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NUPTIAL CHAMBER CONSTRUCTION IN THE SPRUCE BARK BEETLE, *IPS TYPOGRAPHUS* (L., 1758) AND TIMING OF FEMALE ARRIVAL

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Abstract: Observations on early reproduction behavior of the spruce bark beetle, *Ips typographus* (L., 1758), were made in summer of 2014 and 2015. It was discovered that male *I. typographus* may construct their nuptial chambers and accept females as early as one day after spruce log colonization. Two females may be accepted by a single male within a relatively short time period (<5 hours). Implications of these findings are discussed with respect to biological control programs intended to reduce damage caused in spruce forests by this serious pest.

Key words: mating chamber, reproduction, tree colonization

INTRODUCTION

The European spruce bark beetle, *Ips typographus* (L., 1758) is a serious pest of spruce forests in Europe (LOZZIA 1993). Adult spruce bark beetles and their larvae breed in the bark of Norway spruce, *Picea abies*. At low population levels, they attack primarily dead, damaged or weakened trees, and act as phytosanitary agents (SAUVARD 2007). Extreme weather conditions (storms, windthrows, drought) can result in damage of large areas of spruce stands, which subsequently provide suitable breeding sites for great numbers of *I. typographus* (HANEWINKEL et al. 2008, VAKULA et al. 2014). During a population outbreak the bark beetles can attack healthy spruce in large numbers and eventually overcome host tree immunity (MULOCK & CHRISTIANSEN 1986), causing major economic losses in managed forests.

When locating suitable breeding sites, adult beetles respond to species-specific pheromones as well as plant volatiles released from the host trees (SCHLYTER & LÖFQVIST 1986, JAKUŠ & BLAŽENEC 2011a). Upon arrival to a host tree, the beetles carefully inspect surface of the bark in the search for a suitable place to start boring a hole (PAYNTER et al. 1990). Typically, an entrance hole with a nuptial (mating) chamber is excavated into the bark by a male (PFEFFER 1954, SAUVARD 2007) who also starts producing aggregation pheromones to attract more beetles to the attack site (SCHLYTER & LÖFQVIST 1986). When a female finds an entrance hole into the gallery that is already inhabited by a male, a sequence of mutual “pushing behavior” between the male and female takes place (PAYNTER et al. 1990). This pushing behavior eventually leads to one of the two outcomes: the female either "wins" and successfully enters the gallery, or the male blocks the entrance hole with his body and the female eventually resumes her searching behavior on the bark. After successful mating, the female starts boring maternal tunnel from the nuptial chamber and directs her resources into oviposition (PFEFFER 1954, SAUVARD 2007). Under some specific conditions, especially in the absence of the males, a female can bore her own gallery (ANDERBRANT & LÖFQVIST 1988).

Despite decades of intensive research of the spruce bark beetle biology, particularly the response of adults to various semiochemicals (SCHLYTER et al. 1987), the factors affecting reproduction success (HEDGREN & SCHROEDER 2004) and various forest protecting strategies (JAKUŠ 1998, SCHROEDER & LINDELÖW 2002), some aspects of its reproduction remain unknown. This study was conducted to fill the gap in our understanding of the construction of nuptial chamber in *I. typographus* and timing of female arrival under field conditions.

MATERIALS AND METHODS

Construction of nuptial chamber by males

To investigate time needed for construction of nuptial chamber, *I. typographus* adults were collected in a Theysohn trap lured with species-specific pheromones (IT Ecolure; Fytofarm, Slovakia) and placed in close vicinity of Institute of Zoology, SAS in august 2014. The trap was checked for the presence of captured beetles several times a day. *I. typographus* adults were sorted to sex according to bristle density in the anterior part of pronotum (SCHLYTER& CEDERHOLM 1981). Males were placed individually in 1.5 ml polypropylene centrifugation microtubes. Open microtubes with the males were inverted over bark on a spruce log (Ø 26 cm, length 45 cm) and secured with one or two steel pins to avoid beetle escape. Each beetle thus had approximately 0.79 cm^2 of bark surface available to bore an entrance hole to the prospective nuptial chamber. The spruce log was exposed to outdoor conditions in a sheltered area to protect the log from rain and direct sunlight. Microtubes were inspected in about 30-min intervals and the time when males started boring into the bark was noted. Beetles which did not start boring within 3h were removed. This procedure was repeated over 4 consecutive days at different times of the day based on availability of captured beetles.

On the fifth day, the bark was carefully removed and males were removed from their respective galleries. Each gallery was photographed with a ruler to allow proper size estimation. The area of the nuptial chamber in the photograph was colored white, printed on a sheet of standard office paper and cut out precisely following its shape. The weight of the "paper chamber", weighed to

±0.0001 g, was then compared to the weight of white area of a known size (based on the ruler) cut from the same sheet of paper. The size of nuptial chamber in mm^2 was then calculated for each male. Upon removal from their galleries, the time elapsed since the male started boring into the bark and the length of males were recorded to investigate the relationship between time, size of the males and size of their nuptial chambers. The data were analysed by multiple linear regression in R, version 3.2.1 (R CORE TEAM 2015). Nonlinear relationships were considered, but the sample sizes were not sufficient for formulation of robust nonlinear models.

Arrival of females

In July 2015, male *I. typographus* beetles were introduced to bark on a spruce log (Ø 25 cm, length 43 cm) in microtubes over a period of 4 days as described above and kept outdoors in a sheltered area. The time when males started boring into the bark was recorded. On the fifth day, the spruce log infested with male *I. typographus* was placed under the baited Theysohn trap and microtubes were removed to allow new beetles to enter the galleries through the entrance holes excavated by males. The bottom collection unit of the trap was removed so that any captured beetles would fall directly on the spruce log. The log was exposed to newly arriving beetles for 5 hours (8:00-13:00). Then, the entrance holes were covered with microtubes and any beetles found on the surface of the bark were discarded. The bark was carefully removed. Bark beetles found in the nuptial chambers were dissected to confirm sex. The age of the galleries at the time of bark removal and numbers and sex of the beetles recovered from nuptial chambers were recorded.

RESULTS AND DISCUSSION

A total of 13 males successfully established their presence in the spruce bark in our first experiment. Size of the nuptial chamber after 19-100.5 hours of male boring activity ranged between 49.2 to 120.7 mm^2, with no apparent increase in size over the observed time period (Fig. 1). Multiple linear regression analysis did not show any statistically significant relationships between the size of nuptial chamber and size of male *I. typographus* or the duration of male establishment in the bark ($F_{2,10}$=1.051, P=0.3852), although the statistical analysis may suffer from lack of power because the different beetle size groups were not represented proportionately. Because size of the nuptial chamber did not increase with time, spruce bark beetle males can likely prepare it within the first 24 hours after entering the host tree.

Despite differences in methodology, sizes of the nuptial chambers in our experiment agree well with field observations of VAKULA et al. (2014), who recorded nuptial chamber areas of 25.44-126.56 mm^2. On the other hand, TURČÁNI & VAKULA (2007) recorded smaller mating chambers in the laboratory experiments – average size of nuptial chambers for the males in control group reached only ca. 39 mm^2 and for irradiated males about 51 mm^2. This suggests that laboratory-reared bark beetles may be less proficient in construction of mating chambers.

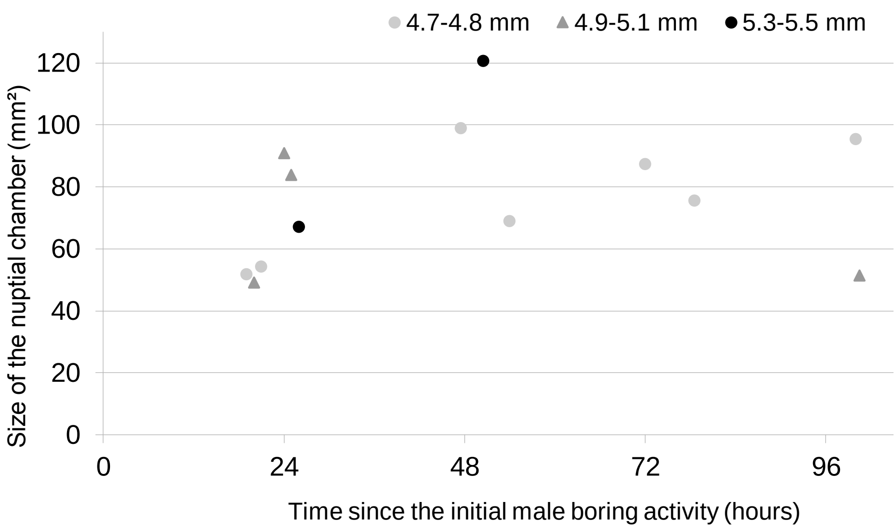

Figure 1. Size of the nuptial chamber in relation to the size of colonizing *Ips typographus* males (see legend) and duration of their establishment in the bark.

Data on arrival of the females (Table 1) suggest that spruce bark beetles male may accept females into their nuptial chamber for mating as early as one day after they colonize the log. Furthermore, we found that males can accept another female in a relatively short time period (within 5 hours) after the arrival of the first female.

The finding of two males in the same nuptial chamber in one case (Table 1) was surprising. It may be possible that the second male was inspecting the entrance hole in the bark in the exact moment when the experiment was terminated and the hole covered by a microtube. However, this would likely prompt "pushing behavior" of the male which already inhabited the nuptial chamber. We did not observe any such behavior at the end of the experiment. It is not clear why two males shared the same nuptial chamber and whether there was any interaction between them. We could not find any similar records of such incidents in the available literature.

In addition to these observations, we also detected that some males left their mating chambers while their entrance holes were exposed to newly arriving beetles. Findings of vacant galleries (galleries with no parent beetles inside), are not uncommon and may depend on the condition of the tree or remaining live tissues in the log. VAKULA et al. (2014) reported that during bark beetle attack of well-irrigated spruce trees, 21% of the newly-created galleries with nuptial chamber contained no adult beetles, whereas all galleries in the control drought-stressed trees contained at least one beetle. No obvious reason for this beetle behavior could be determined, as many of the abandoned galleries did not contain any visible resin.

Table 1. Arrival of female *I. typographus* to spruce logs in relation to time of male colonization.

Time since the initial male boring activity	Beetles recovered from the gallery
1 d (28 h)	2 ♂
1 d (28 h)	1 ♂, 1 ♀
2 d (49 h)	no beetles
2 d (49 h)	1 ♂
3 d (71 h)	1 ♂, 1 ♀
3 d (71 h)	1 ♂, 2 ♀
3 d (73 h)	no beetles
3 d (75 h)	1 ♂, 1 ♀
4 d (93 h)	1 ♂, 1 ♀
4 d (95 h)	1 ♂, 2 ♀
4 d (96 h)	no beetles
4 d (96 h)	1 ♂, 2♀
4 d (98 h)	1 ♂, 2♀

Data presented in this paper suggest that the males can construct their nuptial chamber and can be joined by the females as early as one day since the colonization of the tree, i. e. earlier than 2-4 days after male arrival, as previously reported in literature (PFEFFER 1954). These findings not only broaden our knowledge of *I. typographus* biology, but also have important implications for biological control programs which consider the use of insect pathogens, such as *Beauveria bassiana* (JAKUŠ & BLAŽENEC 2011b). Especially in the case of pheromone traps combined with self-inoculation system intended for introduction of a fungal spores into bark beetle population (VAUPEL & ZIMMERMANN 1996), knowledge of the timing of reproduction events is crucial. *I. typographus* may be killed by *B. bassiana* in less than 4 days (KREUTZ et al. 2004). If the time since beetle inoculation until contact with other beetles (male or female) is too long, the pathogen may reduce the beetle's fitness to a level where it is not able to find a mate and transfer the infection to a new host. Given the relatively short time needed for preparation of nuptial chamber and rapid arrival and acceptance of several females into the male's gallery, as observed in this study, the vitality of *I. typographus* potentially infected by *B. bassiana* should not be compromised.

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