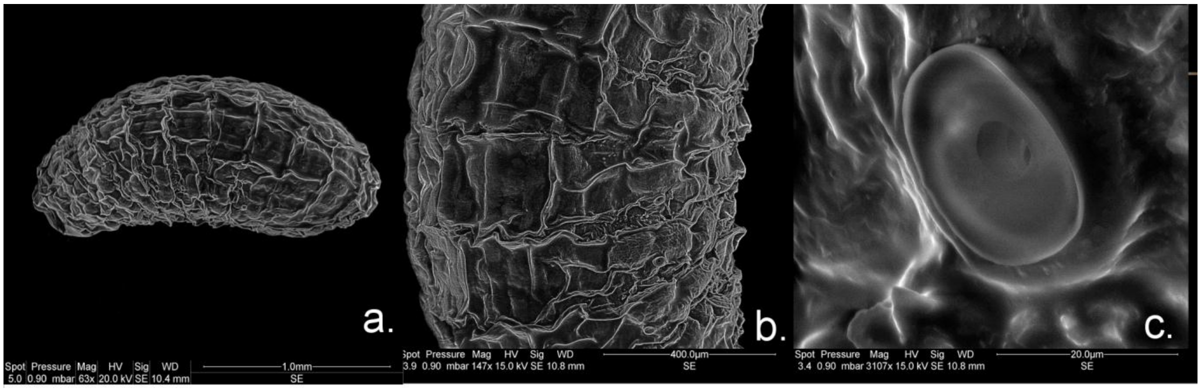


Figure 1. SEM photograph of larva of *R. maculatus*: a. Lateral view, b. Detail of larva, c. Opening of tracheal system of the larva (SEM: Quanta 200 FEI).

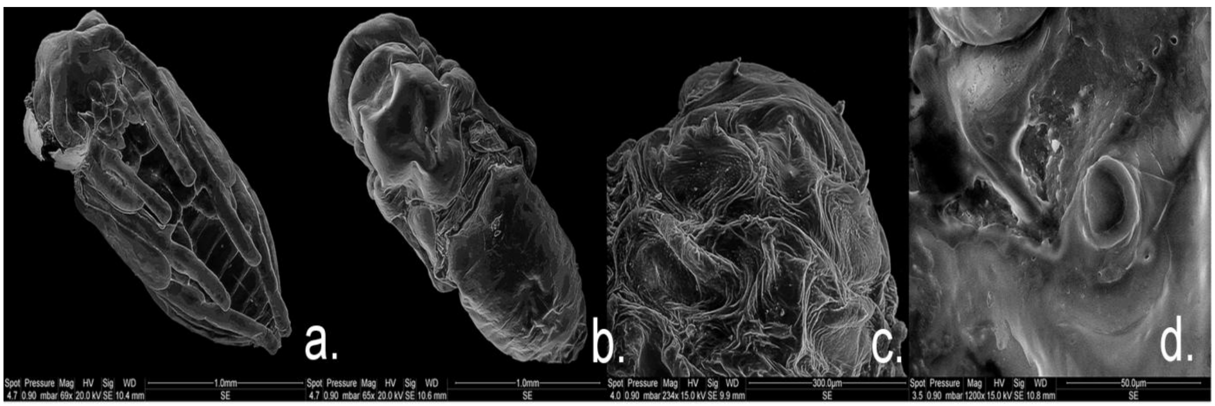


Figure 2. SEM photograph of pupa of *R. maculatus*: a. Ventral view, b. Dorsal view, c. Detail of terminal part of abdomen, d. Opening of tracheal system of the pupa (SEM: Quanta 200 FEI).

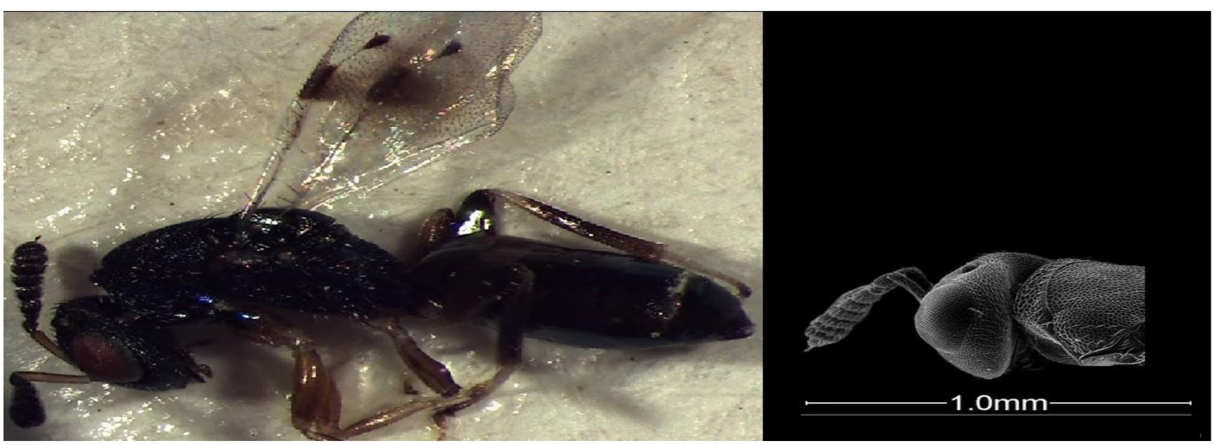


Figure 3. Female of *R. maculatus*: Lateral view of the head showing baculiform sensillum at the apex of calava of antenna (SEM: Quanta 200 FEI).

The larva is white with elongated body, flat on ventral side and convex dorsally. Its abdomen is 10 segmented. There are 9 pairs of spiracles: 1 pair in the mesothorax and 8 abdominal pairs. The head of pupa is depressed and suboval in shape. Its thorax is slightly convex, whereas the abdomen of pupa is oval and longer than the head and thorax together. In adult female, calava of antenna has baculiform sensillum at apex whereas the male antenna is without such structure. Antenna attached slightly above the level of lower margin of eye.

Propodeum with longitidunal carina and lateral rugae, length of marginal vein 2.8-3 times its width. Stigma height was not exceeding marginal vein.

*R. maculatus* was abundant in the field and in the rearing cages. The mortality was calculated between the deposition of eggs and adult emergence. The correlations between the parameters studied have been established. In the field, females of *S. amygdali* deposited 303.8 (±234.3 SD) eggs in 10.8 (±8.4 SD) maternal galleries (Table 2).

Table 2. Descriptive analysis of studied parameters of *S. amygdali* parasitized by *R. maculatus*.

| Parameter | Mean | SD | N |
|---|---|---|---|
| Oviposition (Eggs) | 303,78 | 234,319 | 9 |
| Attack number | 10,78 | 8,438 | 9 |
| Adult number | 152,89 | 93,364 | 9 |
| Attack density | ,070 | ,042 | 9 |
| Multiplication rate | 18,247 | 11,1269 | 9 |
| Parasitoid number | 17,78 | 10,918 | 9 |
| Parasitism rate | 2,187 | 1,5683 | 9 |
| Larval mortality of *S. amygdali* | 39,61 | 24,31 | 9 |

The average density of attack by the pest was 0.07 (±0.04 SD) galleries per $cm^2$. The mean multiplication rate was 18.2 (±11.1 SD). The mean rate of parasitism of *R. maculatus* was 2.1 (±1.6 SD) with a mean larval mortality of *S. amygdali* population 39.6% (±24.3 SD). The death of the larval population of *S. amygdali* cannot be purely due to the presence of *R. maculatus*. Other natural enemies that might have caused death of *S. amygdali* population weren't found. In addition to that, a part of the mortality was suspected due to overpopulation on the infested branches. These results were confirmed by the correlations established between the multiplication rate and attack density of *S. amygdali* (Table 3).

Table 3. Pearson correlation between various studied parameters of the population of *S. amygdali* parasitized by *R. maculatus* (only significant values given).

| | Length | Surface | Oviposition | AN | Adults | AD | MR | Parasitoids | PR | Mortality |
|---|---|---|---|---|---|---|---|---|---|---|
| Length | **1** | | | | | | | | | |
| Surface | | **1** | | | | | | | | |
| Oviposition | | | **1** | | | | | | | |
| AN | | ,761* | ,975** | **1** | | | | | | |
| Adults | | ,819** | ,858** | ,890** | **1** | | | | | |
| AD | | | ,957** | ,936** | ,796* | **1** | | | | |
| MR | -,756* | | | | | | **1** | | | |
| Parasitoids | | | | | ,701* | | | **1** | | |
| PR | | | | | | | ,846** | | **1** | |
| Mortality | | | | | | ,710* | ,837** | | | **1** |

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

The obtained results show positive relationship between the mortality percentage of the pest and its attack density. However, there was a statistically negative correlation between percentage mortality and the multiplication rate of the pest. Thus, an increase in the mortality of larval stages reduces the number of emerged adults. A galance on the data resulting from experiments shows positive relationship between adult pests and parasitoids. Similarly, there is positive relationship between parasitism rate and the multiplication rate of the host. The parasitism increases with the increase in the number of adults and their multiplication rate.

**DISCUSSION**

The almond bark beetle *S. amygdali* is attacked by many natural enemies. Hymenopterous parasitoids constitute one of the most effective natural controls due to their important role in pest control (Russo, 1931; Mendel, 1986; Campos and Gonzalez, 1990; Zeiri *et al.*, 2010; Zeiri *et al.*, 2011). The potential uses of these organisms for applied biological control of bark beetles have been assessed (Moeck and Safranyik, 1984). *R. maculatus* is an oligophagus parasitoid and can attack a wide range of beetle hosts at different population levels. In the present study, the average coefficient of dominance for *R. maculatus* on *S. amygdali* was found to be 10.44%. This indicates an increase of *R. maculatus* parasitism that could be augmented to suppress the almond bark beetle population. However, the abundance of *R. maculatus* on *H. fraxini* was between 2.1% and 3.9% from the total number of reared parasitoids with 20.1% total mortality (Nakladal and Turcani, 2007). The average coefficient of dominance for *R. maculatus* on *S. nitidus* was recorded to be 6.67% (Buhroo *et al*., 2006). Stojanovic and Markovic (2007) obtained *R. maculatus* up to 58% on the samples of *S. multistriatus, S. kirschii, S. ensifer* and *S. pygmaeus* whereas, the average domination of *R. maculatus* on *S. intricatus* was 0.26% ; with a maximum of 7.69% (Markovic and Stojanovic, 2003).

*R. maculatus* was also well studied on *P. scarabaeoides* by Campos and Gonzalez (1990) in Spain. Its longevity and fecundity were influenced by the conditions of feeding. The impact of *R. maculatus* on *P. scarabaeoides* population was most prominent and constitute 7.5% of the total number of parasitoids captured on *P. scarabaeoides* (Gonzalez and Campos, 1990). Mendel (1986) counted 32% *R. maculatus* on *P. scarabaeoides* but it was rare on other beetles such as *S. rugulosus mediterraneus* (3.6%) and *S. multistriatus orientalis* (2.3%). Detailed studies have also been carried out on *R. maculatus* in Egypt showing the important impact of the parasitoid on the population of *S. amygali* (Youssef *et al*., 2006; El-Latif *et al*., 2009). Parasitoids usually locate their host by walking on the bark, paralyzing the larvae by injecting venom, and laying eggs on the paralyzed host. Eggs and larvae develop quickly (Kenis *et al*., 2004). The food and water are important demands on parasitoid's life (Campos and Gonzalez, 1990; Mendel, 1988). The number of eggs laid by females of *R. maculatus* was 44.5 in average and the egg eclosion occurs on the second day from deposition (Campos and Gonzalez, 1990). The larval development takes about 15 days (Russo, 1938; Gonzalez and Campos, 1990).

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