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ORIGINAL RESEARCH ARTICLE

Assessing the laboratory mass rearing of Predator Beetle *Rhizophagus grandis* Gyll. (Coleoptera: Monotomidae)

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Abstract

The aim of this study was to determine the progeny production of predator beetle *Rhizophagus grandis* (Gyllenhal) (Coleoptera: Monotomidae) under laboratory conditions. Different numbers of male and female predators introduced into breeding logs and the numbers of new progenies and their sex ratios were determined in the early and late spring periods. A total of 8776, 13,402, 5742 and 7864 *R. grandis* individuals were reared in 60, 109, 128 and 56 breeding logs in four consecutive years, respectively. Of the prepupae that have dropped from breeding logs to enter the sand in experimental basins, 81.4 % of them have emerged as adults at the end of the experimental periods. The average number of *R. grandis* per log was 146.2, 123, 44.9 and 140.4 according to experimental years, and 181.7 and 93.2, 123, 62.4 and 32.9, 163.8 and 101.5 according to the early and late spring periods in the experimental years, respectively. In the second year of the experiments, only early spring rearings have been performed that have yielded 123 *R. grandis* per log on average. Progeny production of a female *R. grandis* was 18.3, 18.7, 5.8 and 17.6 on average, 2004, 2005, 2006 and 2007 respectively. Different numbers of predator females and males were used in early and late spring experiments in the study. Progeny production was higher in early spring when the experiments were performed with 6 females and 2 males. Of the 35,784 *R. grandis* individuals that were obtained from the experiment, 16.4 %, 28.3 % and 55.3 % were collected from the first, second and third part of the sand environment where the breeding logs kept during experiment.

Keywords Biological control · Progeny production · Predation · Bark beetle

Introduction

Oriental spruce, *Picea orientalis* (L.) Link. is one of the most important tree species (Kayacik 1995) in the northeastern Black Sea region of Turkey and the western parts of Georgia (Davis 1965). *Dendroctonus micans* (Kugelann) (Coleoptera: Curculionidae), which has a devastating effect on oriental spruce forests, was first discovered in 1957 in Georgia and by 1963 had heavily infested the oriental spruce forests of the country (Khobakhidze et al. 1970). It was first recorded in Posof in 1966 in Turkey (Acatay 1968), and then in all oriental spruce stands neighboring Georgian border (Serez 1979; Benz 1984; Alkan 1985). *Dendroctonus micans* has established in all of the spruce forests in Artvin at the end of the 1990s (Eroglu 1995; Alkan 2000; Eroglu et al. 2005), and today it has well established in all of the oriental spruce forests in the Eastern Black Sea region of Turkey (Alkan Akinci et al. 2009, 2014). However, sporadic outbreaks of the pest make it difficult to control (Fraser et al. 2014). Therefore, it is necessary to carry out regular surveys to identify an epidemic risk (Anonymous 2012).

Although there is no effective measure to prevent the natural spread of *D. micans* (Jeger et al. 2017), the management of the pest depends on detailed surveys to determine the extent of the outbreak, salvage logging and sanitation fellings to reduce the beetle population, spot peeling to ensure the removal of all beetle stages under the bark and limiting the

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transport of spruce logs to prevent the spread to far distances, and an effective biological control program that will be performed by the predator beetle *Rhizophagus grandis* (Gyllenhal) (Coleoptera: Monotomidae) (Grégoire et al. 1985; O'Neill and Evans 1999; Evans and Fielding 1994; Alkan-Akinci et al. 2010). In biological control programs that are important components in pest suppression (Pearson and Callaway 2003), managing predators and parasitoids can support the decrease of bark beetle damages (Moeck and Safranyik 1984). The ecological relationship between predator and prey is important in keeping the growing populations of bark beetles under control (Fetting and Hilszczannski 2015; Cebeci and Baydemir 2018; Kocoglu and Ozcan 2018).

Rhizophagus grandis is a totally specific predator and has been found only in *D. micans* galleries (Grégoire 1988; Dohet and Grégoire 2017). It is an important factor in limiting *D. micans* populations (Khobakhidze 1965). Although the predator beetle does not completely eliminate the population of the pest (Anonymous 2012), it has been stated that the amount of *D. micans* decreases with the increasing amount of *R. grandis* at the field conditions (Evans and Fielding 1994). Results from laboratory studies also indicate that *R. grandis* is an important limiting factor on the prey (Merlin et al. 1984; Grégoire et al. 1989; Alkan Akinci and Grégoire 2016). *Rhizophagus grandis* follows its prey in the field wherever it is possible. But there is time lag between prey and predator movements. This time lag has been recorded as 15 years in Holland and 50 years in Belgium (Grégoire 1988). This time lag is about 20 years in Turkey (Serez 1987). *Dendroctonus micans* causes severe damages at outbreak areas during this time lag between the movement of itself and *R. grandis* (Grégoire et al. 1985). *Dendroctonus micans* reaches an epidemic level before the predator arrives in its distribution area, and control of the damage becomes nearly impossible. Therefore, it is important to release *R. grandis* artificially in *D. micans*' distribution area in order to have the chance of effective control by eliminating geographical and numerical differences (Grégoire et al. 1985). It is known that *R. grandis* eventually occupies more than 80 % of its prey's brood systems (Grégoire et al., 1985). Similarily, former studies showed that *R. grandis* individuals have occupied 78–84 % of the *D. micans* galleries in the oriental spruce forests (Özcan et al. 2006; Özcan 2009; Alkan-Akıncı et al. 2010). *R. grandis* is an important component of biological control program and its population should be supported by release studies in the *D. micans* infested regions.

In this study, it is aimed to contribute to the Turkish mass rearing program that is based on log-breeding method and carried out by the Regional Directorate of Forestry in Artvin and Trabzon. Log-breeding method has some drawbacks. Such as yield unpredictability, labour intensiveness, need for space and need for hundreds of thousands of *D. micans* larvae for introducing to the breeding logs are mentioned and discussed in some works (Grégoire et al. 1984a, b; King and Evans 1984; Aksu 2011; Alkan Akinci and Grégoire 2016). Our study will contribute to yield unpredictability, which is one of the most important drawbacks of log-breeding method.

For this purpose, progeny production of *R. grandis* under laboratory conditions, the amount of new progenies obtained according to the different numbers of *R. grandis* pairs introduced into breeding logs in early and late spring periods, their distribution in the sand environment in which the breeding logs were kept, and the sex ratios of new offsprings were assessed. Thus, several aspects of the interactions between predator and prey were evaluated.

Material and method

This experimental study was carried out in the Maçka Biological Control Laboratory of Trabzon Regional Directorate of Forestry from 2004 to 2007. Laboratory temperature was 21 ± 2 °C and humidity was 75 %±5. The results of the experiment were evaluated in two separate periods as early spring and late spring period. The results from *R. grandis* pairs that were introduced during 3–31 March were evaluated as early spring results and 5 April − 7 June as late spring results (Table 1).

Preparation of breeding logs

Wild *R. grandis* adults and wild *D. micans* larvae that were collected from Yeşiltepe oriental spruce forests in Maçka (Trabzon) were used in the rearing experiments. A total of 2642 female adults of *R. grandis*, 1138 male adults and approximately 255,570 *D. micans* larvae were used. Fresh oriental spruce logs that were 16–31 cm in diameter and 22–39 cm in length were prepared. Logs were engraved 1.5–2 cm in width and depth, and 17–20 cm in length by a chisel in the wooden part with leaving the covering bark on the upper surfaces and on the opposite sides of the breeding logs. Then wild *D. micans* larvae were introduced into these engraved parts. The larvae were kept for 5–7 days to feed in the phloem and establish in the breeding logs. After this period, *D. micans* larvae that could not feed and go down in the phloem were collected and removed from the logs (Fig. 1a, b and c).

The engraved parts were filled with cotton and the upper surfaces of the logs were covered with paraffin. Then *R. grandis* pairs introduced into breeding logs. For this purpose, bark was cut in a triangular shape, and predators were introduced to logs where *D. micans* larvae were present. After introducing the predators, cut bark were closed back with sealing material. Prepared logs were placed perpendicularly into the aluminium and polyethylene basins

Table 1 Period of preparation of breeding logs, number of logs prepared, *Rhizophagus grandis* individuals introduced into logs and breeding times

Year	Period	Number of logs	Number of *R. grandis* parents introduced into each logs	Preparation dates of breeding logs	Breeding time (days)
2004	ES*	36	8 ♀ −4 ♂	3–31 March	70
2005	ES	78	6 ♀ −2 ♂	3–31 March	71
2005	ES	31	8 ♀ −4 ♂	3–31 March	71
2006	ES	52	6 ♀ −2 ♂	3–31 March	60
2007	ES	35	8 ♀- 4 ♂	3–31 March	69
2004	LS**	24	8 ♀ −4 ♂	5 April- 7 June	70
2006	LS	53	6 ♀ −2 ♂	5 April- 7 June	59
2006	LS	23	16 ♀ −8♂	5 April- 7 June	59
2007	LS	21	8 ♀- 4 ♂	5 April- 7 June	69

ES*: Early spring, LS**: Late spring

that had sterilized sand at the bottom. The prepared logs were then placed on shelves in the laboratory (Fig. 1d, e and f g, h, i).

Breeding logs were kept in the laboratory during experimental periods that were determined depending on preliminary experiments and works of Keskinalemdar et al. (1986) with Alkan and Aksu (1990). Sterilized sand in the basins were moisturized properly. Experimental periods include a period of time that start from introduction date of *D. micans* larvae to breeding logs untill collection of new progenies of *R. grandis* from sand environment. Breeding time that were determined depending on preliminary experiments and former works include a period of time that start from introduction date of *R. grandis* pairs into breeding logs untill the collection of new progenies from sand environment.

Fig. 1 **a** Setup of the rearing experiment (**a**) introducing wild *D. micans* larvae into the engraved parts, (**b**) engraved parts on the opposite sides of the breeding log and larval frass produced by introduced *D. micans* larvae, (**c**) cleaning larval frass, (**d**) closure of the engraved parts where the larvae were introduced, (**e**) covering upper surface of the log with paraffin, (**f**) *D. micans* larvae feeding in the phloem, (**g**) introducing wild *R. grandis* pairs into the log, (**h**) breeding log in sterilized sand in basin, (**i**) breeding logs on the shelves in the laboratory

Gathering *Rhizophagus grandis* from experimental logs and sex determination studies of new progenies

At the end of the breeding time, new progenies of *R. grandis* that were in the sand were collected and counted with using a brush. The sand in the basin was evaluated in three separate parts. When the logs were removed from the sand, some of the sand was got stucked on the lower surface of the log, that was in contact with the sand. The sand on the log has been assessed as part one (part I). The sand under the breeding log and that was still in basin has been assesed as part two (part II), and the remainder sand in the basin as part three (part III) (Fig. 2). The average weights of the sands in these three parts have been determined.

In addition, the sexes of 6771 *R. grandis* adults obtained from 72 of the breeding logs were determined by a binucular microscope. The sex discrimination of *R. grandis* individuals was determined according to Tosun (2008) that is described by the prominent angled aedegus located at the end of the abdomen in male individuals.

Statistical analyses

Data were analyzed using IBM SPSS statistics version 20.0 for Windows® software. The number of new progenies, the amount of new progenies obtained according to the different numbers of *R. grandis* pairs introduced into the breeding logs were compared between years, and early spring and late spring periods. The distribution of new progenies in the sand environment in which the breeding logs was kept was compared between sand environment parts. The differences between the mean of the two groups comparisons were determined by the independent samples t-test and the differences between the means of more than two groups based on the same variables were determined by one-way Anova.

Results and discussion

Progeny Production of *Rhizophagus grandis* under laboratory conditions

A total of 8776, 13,402, 5742 and 7864 *R. grandis* progenies were obtained in the breeding logs in the experimental years, respectively. The numbers of *R. grandis* progenies in different biological stages that are obtained from the logs are presented in Table 2. Minimum number of new progenies per log was 1 and maximum number of new progenies obtained per log was 477. Accordingly, minimum and maximum number of new progenies per parental female in a log was 0.13 and 103, respectively. Higher number of new progenies per parental female in a log was obtained in early spring experiments (Table 2).

Average breeding time (± SD) was 66.4 (± 6.4) days. At the end of breeding time, 76.3 % of the total progenies were adults, 5.1 % were young adults, 13.2 % were prepupa and 5.4 % were pupae. At the end of the experimental periods, 81.4 % of the progenies have become adults. Grégoire et al. (1989) stated that 72 % of the *R. grandis* individuals obtained at the end of 60–80 days were adults, 18 % were prepupa and 10 % were larvae during their breeding experiments performed in polyester boxes at room temperature in 1986. These results support the validity of our breeding method and the adult rates obtained from breeding experiment. It is stated that the generation time of the predator is 67 days on average when the rearing has been performed at an average temperature of 22 °C and 75 % humidity environment (Keskinalemdar et al. 1986; Alkan and Aksu 1990). At least 25 days at room temperature are needed for incubation of the eggs and larval development and plus 45 days for prepupas to develop into young adults (Merlin et al., 1984). Data from the larval, prepupa and adult stages of *R. grandis* and the timing of the life cycle indicate that this species has an excellent survival strategy. Furthermore, the predator's ability to survive for long periods and having flexible phenology enables consume most development stages of its prey that can be found at any time of the year. The observed delay in prepupal

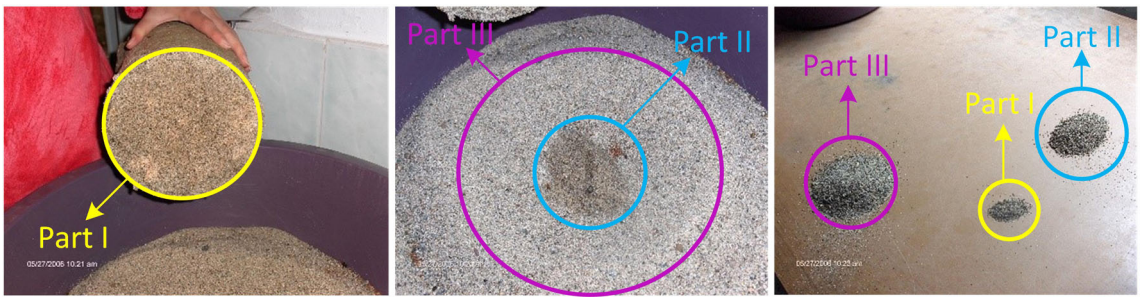

Fig. 2 Parts of sand where *Rhizophagus grandis* progenies are collected

Table 2 Number of *Rhizophagus grandis* progenies according to the years and periods

Year	Period	Number of Parental *Rhizophagus grandis* females	Number of logs	Adults	Young adults	Pupae	Prepupae	Total number of new progenies	The average number of new progenies per parental female	Min. and max. number of new progenies per log	Min. and max. number of new progenies per parental female in a log
2004	ES*	8♀	36	4729	443	744	624	6540	22.7	48–396	6-49.5
2004	LS	8♀	24	1395	147	230	464	2236	11.7	1-244	0.13–30.5
2005	ES	6♀	78	7847	592	571	1347	10,357	22.1	6-369	1-103
2005	ES	8♀	31	1947	95	111	892	3045	12.3	12–236	1.5–29.5
2006	ES	6♀	52	1980	267	78	918	3243	10.4	4-364	0.67–60.67
2006	LS**	6♀	53	1515	143	78	223	1959	6.2	1-164	0.17–20.67
2006	LS	16♀	23	392	16	20	112	540	1.5	3–72	0.19–4.5
2007	ES	8♀	35	5390	113	95	134	5732	20.5	14–477	1.75–59.63
2007	LS	8♀	21	2113	4	8	7	2132	12.7	26–256	3.25-32
TOTAL			353	27,308	1820	1935	4721	35,784			

ES*: Early spring, LS**: Late spring

development stage is important features that enable the predator to exploit its prey effectively (King et al. 1991; Fielding 1992).

The average number of *R. grandis* progenies per log and per female *R.grandi*s according to the experimental years was 146.2, 123, 44.9, 140.4 and 18.3, 18.7, 5.8, 17.6, respectively. In the 3rd year of the study (2006 experiments), the average number of *R. grandis* progenies per log was 3.3, 2.7, 3.1 times lower than the other years, and the new progeny yield of a female *R. grandis* adult was approximately 3.2, 3.2 and 3 times lower than the other years (Table 3).

There was a significant decrease in terms of new progeny yields of *R. grandis* in 2006 experiment. There were enough prey larvae in the breeding logs to be consumed by parental pairs of *R. grandis* so the decrease in the new progeny amounts may not be directly linked to the supply of nutrients. The decrease in the number of progenies may be related to the decrease in egg laying performance of parental females introduced into breeding logs.

In some years sharp decreases have been recorded in the number of new progenies of *R. grandis* that are obtained from mass rearing programs carried out by the Regional Directorates of Forestry. These decreases in almost all the breeding logs can suggest that these results may be developed depending on ineffective conditions that occurred in those particular years. These may be related to the development stage of overwintering *D. micans* and reproduction success of wild *R. grandis* adults. These decreases can be observed only in a limited number of breeding logs of same year.

Despite a fungal isolation and identification has not been performed in the study, fungal origin diseases are known to be the one of the reasons that cause mortality of new offsprings.This situation may be due to a fungal origin disease that affects *R. grandis* individuals at the different biological stages. The entomopathogenic fungus *Beauveria bassiana* (Hyphomycetes) is known to parasitize insect larvae, pupae and adults (Fielding and Evans 1997; Zhao et al. 2008; Davydenko 2018). The disease has been appeared in mass rearing program in Britain, and caused up to 80 % mortality of incubating *R. grandis* pupae and adults (Fielding and Evans 1997). *B. bassina* is one of the main factors in rearing programs (Fielding and Evans 1997; Zhao et al. 2008). Fielding and Evans (1997) state that during British main breeding program it became a constant problem among pupae and adults of R. grandis, and it occasionally caused total mortality.

Table 3 Average number of *Rhizophagus grandis* progenies per year

Year	Mean *R. grandis* progeny per log (Mean±SD)	Mean progeny per female *R. grandis* (Mean±SD)
2004	146.2±94.8	18.3±11.9
2005	123±98.2	18.7±16.4
2006	44.9±53.4	5.8±8.9
2007	140.4±106.7	17.6±13.3

Dendroctonus micans larvae that are used in breeding logs can form multiple galleries during their establishment in the phloem. During the introduction of maternal *R. grandis* adults to the breeding logs, it is possible to introduce these predator adults to a smaller larval gallery through conventional mass breeding method in which predator adults are being introduced to breeding logs by cutting the bark in a triangular shape on the lateral side of the logs. As a result, this kind of introduction may cause low reproductive productivity due to nutrient shortages. However, in recent years, the fact that the maternal *R. grandis* adults are being introduced to the breeding logs not from the lateral side, but from the top into the engraved parts where *D. micans* larvae are introduced, has largely eliminated this disadvantage.

The amount of new progenies obtained according to the different numbers of *Rhizophagus grandis* pairs introduced into the breeding logs

The difference between the number of new progenies from the experiments in 2004 and 2007 were significant in the early and late spring experiments when the experiments were performed with 8 females (Year: 2004; t test, $t = 4.045$; $p < 0.05$; Year: 2007, $t = 1.685$; $p < 0.05$). The average number of new progenies in the early spring experiments in 2004 was approximately 1.9 times higher than the late spring averages, and in 2007 it was 1.6 times higher than the late spring averages (Fig. 3). The difference between the number of new progenies from the experiments performed with 6 and 8 females in early spring in 2005 was significant ($t = -2.932$; $p < 0.05$). The average number of new progenies in the breeding logs that had 6 females was 1.8 times higher than the progenies of 8 females (Fig. 3).

Although there was a significant decrease in the number of *R. grandis* progenies obtained in the 2006 experiments, the difference between the number of new progenies from the experiments performed with 6 females in early and late spring was significant ($t = 4.434$; $p < 0.05$). The average number of new progenies in the early spring period was approximately 1.7 times higher than the late spring period. There was also a significant difference between the average number of new progenies in the experiments that were performed by introducing 6 and 16 females (t test; $t = 2.814$; $p < 0.05$). The average number of progenies in the experiments by introducing 6 females was approximately 4 times higher than introductions of 16 females (Fig. 3).

In this study, the average number of new progenies of *R. grandis* was always higher during the early spring period. Merlin et al. (1984) states that during laboratory rearing in tubes when a female *R. grandis* who laid eggs in a tube was taken into a new breeding tube where no *R. grandis* female had been placed before, this individual began to lay eggs again. However, no information is given about the egg yield of these females. Wild *R. grandis* adults were used in our experiments. They were collected from active *D. micans* galleries in the field. The ones that were used in the early spring period experiments were collected from the field at the beginning of the vegetation period. So during our experiment they may have laid all their eggs in our breeding logs. But the ones that were used in late spring period experiments were collected after the vegetation period have started. So these predator females may have laid some of their eggs in the *D. micans* galleries that were in the field and they may not show higher performance during the experiments as the ones collected at the beginning of the vegetation period.

In addition, when different numbers of *R. grandis* females (6 and 8 females) were used in early spring experiments in the same year, the average number of new progenies was higher in experiments with 6 females. When different numbers of *R. grandis* females (6–16 females) were used in the late spring experiments in the same year, the average number of new progenies was higher in the experiments with 6 females. A total of 80 *R. grandis* adults were obtained from 1 male and 1 female *R. grandis* pair in a rearing study in the log (Keskinalemdar et al. 1986). When the experimental years are evaluated within themselves, a total of 18.3, 18.7, 5.8 and 17.6 new progenies were obtained on average in the experimental years, respectively. The highest number of new progenies per parental female was 22.7 on average in the early spring period and by 8 parental females. Maximum number of new progenies per parental female in a log was 103 in the early spring period and by 8 parental females.

The average number of eggs laid by *R. grandis* females can vary from 30 to 117, and studies conducted in polyester boxes in 1986 yielded 30–70 adults per 1 female *R. grandis* (Grégoire et al. 1989). A pair of *R. grandis* yielded 1-104 and 7-102 larvae with different nutrition amount (Alkan Akinci and Gregoire 2016). Although the number of eggs laid by the predator varies, they lay an average of 70–80 eggs. When more than one female is found in a brood chamber, average numbers of progenies per female decrease. This is evaluated as a regulatory process that lowers egg production when several females are together in a brood chamber (Merlin et al. 1984). Similarly, in our experiment, when we used higher numbers of parental *R. grandis* females in breeding logs, the average numbers of progenies have decreased.

Distribution of the new progenies in the sand environment and their sex ratios

Of the approximately 4450 g sand that each breeding log is located in, 1.12 % was in the first part, 14.61 % in the second and 84.27 % in the third part. Average numbers of *R. grandis* progenies in part I, part II and part III are presented in Table 4. The difference between the average numbers of the progenies

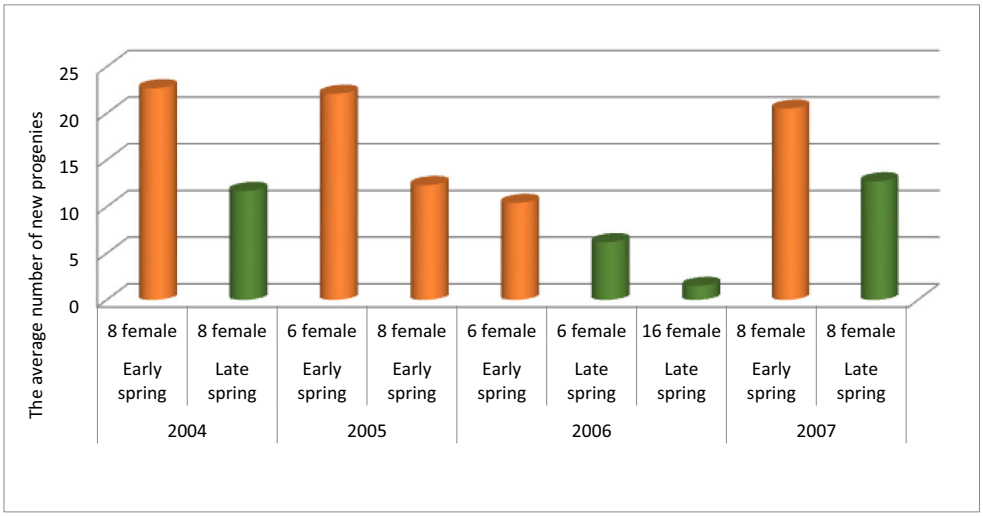

Fig. 3 The average number of new progenies according to the experimental years obtained with different numbers of *Rhizophagus grandis* parental females by early and late spring laboratory rearings

in these three sections were significant ($df = 2$; $F = 58.8$; $p > 0.05$). Of the total *R. grandis* progenies, 16.4 % were collected from the first part, 28.3 % from the second part and 55.3 % from the third part.

The average number of progenies in 1 cm^3 sand was 0.85, 0.11 and 0.04 in the first, second and third part, respectively. Although 55.3 % of *R. grandis* progenies were collected from the third section, progenies existed more frequently in the first two sections, which were observed to remain more humid than the third section. In the mass rearing programs, it is useful to reduce the amount of sand used today in that *R. grandis* progenies use the sand in the third section to a lesser extent. This effort can contribute to ease the labor intensive and time consuming process during sterilization of the sand. However, the optimum amount of sand should be determined by further studies.

Sex discrimination of 6771 *R. grandis* progenies was performed. These progenies were reared in 72 breeding logs by 6 females and 2 males each. Of the total 6771 *R. grandis* progenies, 72.2 % were females and 27.8 % were male adults. The highest ratio of the females in a breeding log was 97.1 % while the highest ratio of males was 62.5 %. Keskinalemdar et al. (1986) states the sex ratio as 3 females: 2 males on average.

Conclusions

Dendroctonus micans is one of the most important forest pests of oriental spruce forests that had caused severe epidemics and tree deaths. Large-scale tree deaths in the wide fronts of expanding populations can cause ecosystem degradation and therefore deforestation. It is important to continue the mechanical and biological control programs against this pest. It is necessary to maintain surveys according to pest risk assessments both in the attacked and unattacked sites. Detection of an infestation in an earlier phase enables better chance of pest management. In this study, which was performed during early spring and late spring periods by introduction of different numbers of predator-prey combinations to breeding logs, results showed that breeding in early spring period and by 6 females and 2 males would provide a higher amount of progenies. Determining the potential predator-prey combinations that can provide the highest number of new progenies contribute to the labor intensive mass rearing studies to be performed effectively. Following our results, practitioners can conduct the mass rearing studies only in early spring, thus they can ensure the release of new *R. grandis* adults in infested areas in an early period. *R. grandis* has the high ability of colonizing its prey's brood systems and establish in infested forests. So, the efforts on realizing the establishment of *R. grandis* in

Table 4 Distributions of *Rhizophagus grandis* progenies in the sand environment

	Number of logs (N)	Mean *R. grandis* progenies (Mean±Std. Deviation)	Min. *R. grandis* progenies (N)	Max. *R. grandis* progenies (N)
Part I	353	16.4±21.8	0	174
Part II	353	28.3±41.4	0	337
Part III	353	55.3±71.6	0	437

infested forests will contribute to population regulation of *D. micans* and maintaining its populations under economical damage threshold.

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Data availability Not applicable.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

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