

Duration of the Development Stages of *Rhizophagus grandis* Gyll. (Coleoptera, Monotomidae) in Laboratory Rearing on *Picea orientalis* Logs in Turkey*

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ABSTRACT

In this study, the duration of the development stages of *Rhizophagus grandis* Gyll. (Coleoptera, Monotomidae) in “log-breeding” method in laboratory conditions was investigated. Rearing studies were carried out in *R. grandis* Biological Control Laboratory of Maçka Forestry Enterprises throughout 28th of April and 14th of July 2009, under the conditions of $22.5^{\circ}\text{C}\pm 2$ constant temperature, $72\pm 5\%$ proportional humidity in perpetual darkness. The first prepupae emergence was observed within 31 days at the earliest and 38 days at the latest following introduction of maternal *R. grandis* mature couples to the logs. Total elapsed time for feeding by maternal *R. grandis* before copulation, their copulation and depositing eggs, embryo development of the eggs and completion of larval stages were 34.5 ± 2.7 days on the average. On rearing logs, depending on the number of obtained *R. grandis* prepupae, the time elapsed between the dates of the first and the last prepupae emergence was minimum 1 day and maximum 29 days. Prepupae emergence have lasted 14.8 days (3-29 days) on the average. A positive linear correlation was found between the quantity of *R. grandis* prepupa and prepupae emergence periods. As the number of prepupae increases, the duration of prepupae emergence gets longer ($r=0.75$, $p<0.003$). In an average of 67.7 ± 2.7 (61-71 days) days following the introduction of maternal *R. grandis* to the logs, 94.7% of the new *R. grandis* adults were obtained.

Keywords: *Rhizophagus grandis*, biological control, mass rearing, development stages

I. INTRODUCTION

Understanding the life cycle of *Rhizophagus grandis* (Gyllenhal) is important in its effective use and the maintenance of its effectiveness as a control agent [1]. The life cycle of *R. grandis* is similar to the life cycle of *Dendroctonus micans* (Kugelann) [2], which can be in any level of developmental stage in any time of the year and therefore the predator has a flexible seasonal development process. Life cycle of *R. grandis* can be divided into two distinct stages. The first one is the stage under the bark that ends with prepupae coming down from the tree to the ground, in which hatched larvae feed on *D. micans* and develop into prepupae; and the second stage in which prepupae, pupae and adult develop in the soil [1].

The prepupae stage is the non-feeding mature larval stage of *R. grandis*. After finishing the feeding period, the larvae enter the prepupae stage and most of them leave the *D. micans*

incubation system and fall down from the tree in order to enter the soil [1]. They turn into pupae in the soil, then finally reach the trees and in the *D. micans* galleries come out as adults ready to begin the new reproduction cycle [3]. *R. grandis* has 3 larval stages. The larvae develop in room temperature, at least, in 25 days. Individuals that develop and become prepupae come out as young adults in 45 days. The first 25 days of this period is the pupation stage and in the last 20 days young adults mature. Adult males hatch earlier than females.

Female predators lay 100 [5], 30-117 [6] eggs in the laboratory. *R. grandis* lay eggs took less than 2 weeks to deposit eggs in the laboratory conditions. There was a big difference between numbers of eggs that were deposited but they usually deposited 70-80 eggs [4]. In this study, researchers aimed to determine the suitable conditions for *R. grandis* rearing in laboratory on logs and tried to determine the time intervals of development stages for this predator so that proper production period, time and period for sensitive prepupae to get down on the soil, and right humidification period for prepupae and pupae in the soil are known.

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II. MATERIAL AND METHODS

Rhizophagus grandis (Gyllenhal) (Coleoptera, Monotomidae) rearing studies were made in *R. grandis* Rearing Laboratory between the 28th of April and the 14th of July 2009, at $22.5^{\circ}\text{C}\pm 2$ constant temperature, $72\pm 5\%$ proportional humidity and in sustained dark room conditions. The material of the study consists of 6,000 *Dendroctonus micans* (Kugelann) (Coleoptera: Curculionidae: Scolytinae) larvae (IV. period), 30 female, 30 male *R. grandis* adults and 13 fresh logs of spruce, 14-20 cm in diameter and 28-34 cm in length.

New offsprings of *R. grandis* prepupae coming out of the logs were observed and recorded. For this purpose, production logs were hung on shelves with nylon threads, 15-20 cm above the aluminium basins they were kept in. In order to count the prepupae coming out of the hung logs, transparent nylon bags that did not cut the air circulation were spread over and under the logs. Nylon bags were regularly taken off, the prepupae in the bags counted and placed on the sand in 2-3 cm deep aluminium and polyethylene basins and logs were covered with nylon bags again. This process was repeated until the last prepupae were seen.

For egg-laying *R. grandis* females on logs, the number of new offspring *R. grandis* prepupae per female and the number of days that prepupae appearance continued were compared using correlation analysis in order to determine if there was a statistical connection. The logs which were prepared for biological observation, were used to observe the development stages of *D. micans* and *R. grandis*. Also, based on the amount of live adult *D. micans* that were acquired from each rearing log and the dates that these started to fly, it was possible to study the effects of meeting predator's need for prey on the production amount. Furthermore, in this study, parametric analysis by square root transformation was applied. For all the statistical evaluation, SPSS (Statistical Package for Social Science) 15.0 statistical software was used.

III. RESULTS AND DISCUSSION

From the 13 logs in which rearing went well, 1,026 *R. grandis* prepupae were obtained in total (Figure 1). Average prepupae number for each log was 78.92 (3-237). From rearing logs

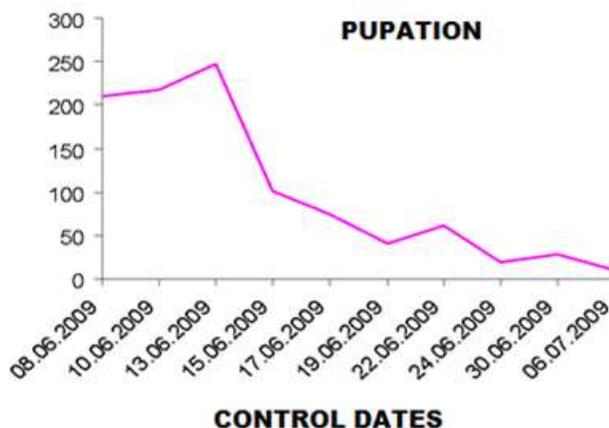


Figure 1. Distribution of *R. grandis* prepupae on rearing logs according to the control dates

number 4, 6 and 9, small number of *R. grandis* prepupae were obtained; 9, 3 and 7, respectively. In addition to this, from the logs numbered 5, 7 and 11, high amounts of *R. grandis* prepupae were obtained: 185, 237 and 202, respectively. From each *R. grandis* egg-laying adult female, approximately 39.46 new offspring prepupae were obtained.

First prepupae were seen on 8 June 2009 and the last ones hatched on 6 July 2009. First prepupae hatched on production logs on the 8th, 10th, 13th, and 15th of June (31 days, at the earliest; and 38 days, at the latest; after egg-laying adult *R. grandis* couples were placed on growing logs). Based on these results, the total period for egg-laying *R. grandis* adults to feed before mating, to mate and lay eggs; and for eggs to become embryo and larvae is 34.54 ± 2.73 days (Figure 1).

Based on the number of *R. grandis* prepupae obtained, the period between first and last prepupae to hatch on the rearing logs was at least 1 and at most 29 days. On log number 6 from which three *R. grandis* prepupae were obtained, prepupae hatched in 1 day. On the rearing logs number 1, 5, 7 and 11, prepupae hatching took 18, 23, 23 and 29 days, respectively. Prepupae kept hatching (except log number 6) for approximately 14.75 (3-29 days). For the egg-laying *R. grandis* females, correlation analysis was used to test whether there was a statistical relation between the amount of new spawn *R. grandis* prepupae and the number of first prepupae hatching days. Correlation analysis resulted in positive linear correlation between new offspring *R. grandis* prepupae amount and prepupae hatching periods. Prepupae hatching period lengthens as prepupae number rises ($r=0.75$, $p<0.003$). Egg laying *R. grandis* adults were placed on the logs approximately 8-7 days after the prior placement

of *D. micans* larvae. First prepupae were observed approximately 34.54 ± 2.73 (31 - 38) days after egg laying adults were placed on the logs. The period between the placement of egg-laying adult *R. grandis* couples on rearing logs and when almost all of the new offspring *R. grandis* adults were obtained was 67.7 ± 3 (61-71) days on average. Approximately 34.54 ± 2.73 days (at least 31, at most 38 days) of this period comprised the placement, feeding, mating and egg-laying of *R. grandis* adults. This also includes the offsprings' embryo development and the time in which they became prepupae, including the completion of their larvae period. Hatching time distribution of new offspring prepupae obtained from logs with normal production is shown in **Figure 1**. Figure 1 shows that the highest rates of new offspring *R. grandis* prepupae hatching occurred in the first few dates of observation (June 8 - June 15 2009). Hatching was very fast during the first 10 days and gradually slowed down after. For most of the production logs, first prepupae hatching was highest on the 31st day after the egg-laying *R. grandis* were placed on logs. 63.74% of first new offspring *R. grandis* obtained were counted 31 days after egg-laying adults were placed on logs. Percentages of prepupae hatching at 33 days, 36 days, and 38 days following the placement of egg-laying adults, were 5.75%, 15.40% and 15.11%, respectively.

On the other hand, there was only a seven-day difference in the separate periods it took for first prepupae to hatch in all the different logs (31-38 days). Prepupae hatching took at least 3 and at most 29 days (Figure 1). Moreover, there was only a seven-day long difference between

the time it took for egg-laying *R. grandis* adult females to feed, mate and lay eggs; and according to the number of eggs these females deposit, egg deposition period varies between a few days (3-5 days) and a few weeks (7-29 days).

During the period that new offspring *R. grandis* prepupae hatching was observed, at the end of production stage, the amount of adult *D. micans* not eaten and flew off from logs were also counted. During the rearing process in which fourth stage *D. micans* larvae were used, first *D. micans* adults flew forty-four (44) days after the larvae were placed on logs. Flight duration of *D. micans* adults that were not eaten by *R. grandis* and flew off can be seen in **Figure 2**.

When the figure is examined, it can be seen that adult *D. micans* flights are highest between the 3rd and 5th day following the first flight and that flight density continuously decreased after this. Comparison of the total amount of new offspring *R. grandis* prepupae and the number of uneaten adult *D. micans* that were left on the logs and flew off within a certain period can also be seen in **Figure 2**. When flight of *D. micans* adults and new offspring *R. grandis* prepupae hatching are compared, it can be seen that *R. grandis* prepupae hatching begins a few days before the flight of *D. micans* adults (Figure 2). First *R. grandis* prepupae hatched two (2) days before the first *D. micans* flight. Within these breeding conditions wherein *D. micans* larvae were 85% in the 4th and 15% in the 5th phase, *D. micans* adult hatching began right after *R. grandis* prepupae hatching was highest and continued until this process was over. In these

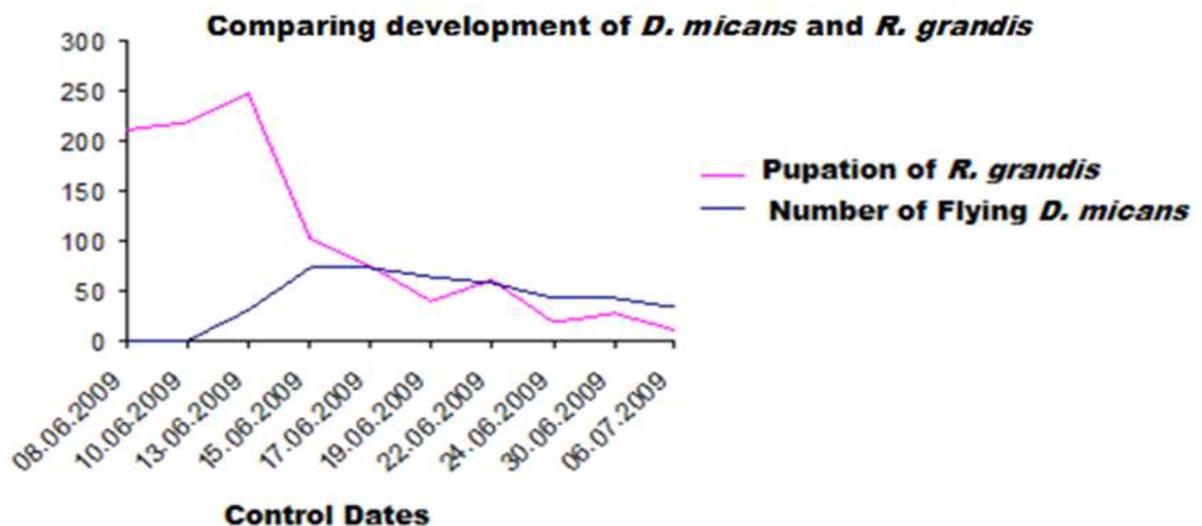


Figure 2. Distribution of amount of new offspring *R. grandis* prepupae amount obtained from the logs and amount of flying *D. micans* adults depending on time and interconnected development of each species

conditions, it was observed that until the prepupae phase that *R. grandis* completed the larvae phase and left the log to get down on the sand, there was always sufficient number of *D. micans* individuals to feed on under the bark.

Previous studies have shown that female predators lay approximately 100 eggs in laboratory conditions [5]. *R. grandis* females lay approximately 30 to 117 eggs and in the rearing studies done in 1986 which made use of polyester boxes, 30-70 adults were rearing per one female *R. grandis* [7]. In the rearing studies which made use of logs, approximately 80 adults were obtained from 1 male, 1 female *R. grandis* couple [8]. The number of eggs differed greatly but such were estimated to be usually 70-80 eggs [4]. In this study, approximately 237 prepupae from two females placed on one production log was obtained. It can thus be said that a female can deposit 120 or more eggs.

A period of with a minimum of 61 days, and a maximum of 71 days elapsed between the time egg-laying *R. grandis* adult couples were placed on logs and the time new offspring *R. grandis* adults were obtained. Average expectancy period for logs was 67.69 ± 3.0 days. It was observed that egg deposition period for female *R. grandis* extended depending on the amount of eggs and that prepupae hatching period also changed depending on this. The laying of too many eggs lengthens the prepupae hatching period. As prepupae numbers increased, the number of days during which prepupae hatching continued also increased ($r=0.75$, $p<0.003$). This result shows that the amount of incubation time for rearing logs may change in relation with *R. grandis* production amount per log.

The amount of time between egg-laying females were placed on logs and larvae became prepupae was approximately 34.54 ± 2.73 days and when average prepupae hatching continuation period was added, the amount is 49.29 ($34.54+14.75$) days at most. The average of this 49.29-day period is 52 days at most [8]. Therefore, embryo development of eggs and larvae period is a total of 45 days at most [8]. It was observed that pupae phase was approximately 8 days and 11-12 days after pupae were seen.

In other *R. grandis* rearing studies, average incubation period for logs from which 94.68% of adults were obtained is 67.69 ± 3.0 days. In another study made in approximately 22°C and 75% humidity, this period was 69 days. In this environment, predator insect completed its generation in approximately 67 days [8].

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