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ORIGINAL RESEARCH ARTICLE

# Investigation of the oviposition and predation impact on *Dendroctonus micans* (Kug.) of wild and laboratory-produced *Rhizophagus grandis* Gyll. on oriental spruce in Türkiye

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**Abstract**

*Dendroctonus micans* (Kugelann) (Coleoptera: Curculionidae) is a primary pest of spruce in Eurasia. It has killed millions of spruce trees especially at the edges of its expanding range. In Türkiye and Caucasus, the beetle's predominant host is oriental spruce, *Picea orientalis* (L.) Link. Sanitary thinning and clearfelling and, when allowed, chemical measures were not sufficient to control *D. micans*. *Rhizophagus grandis* Gyll. (Coleoptera: Monotomidae), a specific predator of *D. micans*, has been mass reared and released at infested stands, and is now considered as the major component in outbreak control. So, managing *R. grandis* from the rearing stage to the release in the infested stands is an important process. The oviposition and impact on *D. micans* on oriental spruce of wild and laboratory-produced *R. grandis* were compared under laboratory conditions in polystyrene boxes containing fresh *P. orientalis* bark and re-hydrated bark powder. Two series of experimental boxes were prepared. Each box contained twenty L2-L3 *D. micans* larvae as well as a pair of wild (series 1), or laboratory-produced (series 2), *R. grandis* adults. A pair of wild *R. grandis* yielded significantly more larvae than laboratory-produced predators. Predation was proportional to the number of predator larvae in the two groups after the first two weeks, but prey supply of the wild predators was nearly exhausted after two weeks. On the third week, predation was still proportional to the number of offspring of the laboratory-produced parents, but this was not the case with the wild predator larvae, as their prey supply had already been almost completely exhausted during the previous period. The offspring of the wild predators decreased after the second week, probably corresponding to cannibalism due to shortage of prey, whilst those of the laboratory-produced predators continued to increase in numbers between the second and the third week.

**Keywords** Offspring production · Oriental spruce · *Picea orientalis* · Predation impact

## Introduction

*Dendroctonus micans* (Kugelann) (Coleoptera: Curculionidae) is one of the most destructive pests of spruce forests throughout Eurasia (Grégoire 1988). It has been first discovered in Türkiye in 1966 at Posof, in oriental spruce, *Picea orientalis* (L.) Link. forests neighbouring Georgia (Acatay 1968). Oriental spruce is native to Türkiye. After the first discovery, *D. micans* has spread westwards and infested all the oriental spruce forests in the Eastern Black Sea Mountains of Türkiye until 2009 (Alkan Akıncı et al. 2014). Millions of cubic meters of trees have been killed at the expanding front of *D. micans* dispersal in Posof, Artvin, Giresun and Trabzon (Alkan Akıncı et al. 2005, 2014; Aksu 2011).

On a global scale, the world's forests are facing overexploitation, expansion of settlements and fragmentation, resulting in loss of biodiversity and ecosystem services. The adverse effects of climate change, including drought, pest outbreaks, wildfires, landslides and hurricanes have

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detrimental impact on the structure and dynamics of forests on a global scale (Hlásny and Turčáni 2009; Ammer et al. 2018). At the regional level, oriental spruce forests in the Black Sea Region of Türkiye have a number of key functions such as soil protection, avalanche and landslide control, clean water and livelihoods for local communities (Alkan Akıncı et al. 2014). Disturbances in the forest environment reduce tree resistance. Host tree resistance is known to strongly affect tree-killing bark beetles (Biedermann et al. 2019). In the new climatic conditions created by global warming, we can expect pests that are currently of minor importance, or endemic pests, to cause significant damage in the coming years (Hlásny and Turčáni 2009) by taking the advantage of successful development, survival and range expansion (Fischbein and Corley 2022). Research indicates that low populations of bark beetles that are in the endemic level can be periodically disturbed by external factors such as droughts and windthrows that triggers endemic pest population's transition to the epidemic level (Hlásny et al. 2019). Biological control is a reliable way to provide long-lasting and environmentally safe pest management. Classical biological control (the introduction of a co-evolved pest-specific natural enemy of non-native origin to control a specific pest that is also non-native) is a well-known method since *Rodolia cardinalis* (Mulsant) (Coleoptera, Coccinellidae) has been used to control *Icerya purchasi* Maskell, 1878 (Hemiptera, Margarodidae) in 1886 (Kenis et al. 2017). One of the most well-known example of successful classical biological control of forest pests is the biological control of *D. micans* by *Rhizophagus grandis* Gyll. (Coleoptera: Monotomidae) in Eurasia (Khobakhidze et al. 1970; Grégoire et al. 1984; 1989; Alkan 1989; Alkan and Aksu 1990; Fielding and Evans 1997; Kenis et al. 2017).

After applying direct control measures against *D. micans*, such as sanitary thinning and clearfelling, which have been insufficient to prevent damage (Jeger et al. 2017), mass rearing programs have been initiated in Georgia since 1963, and later on in France, Türkiye and the UK, to produce *R. grandis* (Kobakhidze et al. 1970; Grégoire et al. 1984; Alkan 1985; Fielding et al. 1991), for releases in infested stands. *R. grandis* is an effective specific predator of *D. micans* (Grégoire 1988; Aksu 2011; Alkan Akıncı and Aksu 2014; Dohet and Grégoire 2017; Özcan et al. 2021), has a high ability in finding the active galleries of the prey, a high fecundity and is very voracious (Grégoire et al. 1984, 1989; Alkan Akıncı et al. 2010; Aksu 2011). *R. grandis* follows *D. micans* as the pest expands its distribution range, but with a time lag that can reach 50 years (Grégoire 1988). In Türkiye, *R. grandis* has been naturally found in a stand neighbouring the Georgian border nearly 20 years after the discovery of *D. micans* (Serez et al. 1987). The predator shows a lower dispersal ability than *D. micans*, which often moves with timber transportations and passes geographic barriers such as the English Channel between the UK and the European mainland, causing outbreaks in newly colonized areas (Fielding and Evans 1997; Gilbert and Grégoire 2003; Alkan Akıncı et al. 2014).

*R. grandis* pairs placed either in freshly cut spruce logs with *D. micans* adults or larvae, or in polystyrene boxes containing bark powder, fresh bark and prey larvae, are being used in rearing programs. Foresters who are running the mass rearing programs in their homeland and researchers from different countries may use different combinations of predator pairs in rearing studies or experiments (Table 1).

A pair of predator produces up to 104 offspring (Keskinalemdar et al. 1986; Grégoire et al. 1989; Alkan Akıncı and Aksu 2014). Two female and one male predators produce 80 offspring (Fielding and Evans 1997) whereas two pairs of predators when used with 750 larvae or with 8 *D. micans* females produced 102 or 134 offspring (Aksu 2011). When higher numbers of predator females and males are used in rearing studies, up to 134 offspring can be produced on a

**Table 1** *R. grandis* rearing data, based on the literature

| Numbers of predators | Total numbers of offspring | Amount / type of prey | Rearing environment | Reference |
|---|---|---|---|---|
| 1♀1♂ | 78 | 300 *D. micans* larvae / L2, L3 | Freshly cut oriental spruce logs | Keskinalemdar et al. (1986) |
| 1♀1♂ | 30–70 | 20 *D. micans* larvae / L2, L3 | Polystyrene boxes containing bark powder and fresh bark | Grégoire et al. (1989) |
| 2♀1♂ | 0–80 | 20 *D. micans* larvae / L2, L3, L4 | Polystyrene boxes containing bark powder and fresh bark | Fielding and Evans (1997) |
| 2♀2♂ | 102 | 750 *D. micans* larvae | Freshly cut oriental spruce logs | Aksu (2011) |
| 2♀2♂ | 134 | 8 *D. micans* females | | |
| 1♀1♂ | 1–104 | 15 *D. micans* larvae / L3, L4 | Polystyrene boxes containing bark powder and fresh bark | Alkan Akıncı and Aksu (2014) |
| 6♀2♂ | 86 | 700–750 *D. micans* larvae / L3, L4 | Freshly cut oriental spruce logs | Özcan et al. (2021) |
| 8♀4♂ | 134 | | | |
| 16♀8♂ | 24 | | | |

spruce log (Özcan et al. 2021). However, higher numbers of predator pairs do not necessarily mean an ever-increasing number of offspring. When the predator combination that produced 134 offspring by 8 females and 4 males was doubled to 16 females and 8 males, the number of offspring decreased to 24 (Özcan et al. 2021). This trend has been recorded by Merlin et al. (1984) in an earlier study. They showed that the mean production per female was inversely proportional to the number of predator females per brood chamber. With 1, 2 and 3 predator females, the mean number of offspring per female decreased from 29.3 to 13.6 and 6.9, respectively.

Both the adults and larvae of the predator prey upon all immature stages of *D. micans* (Grégoire 1988). In laboratory conditions, up to 40 *D. micans* eggs can be consumed by a pair of *R. grandis* adults in a week (Merlin et al. 1984). A predator larva needs to consume the equivalent of a mature *D. micans* larva throughout its immature life (Grégoire et al. 1989). The offspring of a pair of *R. grandis* can consume two-thirds of a *D. micans* brood (Grégoire et al. 1989; Alkan Akıncı and Grégoire 2016).

*D. micans* is present in Artvin oriental spruce forests since 1970 (Özder 1983) and infested all the spruce stands in Artvin until 1995 (Eroğlu 1996). Control measures (sanitation felling, spot peeling and application of chemicals) that have been implemented until 1985 could not prevent outbreaks (Alkan 2000). Then, a biological control program based on laboratory mass-rearing *R. grandis* started in 1985 (Alkan 1989). More than six million predators have been reared in Türkiye so far (Aksu et al. 2014; URL 1). In Türkiye, rearing laboratories belong to the General Directorate of Forestry. Laboratory-produced predators are being released at outbreak areas and areas with higher populations of *D. micans* by the staff of these rearing laboratories. Aksu (2011) mentions a decrease in oviposition when laboratory-produced *R. grandis* adults are used during two consecutive years, so, every third year, wild *R. grandis* adults have been collected and used in laboratory mass rearing studies (Aksu 2011). However, to our knowledge, the oviposition and predation impact on *D. micans* of wild and laboratory-produced *R. grandis* has not been studied so far, even though, in the mass rearing programs, the aim is to maximize the offspring of each pair of predators, to support effective production. Here we report the results of comparisons of oviposition levels and predation impact of wild and laboratory-produced *R. grandis* on oriental spruce, to document possible ways of improving the rearings in Türkiye.

## Materials and methods

The experiments were run in cylindrical crystal polystyrene rearing boxes (5.5 cm in diameter and 2.5 cm high; producer: LP Italiana, Italy, model: L202038) containing oriental spruce bark powder, fresh bark disks, *D. micans* larvae and *R. grandis* adults. Wild *D. micans* larvae and wild *R. grandis* adults were collected from an infested oriental spruce stand in the Taşlıca Forest Sub-District in Artvin. Laboratory-produced predator adults were from stock material maintained in the Biological Control Laboratory of Artvin Regional Directorate of Forestry. Following Grégoire et al. (1984), a pair of predator adults was placed in each rearing boxes. The experiment was conducted from 24 June to 28 July 2015.

Wild *R. grandis* adults were collected from *D. micans* galleries on infested oriental spruce. Laboratory-produced *R. grandis* adults were produced in the spring of 2015 at the Biological Control Laboratory of the Artvin Regional Directorate of Forestry by a staff constituted of foresters and biologists. The "log-breeding" method was used (Aksu 2011), derived from the work of Georgian entomologists (Khobakhidze et al. 1970). The rearing laboratory has a sealed door and air conditioning, and its windows are protected from direct sunlight. Temperature was kept at 19–22 °C and air moisture at 70–75% during the breeding process. Some of these laboratory-produced *R. grandis* adults were released in infested stands, and some were kept as stock material. Some of the *R. grandis* adults from this stock material were used in our experiments.

### Preparation of the experiment

Two series of experimental boxes were prepared. There were 3 batches in each series. Before starting the experiment, polystyrene boxes, fresh phloem disks, rehydrated bark powder and *D. micans* larvae have been prepared. *R. grandis* adults have been separated according to their sexes (Fig. 1a and e). Rehydrated bark powder was put into the polystyrene boxes, together with two phloem disks (diameter: 5 cm), extracted from freshly harvested oriental spruce trees. A total of 20 *D. micans* larvae (L2, L3) were also introduced in each box. All boxes were prepared on 24 June 2015. Pairs of predator were placed into the experimental boxes after two days, on 26 June (date recorded as the first day of the experiment) (Fig. 1f and k).

Wild *R. grandis* pairs were introduced into boxes in the first series and laboratory-produced predator pairs into second series. The experimental boxes were kept for one, two or three weeks in the laboratory (batches one to three, respectively). After these periods, predator pairs were removed from the boxes in the batches on the 8th, 15th

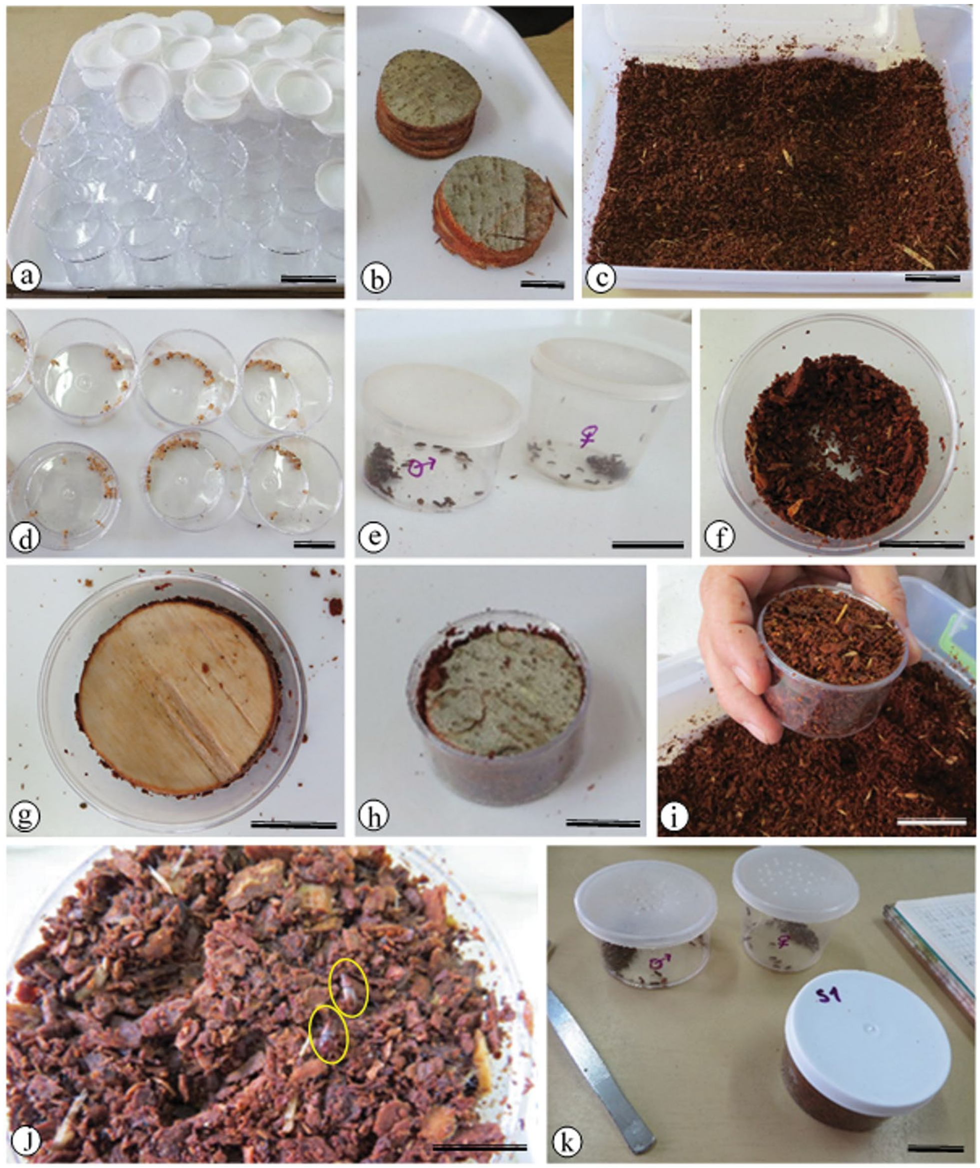



**Fig. 1** Preparation of the experimental boxes (Scale bars: a, c = 5 cm; b, d- i, k = 2 cm, j = 1 cm)

and 22nd day of the experiment, and the batches were kept for an additional 10 days to allow enough time for incubation of the predator's eggs. Then, the number of *R. grandis* larvae and of remaining *D. micans* larvae in each box was recorded (Fig. 2a and c). There were ten replicates for each batch. So there were 30 boxes for the wild predator series and 30 boxes for the laboratory-produced predators. A control group (boxes without predators; ten replicates) was also prepared to evaluate natural mortality.

## Analyses

Data were analyzed using IBM SPSS statistics version 30.0. Independent samples t-tests were used to compare the offspring of the wild and laboratory-produced parents, and numbers of prey larvae consumed in boxes by the two series of wild and laboratory-produced predators. Linear regressions were used to match predation with the numbers of predators present in the boxes.

# Results

## Predator production

The results are presented in Table 2. The mean offspring sizes (± SEM) of the wild pairs were 43.1 ± 3.32 (extreme values: 33–68) larvae after 7 days, 71.0 ± 3.24 (53–83) after 14 days, and 51.8 ± 2.35 (43–68) after 21 days. The laboratory-produced pairs produced at the same time 17.9 ± 2.13 (8–28), 30.4 ± 3.10 (21–49), and 32.2 ± 1.75 (26–44) larvae, respectively (Fig. 3). Significantly more larvae were

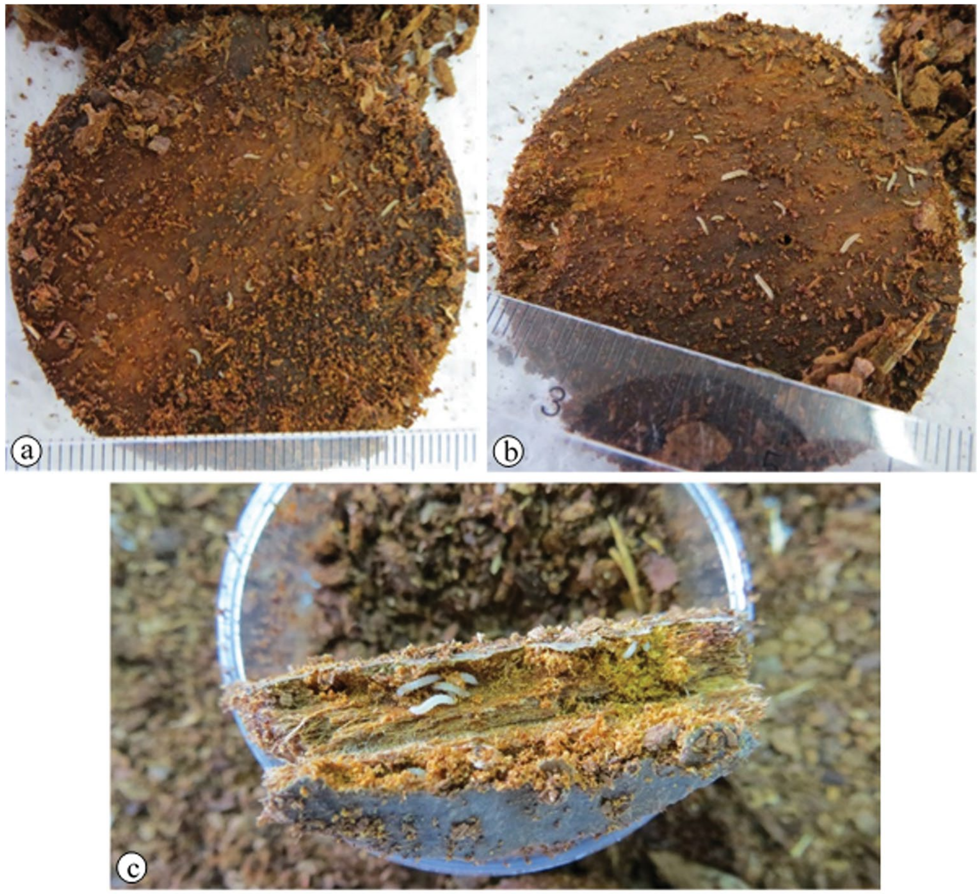


**Fig. 2** Examples of *Rhizophagus grandis* larvae collected from experimental boxes

**Table 2** Numbers of *Rhizophagus grandis* larvae produced per box by wild and laboratory-produced predator pairs

| Origin of the parental adults | Number of *Rhizophagus grandis* larvae per experimental box | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time of the counts | Replicates | | | | | | | | | |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Wild *R. grandis* pairs | day 7 | 49 | 39 | 36 | 35 | 33 | 68 | 38 | 39 | 51 | 43 |
| | day 14 | 65 | 81 | 53 | 78 | 81 | 63 | 66 | 83 | 77 | 63 |
| | day 21 | 44 | 68 | 47 | 52 | 54 | 49 | 49 | 43 | 53 | 59 |
| Laboratory-produced *R. grandis* pairs | day 7 | 12 | 28 | 24 | 14 | 8 | 16 | 19 | 22 | 25 | 11 |
| | day 14 | 45 | 22 | 32 | 34 | 49 | 27 | 28 | 21 | 24 | 22 |
| | day 21 | 44 | 32 | 28 | 30 | 26 | 27 | 31 | 33 | 32 | 39 |

produced by the wild adults than by the laboratory-produced predators, after each period of time (respectively, day 7: $t = 6.385$; $p < 0.001$; day 14: $t = 9.067$; $p < 0.001$; day 21: $t = 6.687$; $p < 0.001$) (Table 3).

The offspring of the wild predators decreased between day 14 and day 21, whilst the offspring of the laboratory-produced predators continued to grow slightly (Fig. 3).

## Predation

As there was no prey mortality in the control groups, the number of *D. micans* larvae that disappeared in the experimental boxes can be attributed to predation. The numbers of *D. micans* larvae preyed upon at the end of the experiment are presented in Table 4. An average of $12.6 \pm 0.65$ (9–16) (63%), $19.7 \pm 0.15$ (19–20) (98.5%) and $19.9 \pm 0.10$ (19–20) (99.5%) prey were consumed by each pair of wild predators and their offspring during the first, second and third week, respectively. The laboratory-produced pairs and their offspring consumed on the average $7.8 \pm 0.55$ (6–11) (39%), $14.2 \pm 0.57$ (11–17) (71%), and $19.2 \pm 0.33$ (17–20) (96%) prey larvae, respectively, during the same periods. Significantly more *D. micans* larvae were preyed upon by the wild predators and their offspring than by the laboratory-produced predators and offspring, after the first two periods (respectively: $t = 5.605$; $p < 0.001$ at Day 7; $t = 9.267$; $p < 0.001$ at Day 14; $t = 2.049$; $p = 0.066$ at Day 21) (Table 5).

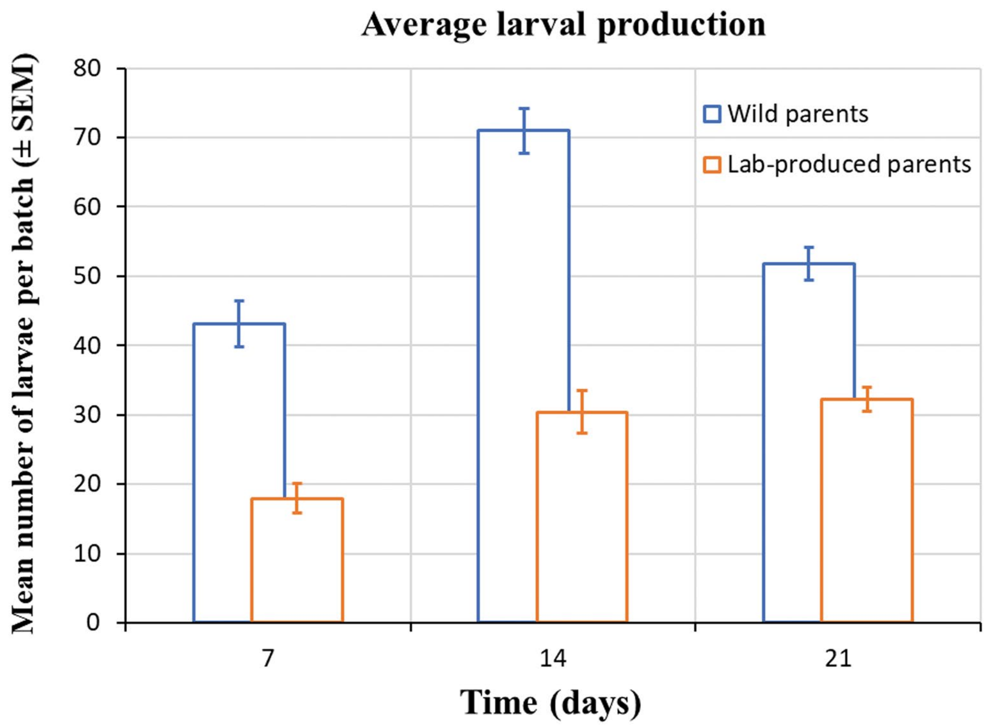



**Fig. 3** Mean numbers of larvae (±SEM) produced in the experimental boxes

**Table 3** Result of the t-tests of the comparison of the larvae produced by wild *R. grandis* adults by laboratory-produced adults

| Time of the counts | Number of *R*. grandis larvae at counting days (mean±SEM) | | Levene's tests for Equality of Variances | | t-tests | |
|---|---|---|---|---|---|---|
| | Wild | Laboratory-produced | F | *p* | t | *p* |
| Day 7 | 43.1±3.32 | 17.9±2.13 | 0.784 | 0.388 | 6.385 | $p<0.001$ |
| Day 14 | 71.0±3.24 | 30.4±3.10 | 0.388 | 0.541 | 9.067 | $p<0.001$ |
| Day 21 | 51.8±2.35 | 32.2±1.75 | 0.628 | 0.437 | 6.687 | $p<0.001$ |

**Table 4** Numbers of prey larvae consumed in boxes by the two series of predators

| Origin of the parental adults | Numbers of prey larvae consumed in boxes | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time of the counts | Replicates | | | | | | | | | |
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Wild *R. grandis* pairs | day 7 | 14 | 13 | 11 | 9 | 12 | 16 | 12 | 11 | 15 | 13 |
| | day 14 | 20 | 20 | 19 | 20 | 20 | 19 | 19 | 20 | 20 | 20 |
| | day 21 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 19 | 20 | 20 |
| Laboratory-produced *R. grandis* pairs | day 7 | 9 | 11 | 6 | 8 | 6 | 7 | 10 | 6 | 8 | 7 |
| | day 14 | 16 | 12 | 15 | 15 | 17 | 14 | 15 | 11 | 14 | 13 |
| | day 21 | 20 | 20 | 19 | 20 | 18 | 17 | 19 | 20 | 19 | 20 |

**Table 5** Result of the t test of *D. micans* larvae preyed upon by the wild predators and their offspring vs. laboratory-produced predators and their offspring

| Time of the counts | Number of *D. micans* larvae consumed at control days (mean±SEM) | | Levene's tests for Equality of Variances | | t-tests | |
|---|---|---|---|---|---|---|
| | Wild | Laboratory-produced | F | *p* | t | *p* |
| Day 7 | 12.6±0.65 | 7.8±0.55 | 0.173 | 0.682 | 5.605 | $p<0.001$ |
| Day 14 | 19.7±0.15 | 14.2±0.57 | 8.363 | 0.010* | 9.267 | $p<0.001$ |
| Day 21 | 19.9±0.10 | 19.2±0.33 | 9.162 | 0.007* | 2.049 | $0.066^{ns}$ |

* Equal variances not assumed

$^{ns}$ non-significant

Predator density was higher in the batches with wild pairs and their offspring than in the batches with laboratory-produced pairs and their offspring.

At day 7, the maximum offspring size produced by the laboratory-produced pairs was 28 larvae (box 2). It was lower than the minimum offspring size produced by the wild batch (33). The maximum offspring size from the wild batch was 68 larvae (box 6) after the first week (Table 2). Predation efficiency was highest in this box (16 *D. micans* larvae consumed) (Table 4).

At day 14, the maximum offspring size from the laboratory-produced pairs was 49 larvae (box 5). It was lower than the minimum offspring size from the wild batch (53). The maximum offspring size from the wild batch was 83 larvae (box 8) (Table 2). All the *D. micans* larvae were consumed in this box (Table 4). A maximum of 17 larvae were consumed by a laboratory-produced batch (Table 4).

At day 21, the maximum offspring size from the laboratory-produced pairs was 44 larvae (box 1). It was almost the same as the minimum offspring size from the wild batch (43). The maximum offspring size from the wild batch was 68 larvae (box 2) (Table 2). Nearly all the *D. micans* larvae were consumed (Table 4).

Predation in each box increased with time and was linearly proportional to the number of predator larvae in the laboratory-produced series (Day 7: $y=0.3963x$; $R^2=0.8917$; $p<0.001$; Day 14: $y=0.4412x$; $R^2=0.9613$; $p<0.001$; Day 21: $y=0.584x$; $R^2=0.9822$; $p<0.001$), and, during the 14 first days in the wild series (Day 7: $y=0.286x$; $R^2=0.9844$; $p<0.001$; Day 14: $y=0.0329x+17.363$; $R^2=0.4858$; $p=0.025$) but, after nearly reaching a plateau in Day 14, it then became independent from the number of predator larvae present as all prey had been consumed (Day 21: $y=0.0177x+18.984$; $R^2=0.1729$; $p=0.232$). This evolution in time is represented in Fig. 4 (day 7), Fig. 5 (day 14) and Fig. 6 (day 21).

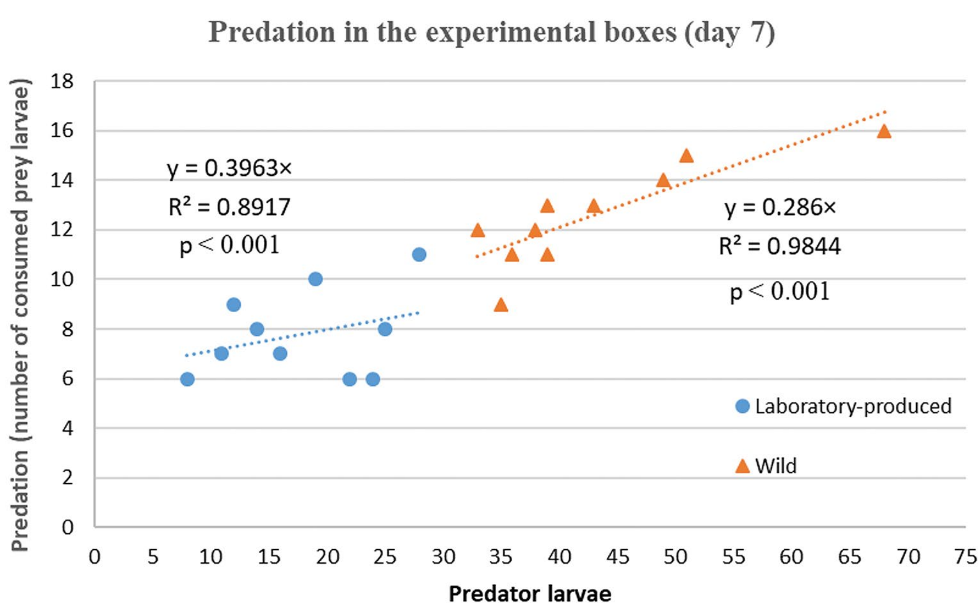
## Discussion

In this study, we compared the oviposition and predation impact on *D. micans* of wild and laboratory-produced *R. grandis* on oriental spruce in Türkiye. We compared brood production over time between wild and laboratory-produced predators, analyzed predation against time in both groups and examined relationships between predation and the numbers of predator larvae in boxes during three time periods.

### Difference against time in brood production between wild and laboratory-produced predators

The wild predators produced more offspring than the laboratory-produced predators during the three time periods of the study. This finding supports practice developed by Aksu (2011) to collect wild *R. grandis* adults in the forest every third year to maintain higher mass-rearing yields (see the Introduction). Volatile compounds present in the larval frass of *D. micans* have been identified as the cause of oviposition in *R. grandis* (Baisier and Grégoire 1988; Baisier et al. 1988; Grégoire et al. 1991), and a number of oxygenated monoterpenes have been identified as oviposition stimulants for *R. grandis* (Dohet and Grégoire 2017). Our results suggest that this blend of oviposition stimulants could differ quantitatively and/or qualitatively between laboratory-produced and wild predators.

**Fig. 4** Relationship between predation and the numbers of predator larvae per box after 7 days



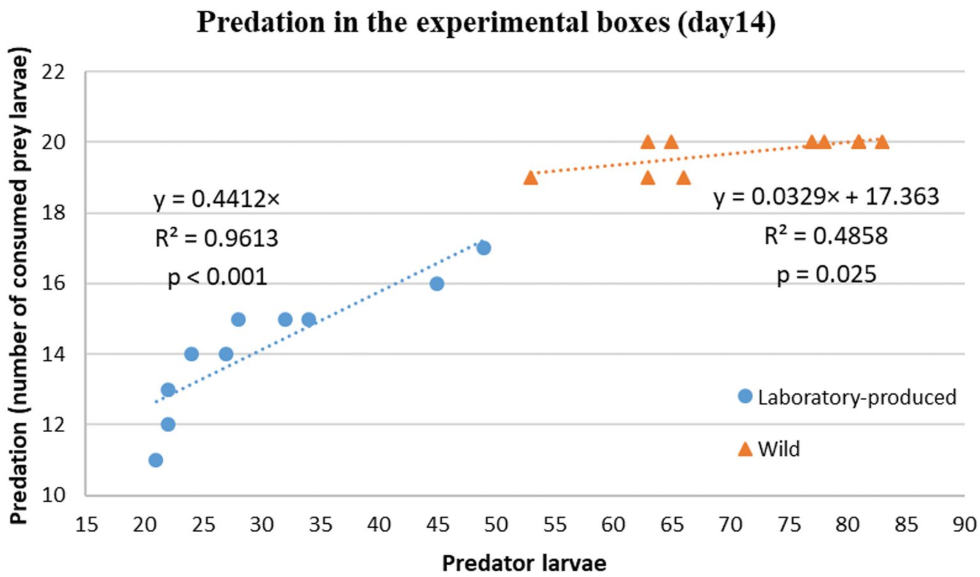



**Fig. 5** Relationship between predation and the numbers of predator larvae per box after 14 days

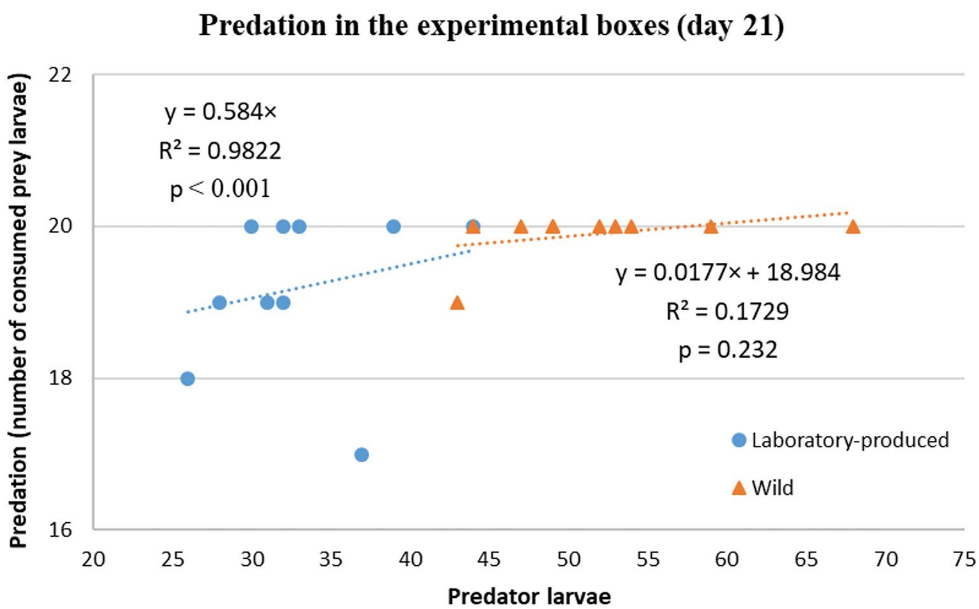



**Fig. 6** Relationship between predation and the numbers of predator larvae per box after 21 days

## Relationships between predation and the numbers of predator larvae in the boxes

Predation was proportional to the number of predator larvae in the two groups after the first two weeks, but prey supply of the wild predators was nearly exhausted after two weeks. On the third week, predation was still proportional to the number of offspring of the laboratory-produced parents, but because there were more predator larvae in the wild series, the prey supply was nearly exhausted after 14 days, whilst there were still on the average six *D. micans* larvae in the laboratory-produced serie on Day 14. The decrease of predator larvae in the wild series between Day 14 and Day 21 can be explained by cannibalism in the absence of prey. Baisier et al. (1984) developed a model based on experimental data, showing that, under conditions of prey shortage, mortality by cannibalism increases exponentially with the number of predator larvae present together. King et al. (1991) also frequently observed cannibalism among *R. grandis* larvae when intraspecific competition was artificially induced in the laboratory. If there had been more prey larvae at the start in the boxes, the wild predators would probably have produced even more offspring.

Cannibalism is considered as a common trait in the population dynamics of a wide range of animal species. Michaud (2003) reports cannibalism among different species of coccinellids. Larvae of the studied coccinellid species cannibalized siblings and non-siblings in response to reduced food availability. Cannibalism is frequently observed among mass-produced coleopteran predators, in particular among carabids (Riddick 2023).

## Conclusion

Basically, three practical conclusions may be drawn from these results:

a) Wild predators, when available, should be used in the rearings;
b) A sufficient number of prey larvae should be provided per rearing boxes;
c) When only small numbers of prey larvae are available, however, and if the supply of parent predators allows, it might become cost-effective to prepare more oviposition boxes with a smaller number of prey.

**Author contributions** HAA and JCG conceived research; HAA conducted experiments; HAA and JCG analyzed data; HAA wrote the manuscript; JCG made comments and suggestions on the manuscript; Both authors read and approved the manuscript.

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### Declarations

**Conflict of interest** The authors declare that they do not have any kind of conflict of interests.

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