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The sex ratio of spruce bark beetles – *Ips typographus* (Coleoptera: Curculionidae: Scolytinae) captured in different types of traps

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Abstract: In forest protection, various types of trap systems are used against spruce bark beetles to treat the foci of infested areas. Traditionally, these include pheromone traps, treated trap trees, and recently also the application of Storanet insecticide nets used for the sanitation of infested wood (trees), which also serve as treated trap trees when pheromones are applied, and the Trinet P system, a variation to a treated trap tree – a tripod. On an aluminium tripod, there is a Storanet insecticide net baited with a pheromone dispenser similar to other types of trap systems. The sex ratio proves to be a significant aspect in the assessment of individual types of trap systems. It is more favourable to catch a larger number of female individuals given that they bear the future development of the population. With a higher number of captured male individuals, the number of females per one male in the insect gallery increases, and the population cannot be reduced sufficiently. The results show that all three types of measures can be characterised by a lower ratio of captured males (approximately 40%) and a higher representation of females (around 60%). Differences in individual types of trap systems are insignificant.

Keywords: pheromone trap; Storanet; trap system; treated trap tree

Ips typographus is the most important pest of Central and Northern Europe, damaging thousands to millions of cubic meters of spruce stands. Information on the importance of the spruce bark beetle can be found in Skuhravý (2002), including details on the various outbreaks of this pest. The outbreaks in Europe were also presented by Grégoire and Evans (2004).

It may seem that the number of captured individuals is the most important success criterion when using pheromone traps or other protective equipment for catching spruce bark beetles (leaving aside efficiency related to the population density in the given location). However, is it the only crucial factor? Aggregation pheromones produced by bark beetles attract both sexes on principle (Zahradník et al. 1993), with the opposite sex being attracted more than individuals of the same sex as those producing the pheromone. With polygamous bark beetles (e.g. spruce bark beetles), it is the male, and with monogamous ones (such as striped ambrosia beetles), it is the female.

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There are two types of information carried by the aggregation pheromone. The sex which produces the pheromone is informed that 'this is a suitable source for founding a new generation', while the opposite sex receives instructions to mating. With polygamous spruce bark beetle, where the male individual usually has 2–3 females, it is thus important to consider the sex ratio in the trap system. If the number of males is reduced, it can simply result in a higher number of females per one male in the insect gallery which may not be very significant for the future development of the population. (Naturally, there can also be a situation when the number of males decreases significantly and not every female can find a male. Furthermore, the defensive facility of spruce can also come into play when spruces – if strong enough – use resin to flood pioneer male beetles, so the tree prevents the attack.) However, catching female beetles still seems primarily more significant as it reduces the population growth.

After the identification and artificial synthesis of aggregation pheromones of *Ips typographus*, pheromone traps of various types were introduced into the forest protection system. They were first used for mass trapping during the outbreak in Sweden (Bakke, Strand 1981; Bakke et al. 1983; Vité 1989; Raty et al. 1995). Due to the lack of traps and inaccessible areas for trapping, where it was not possible to carry out regular inspections, poisoned traps – stem or log treated with insecticide and baited with a pheromone dispenser – were introduced into the forest protection system. Recently, they have been replaced with the Trinet P system, where the treated trap trees have been replaced with an aluminium tripod covered with Storanet insecticide net. Insecticide nets have been used in Central Europe for less than ten years to protect the landings of infested wood. After application to the landing and subsequent baiting with pheromone dispensers, they also acted as poisoned trap trees. Exceptionally, they were also used for the control of individual stems.

Therefore, the sex ratio of captured individuals is very important for the population development and other impacts on forest stands. When using treated trap trees, the Trinet P system and the application of Storanet insecticide nets, the resistance of *Ips typographus* to insecticides may occur, especially to synthetic pyrethroids that are used in this case. Different sensitivity of males and females to pyrethroids may play a significant role in the development of resistance, and this offers another opportunity for research.

The aim of this study is to assess differences in the number of captured males and females in various commonly used trap types. Not only the total number of captured individuals, but also the sex ratio of captured specimens is important. For every male, there are typically 1–3 females (depending on the population phase – latency *vs.* outbreak). When a high number of males is captured, the male-to-female ratio may increase, which would have a lower impact on reducing the population.

It can be hypothesised that there are differences in the sex ratios of captured spruce bark beetles across different types of trapping devices. All traps are baited with a pheromone dispenser, but the trapping methods, and consequently the contamination, differ, so different sex ratios among the captured individuals can be expected.

MATERIAL AND METHODS

The experiments were conducted in the Brdy area (Czech Republic) at an altitude of 600–620 m a.s.l. in spruce stands which are approximately 90 years old, with a higher incidence of *Ips typographus* on a recently clear-felled area. The area is owned by Vojenské lesy a statky, s. p., Hořovice division, Obecnice forest administration unit. The experiments were commenced on May 2, 2018, and finished on August 30, 2018. Inspections were done at regular intervals of 14 days when captured beetles were collected – those from pheromone traps were collected directly, while logs covered with the Storanet insecticide net – one-meter length of treated logs packaged by Storanet nets (a substitute for Trinet P with respect to easier collection) and poisoned trap trees (one-meter logs treated with the 'Vaztak Les' insecticide in 1% concentration) were placed on frames with 15-centimetre sideboards and a dense net on the bottom which prevented dead beetles from falling through. The diameter of all the logs used was 20–25 cm. The three experimental variants had seven replications.

The sex of the collected sample was identified in dissections of all caught individuals (if there were fewer than 100 of them), and in the case of larger samples, random samples of 100 individuals were selected. Dissections were made after beetles were treated in 10% potassium hydroxide, their

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abdomens were removed, and either the presence or absence of the male genitalia was detected. Results were reported in per cent as individual samples differed in size, as mentioned above. The sex ratio can also be determined by morphological characters, by the density of hair on the front (Schlyter, Cederholm 1981; Anderbrant et al. 1985; Anderbrant 1990), but this method is not reliable. In particular, as the beetles age, hair abrasion occurs, and the differentiation of hair density changes, making the sex determination unreliable. Bakke (1970) provided an alternative method for establishing the sex of live males. Bednarz and Kacprzyk (2012) used the higher weight of female beetles from galleries due to the presence of maturing eggs. Zahradníková (2014) tested the assessment of *Ips typographus* sex ratio by using principal component analysis (PCA) and cluster analysis (CLU) statistical methods.

The only known reliable method is genital dissection (Annila 1971; Botterweg 1983; Lobinger 1996; Faccoli, Buffo 2004). The sex parameter does not correlate with any studied morphological parameter (weight, length and width of elytra, length and width of pronotum). Three types of trap system were included in the experiments: (*i*) Theysohn pheromone slot trap; (*ii*) log covered with Storanet insecticide net; (*iii*) treated trap tree. The Pheagr IT pheromone dispenser was used as a lure.

The data were evaluated in the NCSS statistics programme (Version 10.0.6, 2015), where the exploratory data analysis and, subsequently, one-factor analysis of variance, i.e. ANOVA (α = 0.05) were done. Additionally, the Tukey-Kramer test for multiple comparisons was carried out. In the Statistica programme (Version 12, 2013), a box plot was made.

RESULTS

The exploratory data analysis confirmed normality (P = 0.7073). The one-factor ANOVA (α = 0.05) rejected the null hypothesis that there would be no statistically significant difference (P = 0.00001) in the numbers of captured male and female beetles between individual types of trap systems (the pheromone trap, the poisoned trap tree and the log wrapped in the Storanet insecticide net) (Figure 1).

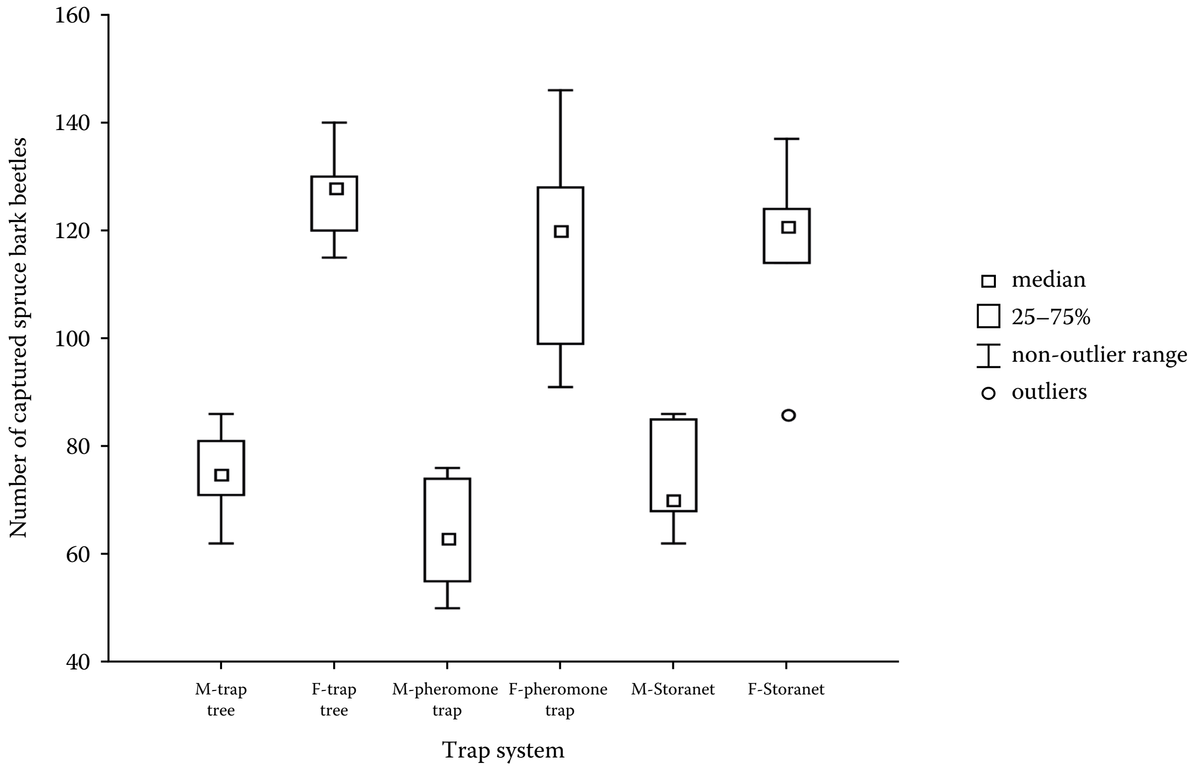

Figure 1. Comparison of numbers of captured male and female spruce bark beetle in individual types of trap system

M-trap tree – captured males on the poisoned trap tree; F-trap tree – captured females on the poisoned trap tree; M-pheromone trap – captured males in the pheromone trap; F-pheromone trap – captured females in the pheromone trap; M-Storanet – captured males on the insecticide net; F-Storanet – captured females on the insecticide net

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Table 1. Tukey-Kramer test for multiple comparison (MSE = 148.7302; critical value = 4.2605)

Type of equipment	Mean	Different from the group
F-pheromone trap	126.42860	M-pheromone trap, M-trap tree, M-Storanet
F-trap tree	116.85710	M-pheromone trap, M-trap tree, M-Storanet
F-Storanet	117.14290	M-pheromone trap, M-trap tree, M-Storanet
M-pheromone trap	75.00000	F-pheromone trap, F-trap tree, F-Storanet
M-trap tree	62.85714	F-pheromone trap, F-trap tree, F-Storanet
M-Storanet	74.00000	F-pheromone trap, F-trap tree, F-Storanet

MSE – mean square error; F-pheromone trap – captured females in the pheromone trap; F-trap tree – captured females on the poisoned trap tree; F-Storanet – captured females on the insecticide net; M-pheromone trap – captured males in the pheromone trap; M-trap tree – captured males on the poisoned trap tree; M-Storanet – captured males on the insecticide net

In the Tukey-Kramer test for multiple comparisons, it was revealed there are always statistically significant differences (P = 0.0087) in the numbers of captured female and male beetles between all types of trap systems (Table 1).

DISCUSSION

Different types of trap systems are used to reduce the population of *Ips typographus*. Trap trees have been used for the longest time – cutting and debranched healthy spruces aged approximately 70 years and older, which were introduced into the forest protection system against this pest already in the first half of the 19th century by Heinrich Julius von Uslar, chief administrator of the forest district in Herzberg (Ratzeburg 1837; Skuhravý 2002). Trap trees are limited in capacity. It depends on the dimensions of the trap tree (typically, a trap tree of 20 m in length and an average diameter of 20 cm is used) and the infestation intensity. In cases of heavy infestation, there is one hole per 1 dm^2. Under these conditions, approximately 1 000 males can be found on a single trap tree, and with a sex ratio of 1 : 2, there will be an additional 3 000 females (Zahradník 1994). *Ips typographus*, by means of its chemical communication through the production of different chemical substances, maintains the necessary spacing, ensuring the completion of the development of the new population (Schlyter, Löfqvist 1986; Schlyter et al. 1987a, b; Byers 1989; Klimetzek, Vité 1989).

The effectiveness of individual defensive measures was mostly always evaluated with a focus on the type of trap system (Skrzecz et al. 2015). In the case of pheromone traps, the effectiveness between their individual types was evaluated (Klimetzek, Vité 1978; Klimetzek et al. 1979; Regnander, Solbreck 1981; Lindgren 1983; Niemeyer 1985a, b; Weslien, Bylund 1988; Zahradník et al. 1991). Comparisons of individual types of trap systems were carried out rather exceptionally (Vité 1989; Raty et al. 1995; Jeniš, Vrba 2007; Lubojacký, Holuša 2011). The ratio of sexes caught in individual types of trap systems was rarely monitored.

Grodzki and Skrzecz (2017) monitored the effect of the application of the Trinet P system on the infestation of neighbouring stands. In stands where the Trinet P system was applied, the tree infestation decreased. The ratio of infested trees with the Trinet P system and control was 20 : 80.

The sex ratio of spruce bark beetle is a significant aspect influencing the growth of its population in forest stands. Despite this fact, little attention has been given to this topic. It has mostly focused on two main areas: (*i*) the sex ratio on tree trunks (during the incubation period or insect invasion) and (*ii*) the sex ratio of captured beetles in pheromone traps.

Based on dissections, Lindelöw and Weslien (1986) revealed that male beetles are the first to leave infested trees, while females start to outnumber them gradually and, in the end, there are 62% of females. At the later stage of the emergence, the sex ratio is 1 : 2 with females prevailing. Freely flying beetles have a sex ratio of 1 : 1. The difference can be explained by the initial prevalence of males at the early stage of emergence. On newly infested trees, the typical sex ratios range from 1 : 2 to 1 : 3 (males to females). Some males die in the effort of forming nuptial chambers as the tree produces resin in self-defence (Zumr 1985). Pfeffer (1955) stated that the sex ratio is related to the outbreak intensity. In the outbreak, one-sided galleries frequently occur, with the sex ratio of 1 : 1.

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The sex ratio captured on traps is determined by the natural sex ratio of bark beetles at what they infested the trees, which is usually 1 : 2–3, exceptionally 1 : 1. It is affected by the phase and intensity of the outbreak. Lobinger (1996) reported changes in the sex ratio in the period 1990–1995, when the outbreak of *Ips typographus* occurred. Before the start of the outbreak in 1991, the sex ratio (males to females) was 27.95 : 72.05, at the beginning and during the subsequent outbreak (1992–1994) it fluctuated between 33.68–49.45 and 65.32–50.55. They also evaluated changes in the sex ratio in individual years, which fluctuated in the range of 33.40–52.80 to 66.60–47.20 during the monitored years. This can also affect the sex ratio in the trap system.

Several authors dealt with the sex ratio of the spruce bark beetle (*Ips typographus*) caught in pheromone traps.

Brutovský (1986) concluded that there is a significant difference between landing (tubular) pheromone traps and pheromone window traps (which are currently used in the Czech Republic). While landing pheromone traps had the share of male beetles in the overall capture ranging from 10% to 20%, the window ones usually showed the result of 30–40%. This is typical of polygamous bark beetles. In monogamous bark beetles like the striped ambrosia beetle (*Xyloterus lineatus*), it is the opposite way – most captured individuals are males, with considerable prevalence of 78–97%. In this case, there is also a difference between landing and pheromone window traps (Novák, Zahradník 1988). Aggregation pheromones always lure both sexes – primarily, they communicate information to the suitable material for reproduction; secondly, they lure the opposite sex to mating. In polygamous species, pheromones are produced by males, while in monogamous by females. This explains the differences in captured male and female beetles (Zahradník et al. 1993).

Facoli and Buffo (2004) assessed changes in the sex ratio of barrier traps in 2000–2001 over the course of that year's catches. During the first three weeks, the number of captured males fluctuated between 30% and 50%, later it stabilised at 33%.

Lubojacký and Holuša (2011) focused on the sex ratio of spruce bark beetles (*Ips typographus*) captured in window traps (Theysohn) and poisoned trap trees in the tripod form (Tripod). While pheromone traps indicated a significant prevalence of female beetles with a sex ratio of approximately 2 : 1, poisoned trap trees only had a moderate prevalence of captured females over males.

Similar results were obtained when using the tripod form of poisoned trap trees and traditional trap trees (Lubojacký, Holuša 2014).

Lubojacký and Holuša (2013) also studied the sex ratio of the double-spined bark beetle (*Ips duplicatus*) captured in window traps (Theysohn) and poisoned trap trees in the tripod form (Tripod). The results were very similar to those observed with *Ips typographus*. While pheromone traps indicated a significant prevalence of female beetles with a sex ratio of approximately 2 : 1, poisoned trap trees only had a moderate prevalence of captured females over males.

The determined sex ratio is not only relevant in direct control methods against *Ips typographus*. The use of poisoned traps, including the Trinet P system and Storanet insecticide nets, can lead to the selection of resistance regarding the method of contamination; the risk here is perhaps greater than in the case of bark wood treatment with insecticides. The sensitivity of males and females, which may be different, is not known yet. From this point of view, the ratio of captured individuals in different types of trap systems is significant, as it apparently also represents the proportion of individuals flying into poisoned traps, the Trinet P system and Storanet insecticide nets, from where *Ips typographus* individuals can fly away before they are killed and thereby create conditions for the emergence of resistance. This issue needs to be addressed further.

CONCLUSION

The results obtained in this project demonstrate that the sex ratio of captured spruce bark beetles shows no differences based on the type of trap system used (pheromone trap, poisoned trap tree, Storanet). Captured female beetles always outnumber captured male ones.

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