Some biological traits of the parasitoid wasp *Rhopalicus tutela* (Hymenoptera: Pteromalidae) in spruce forests of Moscow Region, Russia

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Abstract

Some biological traits of *Rhopalicus tutela* (Walker), a parasitoid of the European spruce bark beetle *Ips typographus* (L.), were studied both in forests of Moscow Region, Russia, and under laboratory conditions. Females of *R. tutela* have mature eggs after overwintering and thus do not need additional feeding to lay eggs. Under laboratory conditions at 8 °C, the life expectancy of males is 58 ± 44 days and that of females is 36 ± 45 days. An increase of temperature shortens the developmental period of *R. tutela*, which can proceed without diapause. The duration of one generation in the laboratory at 22–24 °C is 14–16 days. In spruce forests of Moscow Region, *R. tutela* has two or three generations per year.

Keywords: *Rhopalicus tutela*, *Ips typographus*, parasitoid, biological trait, laboratory insect culture, biological protection, spruce forest

Introduction

Rhopalicus tutela is a polyphagous ectoparasitoid of many genera and species of bark beetles (Curculionidae: Mesoptiliinae, Molytinae, Scolytinae) (Noyes, 2019) and also of *Mikiola fagi* (Hartig) (Diptera: Cecidomyiidae) (Tselikh, 2012). Biological traits of *R. tutela* were previously studied by many domestic and foreign researchers (Bouček, Půlpán and Šedivý, 1953; Hedqvist, 1963; Kharitonova, 1972; Girits, 1975; Kolomiets and Bogdanova, 1980; Eck, 1990; Krüger and Mills, 1990; Hougardy and Gregoire, 2003, 2004). This parasitoid can be successfully bred under laboratory conditions and thus potentially used as a prospective agent for biological protection of spruce forests against *Ips typographus* (L.) (Coleoptera: Curculionidae: Scolytinae). The first experiments on cultivation of *R. tutela* under laboratory conditions on spruce logs showed positive results (Chilakhsaeva, 2017). Nevertheless, to develop methods of mass reproduction of the parasitoid, a better and more complete knowledge of the basic biological characteristics of this species is needed. The present study was aimed at studying phenology, as well as fertility and life expectancy of parasitoids in the laboratory.

Materials and methods

Observations were made on standing and windfall trees of middle age which were infested with *I. typographus*. Work was carried out in the spruce woods with old local outbreaks of *I. typographus* in the Pushkino District of Moscow Region and in the laboratory of the All-Russian Research Institute of Silviculture and Mechanization of Forestry (ARRISMF). A total of 256 adults of *R. tutela* were collected and studied during 2013–2016 and in 2019.

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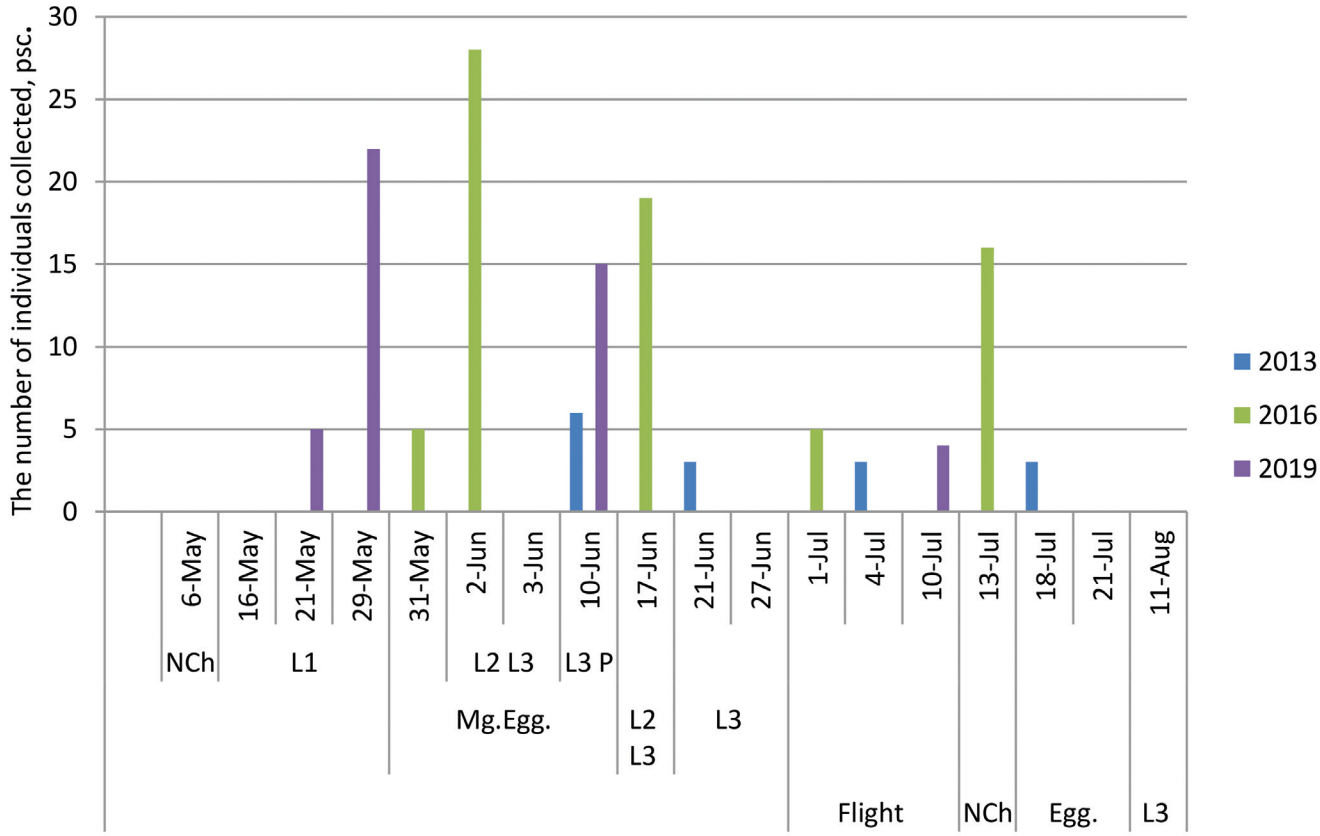

Fig. 1. The occurrence of *Rhopalicus tutela* in the forest on trees populated by *Ips typographus* beetle for three years of observations and the development of *Ips typographus.* For *Ips typographus*: Flight — flight period; L1; L2; L3; — larva's growth 1, 2 and 3 stages; Mg.Egg. — Maternal galleries and egg laying; NCh — Nuptial chamber; P — pupa.

Adult parasitoids were picked in test tubes on bark of *I. typographus*-infested trees in autumn and spring to rear *R. tutela* in spruce logs in the laboratory. The collected bark was put in plastic bags and stored in a fridge at 0–1 °C, and if needed taken out of the bags and kept in the laboratory at room temperature for parasitoid emergence.

Several specimens of adult parasitoids collected in the forest that emerged from the spruce bark were placed in 100 ml Erlenmeyer flasks with damp filter paper. The flasks were loosely plugged with cotton corks and kept at 8 °C and 15 °C with a light regime of 16 hours. The parasitoids were fed every other day by filter paper saturation with water and honey solution (1.5 : 1) drops. The parasitoids that had been kept for feeding at 8 °C were exposed to room temperature for 30–40 minutes. Dead parasitoid specimens were counted daily and preserved in 70 % ethanol.

In the laboratory, *R. tutela* were reared in *I. typographus*-infested spruce logs. For this purpose, 20–27 cm long and 9.5–12 cm diameter logs with cross sections coated in paraffine wax were infested with *I. typographus*. The spruce logs were placed in a separate room. During the experiment, the logs were dampened with water daily. When larvae of *I. typographus* reached the second or third instar stages, *R. tutela* males and females were released to them. The emerged parasitoids were collected in Erlenmeyer flasks and stored at 8 °C to be used in the next experiment.

Female fertility was identified through *R. tutela* female dissections and mature egg count. Simultaneously, female and male size and female maximum gaster width were measured. To observe the developmental stages of parasitoid larvae, they were placed in glass test tubes filled with a layer of sawdust 1 cm thick, moistened with water. The tubes were plugged with paper corks and kept in darkness at 25 °C.

An MBS-10 binocular microscope was used to study the collected specimens. We used MS EXCEL 2010 statistical analysis package (regression) to find out if there is any link between *R. tutela* abdomen width and its fertility.

Results and discussion

In spruce forests of Moscow Region, *R. tutela* is found on trees infested with *I. typographus* and the six-toothed spruce bark beetle *Pityogenes chalcographus* (L.). In such trees, we observed the presence of *R. tutela* only in the area of *I. typographus*, despite the fact that habitats of these two species of bark beetles are adjacent and their galleries cross. In trees declining due to damage by *I. typographus* and also infested with other bark beetles, *R. tutela* also parasitized larvae of the lesser spruce shoot beetle *Hylurgops palliatus* (Gyllenhal).

Fig. 2. Females of *Rhopalicus tutela* on the bark, below — the position of the female during egg laying.

According to our observations, adult *R. tutela* start to emerge in late May and fly until mid-August; in warm weather, the species can be found as late as mid-September. Its massive flight begins in June, when *I. typographus* develops larvae of the second and third instars (Fig. 1).

Swarming of *R. tutela* occurs in sites with host infestations on thin bark. The mean bark thickness is 1.39 ± 0.1 mm (minimal — 0.5 mm, maximal — 2.1 mm) (Krüger and Mills, 1990). Before mating the male mounts the female for about 3 minutes. Mating lasts 1–1.5 minutes. *R. tutela* males are rather aggressive in keeping competing males off the females. After mating the *R. tutela* female seeks bark beetle larvae by moving quickly in the chosen area and spinning, knocking gently with her antennae on the bark. Once a host larva is found, the female penetrates into the bark with her ovipositor and lays an egg on the larva (Fig. 2). The mean ovipositor length is 2.2 ± 0.2 mm (Krüger and Mills, 1990). *R. tutela* is able to lay several eggs on one host larva (Krüger and Mills, 1990; Hedqvist, 1963). The most active emergence was observed from 12 : 00 to 14 : 00 in warm sunny weather. In nature, after wintering, we observed a different ratio of males and females. During flight in different years this ratio was equal from 1♂ : 3♀ to 3♂ : 1♀. After mating the female paralyzes the host larva and lays an egg on or near it. *R. tutela* prefers to parasitize second and third instar larvae of *I. typographus* and sometimes also its pupae. The parasitoid larva consumes the bark beetle larva completely (Fig. 3). Older parasitoid larvae

Fig. 3. Larva parasitizing on the larva of the spruce bark beetle (June 4, 2013).

Fig. 4. Larva of *Rhopalicus tutela* before pupation.

Fig. 5. Pupa *Rhopalicus tutela*: a — on the sixth day after pupation, b — on day 13 — before hatching imago (pupa size 2.05 mm), c — on day 15 — female imago.

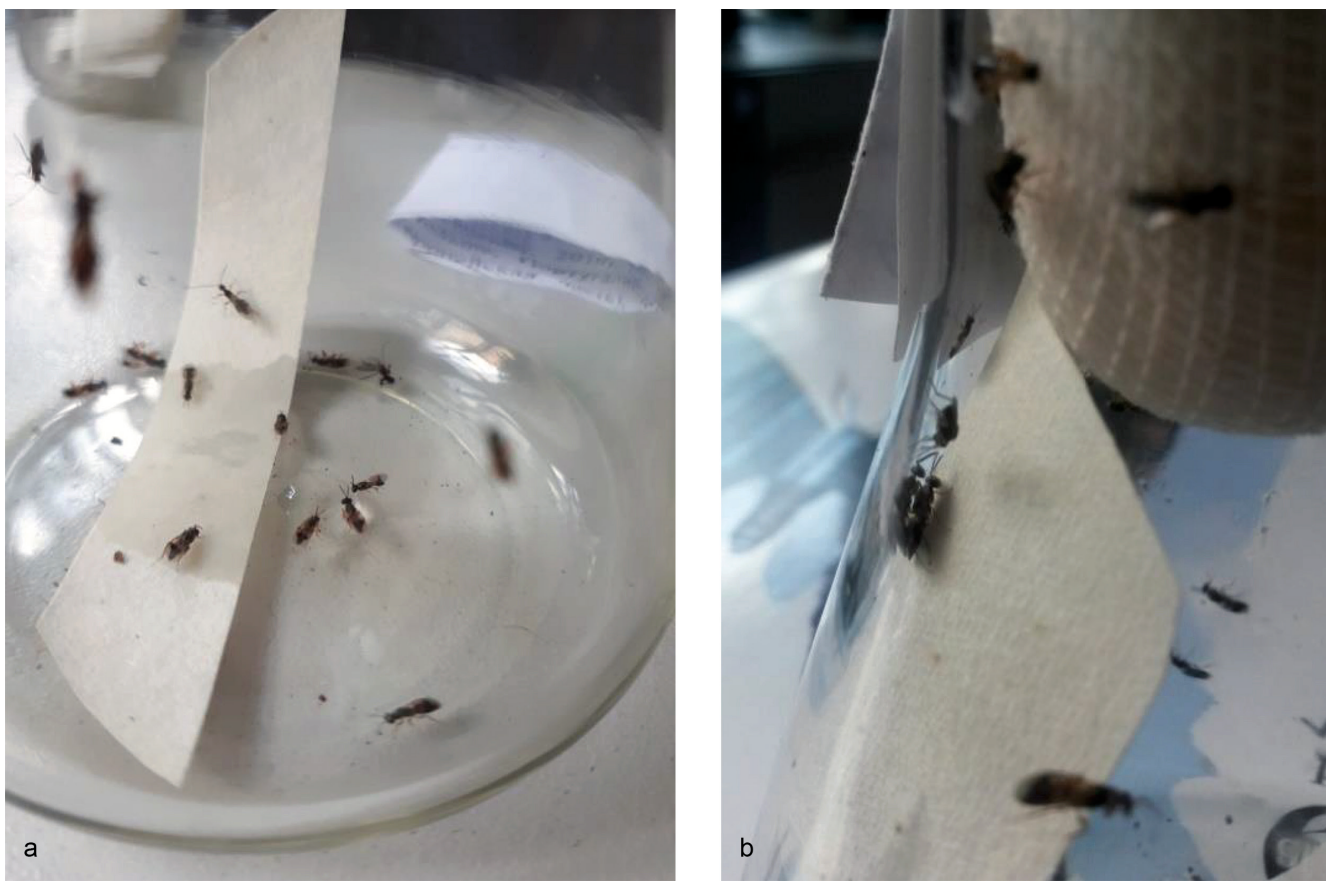

Fig. 6. The maintenance of *Rhopalicus tutela* in the laboratory: a — feeding with honey solution; b — mating.

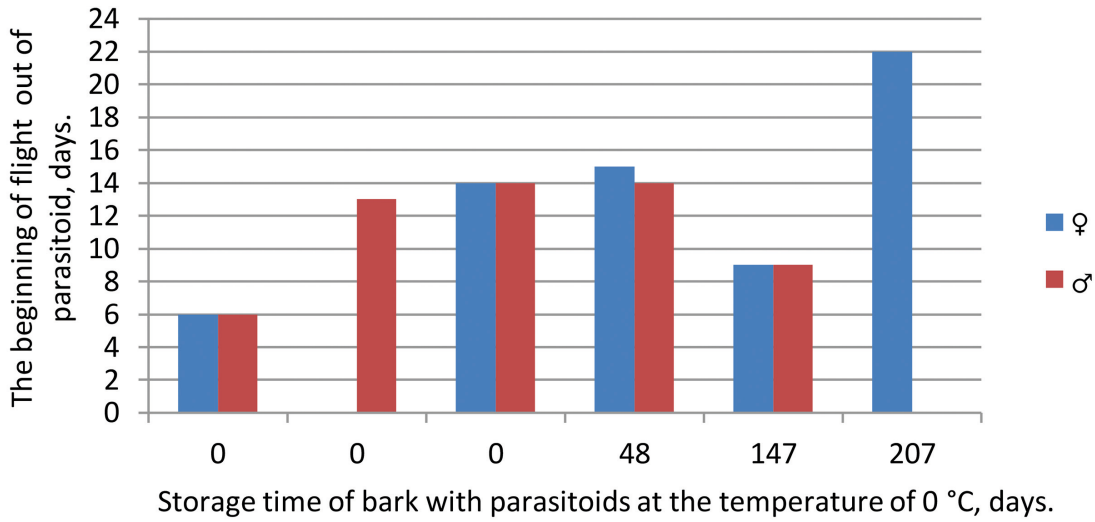

Fig. 7. The beginning of the flying out *of Rhopalicus tutela* depending on the duration of storage of the bark at a temperature of 0 °C.

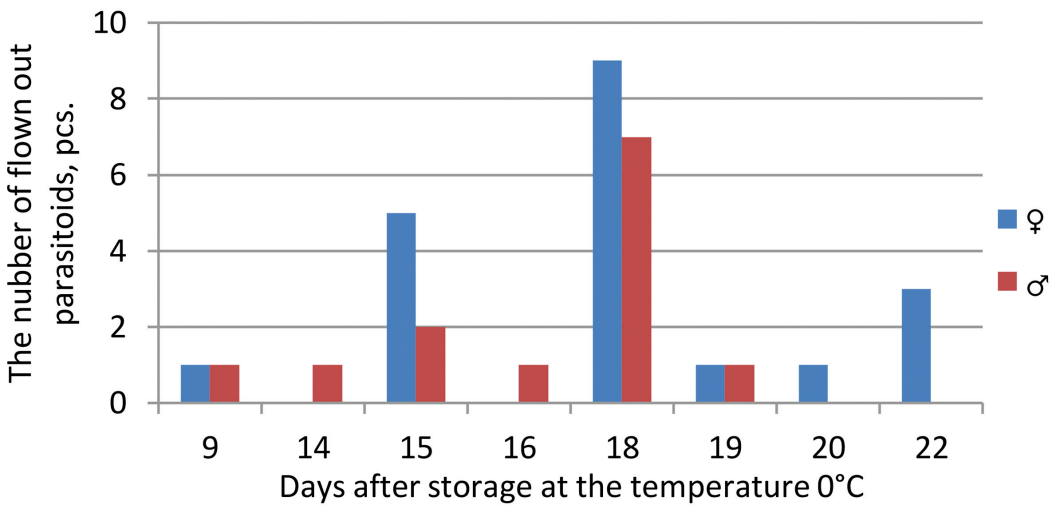

Fig. 8. The appearance of females and males of *Rhopalicus tutela* after storage of bark at a temperature of 0 °C.

ZOOLOGY

are fusiform, slightly curved. Their bodies consist of 13 segments and a head capsule (Fig. 4). Often it positions itself in the *I. typographus* pupal cradle. During collection adult larvae easily drop out of the bark. The pupae of the parasitoids are formed without a cocoon; at first they are white, then gradually become dark as they develop (Fig. 5).

The last instar larvae overwinter and then pupate in spring. Imagines hatch from pupae and then chew their way out through the bark to emerge. The species has two generations per year. Development of a third generation of *R. tutela* is possible in warm autumn in open sunny places. We observed its emergence in mid-September.

In the laboratory, adult *R. tutela* readily feed on water and honey solution. Males are active and mate with females, aggressively competing with each other for them (Fig. 6). Rearing on spruce logs produced three generations of *R. tutela*.

Parasitoids fly out after 9–22 days from spruce bark collected in the fall, after 6–14 days from the bark collected at the end of April and in early May. Usually males and females emerge simultaneously but sometimes males emerge a day earlier. A duration of 0 °C temperature exposure has no impact on the diapause period of *R. tutela* larvae (Fig. 7). Mean *R. tutela* emergence time after lab storage of bark at 0 °C temperature was 16.6 ± 4.1days. The maximal number of parasitoids emerged on the 18th day (Fig. 8).

On spruce logs parasitoids started to emerge 14 to 16 days after infestation with *I. typographus*. *R. tutela* adult flight lasts 5–8 days; first the bark beetle flies out, then parasitoids. The simultaneous flight of both individuals is observed in the late period of flight of the bark

Fig. 9. Eggs of *Rhopalicus tutela*.

beetle and at the beginning of the flight period of *R. tutela*. The ratio of males and females flying out of the bark varies from 1♂ : 1♀ to 1♂ : 3♀. When breeding on logs, the mean sex ratio is 2♂ : 1♀.

R. tutela females at the moment of emergence immediately have mature eggs inside. Grown from spruce logs in the laboratory, 64 % of females had mature eggs inside. Of those females collected in the forest during the development of the second and third stages of bark beetle larvae, 53 % had mature eggs inside.

Eggs have a milky white color. Mean length is 0.67 ± 0.11 mm; mean width is 0.15 ± 0.07 mm (n = 90) (Fig. 9). A female can keep up to 10 eggs and on average 3 eggs. The mean number of eggs in the females collected in the forest and reared in the laboratory was similar (Table 1).

Table 1. *Rhopalicus tutela* fertility

Origin of females (where grown/ collected)	Number of females, pcs.	Number of eggs, pcs.	
		Mean ± SD*	Min–max
Females collected in the forest	30	3 ± 1.3	1–5
Females reared in the lab	25	2.8 ± 2.1	1–10

* SD — standard deviation

The sizes of males and females of *R. tutela* are summarized in Table 2.

The relationship between abdomen width and fertility was poor for females collected in the forest (Fig. 10). The statistical correlation coefficient and model parameters are not significant (r = 0.18; Student's t-test is 0.875 < t crit.). The relationship between the width of the abdomen of lab-reared females and fertility is poor as well (r = 0.29 — it is slightly higher but statistically insignificant, Student t-test criterion equals 1.45). Nevertheless, it indicates that fertility rises as abdomen width grows. A similarly poor link was found in studies of *R. tutela* body size and its fertility interaction.

Rhopalicus tutela lifetime at 8 °C is higher than at 15 °C (Table 3). Males live longer than females. We took some females from this experiment for rearing, thus their lifetime was not defined accurately. Nevertheless females lived over 26 days at 8 °C and over 10 days at 15 °C. Parasitoids that emerged from the last experiment logs lived at 8 °C from September until February. The average female lifetime at 8 °C is 36 days, while the maximum is 153 days.

The development of *R. tutela* is highly dependent on temperature, as indicated by many studies. *R. tutela* development duration according to many studies is shown in Table 4.

An increase in temperature shortens development periods, as development can proceed without diapause. As a result of our experiments in the lab at 22–24 °C, 14–16 days elapse from settlement to the start of emergence out of the bark of parasitoids.

We produced three generation of *R. tutela* in the lab from June to early September on spruce logs. A low temperature impacts development duration but does not affect the duration of diapause. Females of *R. tutela* do not need a preoviposition feeding period to lay eggs. However,

Table 2. *Rhopalicus tutela* male and female body length

Origin of females (where grown/collected)	Female body length, mm			Male body length, mm		
	Number of females, pcs.	Mean length ± SD	Min–max	Number of males, pcs.	Mean length ± SD	Range
Forest collected specimens	30	4.35 ± 0.58	2.95–5.75	46	3.84 ± 0.78	1.75–4.95
Lab reared specimens	25	3.98 ± 0.70	2.75–5.10	32	2.63 ± 0.27	2.10–3.20
All specimens	55	4.18 ± 0.66	2.75–5.75	78	3.34 ± 0.86	1.75–4.95

Table 3. Lifetime of *Rhopalicus tutela* at various temperatures

Temperature	Number of males in experiment, pcs.	Male lifetime, days		Number of females in experiment, pcs.	Female lifetime, days	
		mean ± SD	min–max		mean ± SD	min–max
8 °C	55	58 ± 44	2–153	76	36 ± 45	3–153
15 °C	6	9 ± 2	7–10			

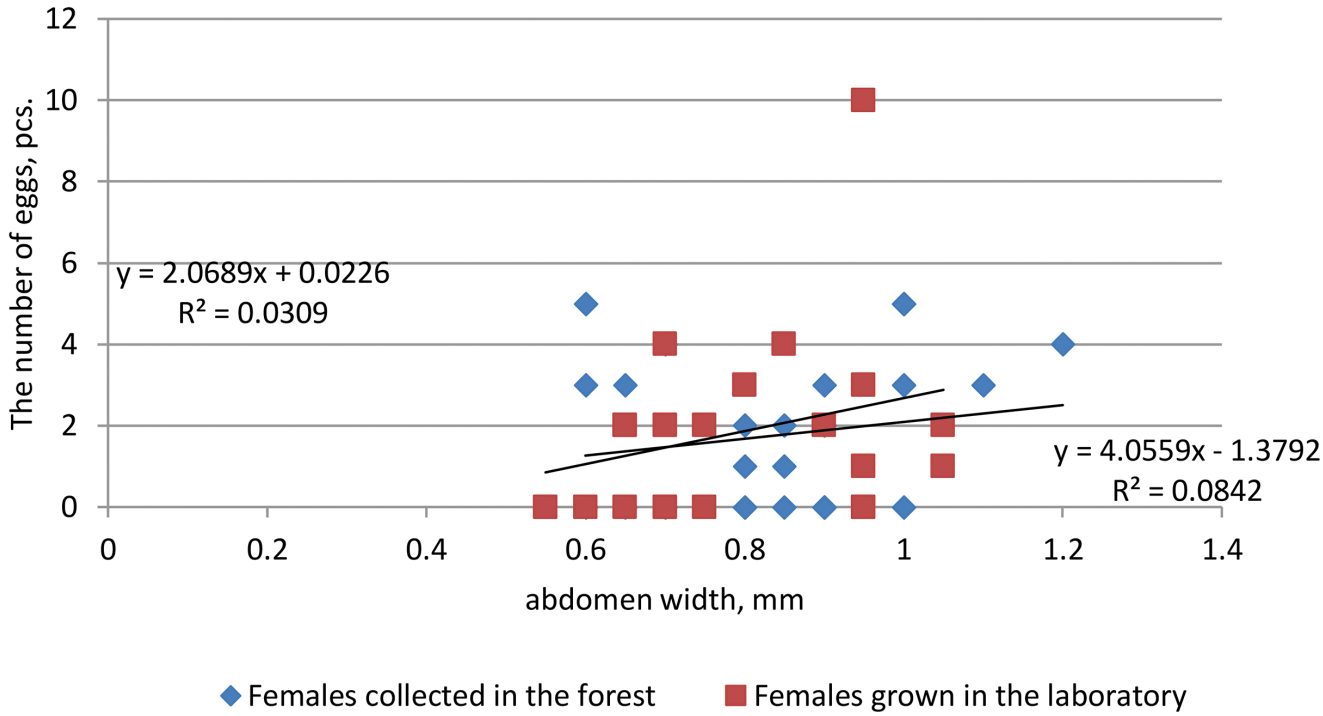

Fig. 10. Dependence of the fertility of females *Rhopalicus tutela* on the width of the abdomen.

Table 4. *Rhopalicus tutela* duration of development in lab from eggs to adults at different temperatures

Temperature, C°	Duration development, days			Source
	eggs	from larva to pupa	from egg to imago	
14–16			42–48	Györfi, 1942
15	4.3 ± 0.4	19.3 ± 0.5	33.8 ± 1.5	Krüger and Mills, 1990
16–18			30–35	Györfi, 1942
20–22			20–27	Györfi, 1942
22			20	Hougardy and Gregoire, 2004
25	2.6 ± 0.3	5.8 ± 0.7	14.8 ± 0.5	Krüger and Mills, 1990

the preoviposition period is momentous. It was found that with additional feeding *R. tutela* lifetime is prolonged and females lay more eggs; the period before oviposition lasts 3.3 ± 1 days and from the second to eighth day females lay a stable number of mature eggs (Hougardy and Gregoire, 2004). It is also suggested with presence of the host, egg formation is faster and larvae size affects sex ratio. Body length does not affect viability; nevertheless, larger species live longer without additional feeding (Hougardy and Gregoire, 2004). We did not find a significant relationship between body size and fertility, however, such a relationship was shown by Hougardy and Gregoire (2004).

Conclusions

Rhopalicus tutela is one of the most common spruce bark beetle parasitoids in Moscow Region. It can be considered as a prospective agent for biological protection of spruce forests in the region. The development of several generations per year, a short life cycle, fertility, and the presence of mature eggs after overwintering of *R. tutela* make it possible to rapidly increase its number through artificial breeding.

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