The influence of irradiation on the behaviour and reproduction success of eight toothed bark beetle *Ips typographus* (Coleoptera: Scolytidae)

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ABSTRACT: Irradiation experiments on *Ips typographus* (L., 1758) with doses of 15 and 30 Gy (cobalt 60) were conducted in 2006. The effect of irradiation on the mating behaviour and reproduction success of the bark beetle has been evaluated in rearing experiments in the laboratory. The effect of treatment on basic rearing parameters – (a) galleries per log, (b) mating chambers per log, (c) galleries with mother tunnels, (d) galleries with larvae tunnels, (e) mother tunnels – was not statistically significant – (a) F = 0.609, d.f. = 4, P = 0.560; (b) F = 1.883, d.f. = 4, P = 0.194; (c) F = 1.322, d.f. = 4, P = 0.303; (d) F = 0.373, d.f. = 4, P = 0.697; (e) F = 0.519, d.f. = 4, P = 0.608, ANOVA. The comparison of detailed rearing parameters showed statistically significant differences in the size of the mating chamber only, when both irradiated variants produced a larger mating chamber than did the control beetles (F = 5.113, d.f. = 4, P = 0.025, ANOVA). Irradiation changed the behaviour of males, so that they moved significantly faster than males in control (n = 18, P = 0.001, t-test), and 15 Gy irradiated males were significantly more successful in competition for females than 30 Gy irradiated males (F = 8.067, d.f. = 6, P = 0.015, ANOVA). In contrast, the number of eggs produced by females was significantly lower in the 15 Gy (F = 5.13, d.f. = 17, P = 0.029, ANOVA) and 30 Gy (F = 5.292, d.f. = 17, P = 0.028, ANOVA) irradiated variants as compared to the control and, the number of hatched eggs was significantly higher in the control group than in the 15 Gy (F = 5.415, d.f. = 17, P = 0.025, ANOVA) and 30 Gy (F = 6.916, d.f. = 17, P = 0.014, ANOVA) variants. The results are discussed below.

Keywords: *Ips typographus*; irradiation; sterile insect technique; reproduction success

The use of the sterile insect technique (SIT) for suppressing pest populations was proposed initially by Knipling (1955). Since that time the effectiveness of using the sterile insect technique (SIT) has been demonstrated by various models and applied operationally and successfully against many insect species. This technique is based on flooding the feral population with large numbers of sterile individuals (mostly males). When there is a high flooding ratio of sterile over feral individuals in the field, and the sterile individuals are fully competitive, the probability of mating of females by fertile males declines. However, to be effective the released mass-reared and sterile males have to successfully transfer their sperm carrying dominant lethal mutations to a large majority of females in the target population.

The treated areas may largely be influenced by the migration of non-sterile males. As a result, SIT is only effective when applied on an area-wide basis addressing the pest simultaneously over large forest areas. The effectiveness of integrating compatible pest control methods is significantly increased by coordinated implementation over larger con-

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tiguous areas to address whole target pest populations (Knipling 1979). This area-wide integrated pest management (IPM) approach to pest management is gaining acceptance for some key insect pests (Tan 2000). The case for an area-wide IPM approach arises for these key pests as they cannot be effectively controlled at the local forest level without the systematic use of insecticides that disrupt natural enemies and cause a negative impact to ecosystems.

Among biologically-based methods, the SIT is the most target-specific and non-disruptive method. Unlike some other biologically based methods it is species-specific, does not release exotic agents into new environments and does not even introduce new genetic material into existing populations as the released organisms are not self-replicating.

The efficiency of this method depends on several parameters which have to be evaluated prior to field release of irradiated insect species, *I. typographus* males in this particular case. One of the most important parameters is an optimal irradiation dose. Too high dose may cause the mortality of males, whereas too low dose may lead to their incomplete sterility. The irradiation may also change the behaviour of males and, if the behaviour is not competitive with that found in wild males, the release of sterile males may have a negligible effect. An additional important parameter is the sub-sterilizing dose in F1 generation which guarantees a higher efficiency of method in F2 generation.

Thus, the goals of this study were as follows:

– To assess the effect of sub-sterilizing doses (15 and 30 Gy) applied to emerging *I. typographus* males on F1 generation of the bark beetle, particularly with regard to their reproduction success.
– To analyze mating behaviour in males irradiated with different doses (15 and 30 Gy) when competing for non-irradiated females.

MATERIAL AND METHODS

We established continual rearing of *I. typographus* adults in June 2006. The beetles were collected in the Štiavnické vrchy Hills (GPS co-ordinates: N: 48°28.175'; E: 18°52.241'), West Carpathians, Central Europe, in their endemic populations in spruce monocultures, where the parasitism and proportion of pathogens are usually low. Infested logs were brought to the laboratory and put into the light boxes. The hatched and emerged individuals were captured daily in plastic jars and kept in refrigerator. After several days, when sufficient number of individuals was stored, we prepared several plastic bottles with individuals and carried out sexing. Adults' sex separation was done manually for these experiments according to Schlytter and Cederholm (1981). Separation was based on the great density of bristles on the pronota in females and/or bigger frontal projection typical for males. Separation of sexes of living, hand-held beetles was done using a stereomicroscope (magnification 42 times). Prior to examination, beetles were stored at a temperature below 5°C. If insufficient number of males were collected from the light boxes, the beetles collected by pheromone trapping were added to the experiment as well. The pheromone traps were installed in the close vicinity of spruce stands where logs for experiments were collected.

Irradiation doses of 15 and 30 Gy were used (cobalt 60). Because of possible problems concerning the rearing of irradiated individuals, the rearing was done in large numbers of individuals and each dose was carried out in five replications. Ten irradiated or non-irradiated (control rearing) males and 20 non-irradiated females were released to each of the rearing boxes at each variant.

Logs for irradiation experiments were taken one week prior to the beginning of the experiment. The log cuts were coated with paraffin to slow down the rate of desiccation. The irradiation experiments started in July (the 2nd flight). The rearing of individuals took place in rearing boxes (Fig. 1). The logs were about 30 cm in length and 20–30 cm in diameter. To avoid problems with the variability of trees, all logs were taken from one tree. Rearing was performed at a stable temperature of about 24°C and in the conditions of a long day (15 hours).

Estimation of the effect of sub-sterilizing doses on F1 generation was done indirectly, by:

(A) evaluation of basic parameters: (1) number of galleries per log, (2) number of mating chambers per log, (3) number of galleries with mother tun-

Fig. 1. Spruce log within rearing box. (Image: authors)

nels, (4) number of galleries with larvae tunnels, (5) number of mother tunnels;

(B) evaluation of detailed rearing parameters: (6) size of mating chambers, (7) length of mother tunnels, (8) number of eggs per mother tunnel, (9) number of larvae per mother tunnel and (10) length of larvae tunnels in treated versus non-treated rearing.

The sandwich experiments were established in the laboratory using males and females obtained via the procedure described above. The nine pieces of bark, each 20 × 20 cm in size, were prepared from the tree felled two weeks prior to the start of the experiments. Nine "sandwich" segments were prepared as shown in Fig. 2. The sandwich consisted of the lower glass, bark and upper glass (glass 30 × 30 cm in size). The bark and upper and lower glass were separated by plastic pieces (6–7 mm wide). To eliminate high humidity inside the sandwich, an opening was made on one of the sides of each sandwich. The opening was covered by a tissue which prevented beetles from escaping and which also allowed airing the space (Fig. 3). In spite of this, condensed water accumulated on the glass. We dried it using cotton wool on sticks via a temporary opening. With these measures, it was possible to keep fungi under control throughout the experiment.

The six males irradiated with the same doses as in the experiment above (control, 15 and 30 Gy) were released to each of the sandwich boxes and their behaviour was observed during the subsequent days.

Their behaviour in respect of their motion, time needed for the preparation of entry holes and the establishment of mating chambers has been evaluated.

A total of eighteen non-irradiated females were released into sandwich experiments 2 days later than males, when entry holes were already established. The competitiveness for females was evaluated based on the number of mother tunnels produced by females attracted by irradiated or non-irradiated males. The length of mother tunnel was an additional criterion for assessment of possible reproduction success. The number of eggs, as well as the number of hatched larvae, was evaluated. The behaviour of larvae was evaluated after larvae had hatched. The experiments continued for 5 weeks, as long as hatched larvae were able to continue feeding.

Data analysis

To test differences in averages of evaluated parameters one-way ANOVA and/or a pair *t*-test (movement of males, preparation of entry holes) was used. Treatment (different doses of irradiation) was considered as an effect in both parametric tests. Data were ln-transformed prior to statistical analysis.

RESULTS AND DISCUSSION

Evaluation of rearing parameters

The effect of treatment on basic rearing parameters – (a) galleries per log, (b) mating chambers per log, (c) galleries with mother tunnels, (d) galleries with larvae tunnels, (e) mother tunnels – was not statistically significant – (a) $F = 0.609$, d.f. = 4, $P = 0.560$; (b) $F = 1.883$, d.f. = 4, $P = 0.194$; (c) $F = 1.322$, d.f. = 4, $P = 0.303$; (d) $F = 0.373$, d.f. = 4, $P = 0.697$; (e) $F = 0.519$, d.f. = 4, $P = 0.608$, ANOVA (Fig. 4). However, males irradiated with the dose of 15 and 30 Gy were more efficient than those non-irradiated (number of galleries per log, mating chamber per log, galleries with mother tunnel and galler-

Fig. 2. Design of rearing sandwich used in some experiments. (Image: authors)

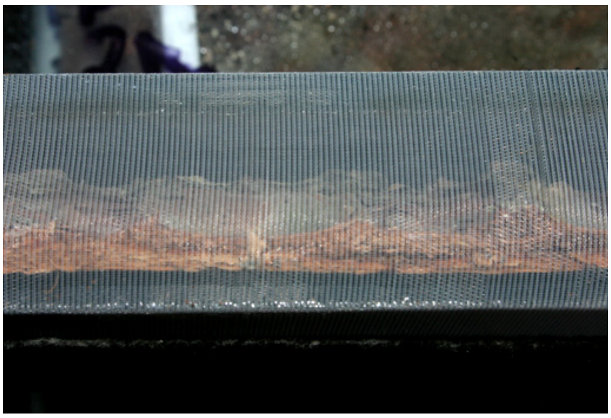

Fig. 3. Detail of ventilation opening on rearing sandwich. (Image: authors)

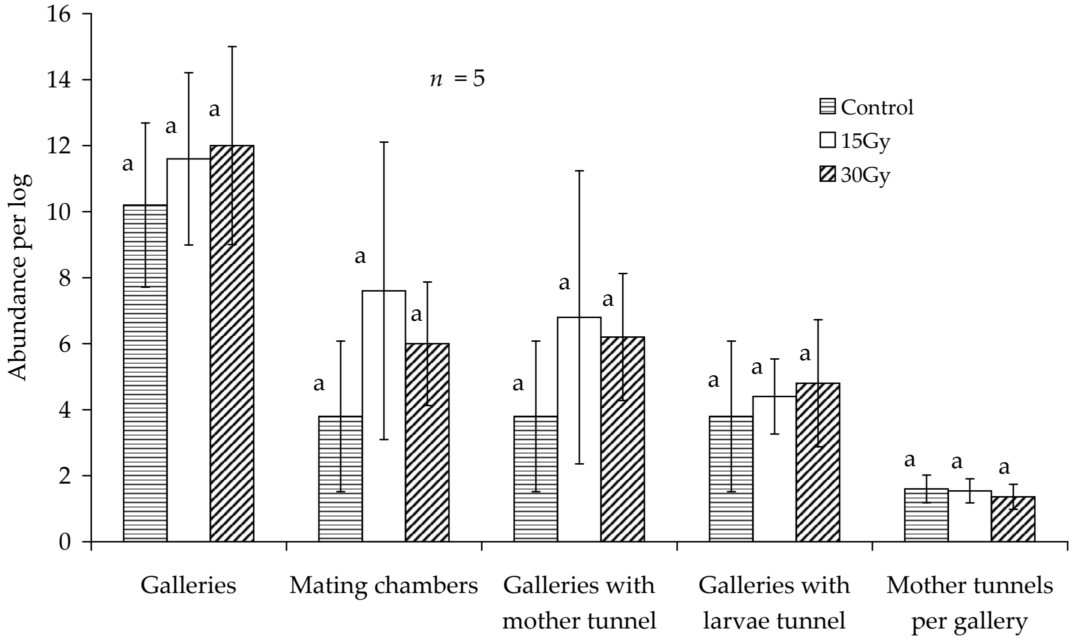

Fig. 4. Comparison of basic rearing parameters in irradiated and control treatments (mean value per log ± standard deviation). The bars flagged with the same letters are not significantly different

ies with larvae tunnel). Number of mother tunnel reached similar values in all the treatments.

The comparison of detailed rearing parameters (Fig. 5) showed statistically significant differences in the size of the mating chamber only, when both irradiated variants (15 and 30 Gy) produced a larger mating chamber than did the control beetles ($F = 5.113$, d.f. = 4, $P = 0.025$, ANOVA). Irradiated males seemed to be more active than non-irradiated males. The number of eggs per mother tunnel and number of larvae per mother tunnel were very similar. In irradiated treatments, females produced fewer eggs and fewer larvae hatched than in control treatments. It would seem that the irradiated males reached a lower reproductive rate than the control males.

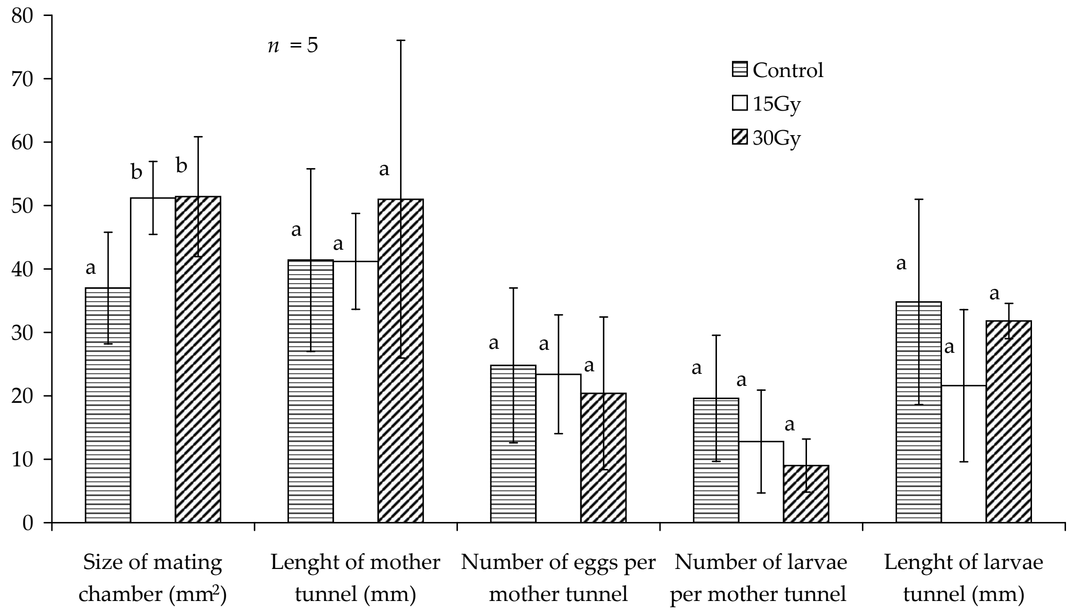

Fig. 5. Comparison of detailed rearing parameters in irradiated and control treatments (mean value per log ± standard deviation). The bars flagged with different letters are significantly different

Behaviour of irradiated and non-irradiated males

Movement of males

Males irradiated by both 15 and 30 Gy were significantly more active than the control males. The distance walked by males irradiated with the 30 Gy 10 minutes after their release was the longest, approximately four times longer (84 cm in average, n = 18) than that found in non-irradiated males (23 cm in average, n = 18). The difference was also statistically significant (n = 18, P = 0.001, t-test). The distance walked by 15 Gy irradiated males was slightly longer than that found in non-irradiated individuals (32 versus 23 cm). Also this difference was statistically significant (n = 18, P = 0.020, t-test). The irradiated males walked over the sandwich area faster and were looking for a suitable place to construct entry holes. Often, they stopped crawling at a suitable place, but continued to move after a short time.

Preparation of entry holes

The time from the release to the start of preparation of entry holes was almost the same for both irradiated and non-irradiated males. In spite of the fact, that the irradiated males walked a significantly longer distance prior to the beginning of the preparation of entry holes, they started to prepare entry holes generally faster than the non-irradiated males. The difference was not statistically significant however (n = 3, P > 0.05, t-test). The 30 Gy irradiated males also bored faster than those irradiated with 15 Gy and non-irradiated and they entered the phloem faster, but the difference between the groups was not statistically significant (n = 3, P > 0.05, t-test). The average number of entry holes/per sandwich was the highest in 15 Gy irradiated males (5.00, n = 3), followed by non-irradiated males (4.67, n = 3) and 30 Gy irradiated males (4.00, n = 3), but difference was not statistically significant (F = 1.000, d.f. = 2, P = 0.422, ANOVA).

Establishment of mating chambers

The mating chambers were formed faster and in a higher number by non-irradiated males (4.00) then irradiated ones (3.33 for 15 Gy and 3.67 for 30 Gy), the differences was not statistically significant (F = 0.047, d.f. = 2, P = 0.955, ANOVA) however. We can conclude the doses of 15 and 30 Gy do not have a significantly negative effect on the behaviour of irradiated males in the preparation of entry holes and the creation of mating chambers. It seems that irradiated males are more competitive in comparison with non-irradiated males in these parameters.

Competition for females

As the results indicate, the number of mother tunnels was the highest in the case of 15 Gy irradiated males (3.30 mother tunnels per mating chamber, n = 7), followed by non-irradiated males (2.67 mother tunnels per mating chamber, n = 7) and 30 Gy irradiated males (1.92 mother tunnels per mating chamber, n = 7). The differences among the variants were significant (F = 4.895, d.f. = 2, P = 0.020, ANOVA). The mechanism of competition for females is not quite clear in this experiment. The lowest number of entry holes might be the main advantage for 15 Gy irradiated males and, thus, more females searched for fewer males. In other variants, this fact did not play any role and non-irradiated males attracted more females than 30 Gy males, despite the fact that the former ones were more abundant than the latter ones. It is possible that 15 Gy irradiated males are more active in competition for females and thus more attractive to females, which might be advantageous when they are released in nature.

The length of mother tunnels in galleries formed by irradiated versus non-irradiated males

The total length of mother tunnels was the longest in the case of non-irradiated males (3.97 cm per mother tunnel, n = 19), and they were significantly longer than 15 Gy (2.52 cm per mother tunnel, n = 23) and 30 Gy (2.15 cm per mother tunnel, n = 12) irradiated males (F = 6.875, d.f. = 17, P = 0.002, ANOVA). The difference in the length of mother tunnels formed by the groups of irradiated males was not significant (F = 0.707, d.f. = 17, P = 0.407, ANOVA). We might predict that the activity of females is connected with the ability of males to mate with females and with maturation and production of fertile eggs. The mating success in irradiated males is probably lower and thus females bored shorter mother tunnels.

The number of eggs laid in galleries formed by 15 and 30 Gy irradiated versus non-irradiated males

The total number of eggs laid by *Ips typographus* females was generally lower than that in natural conditions. The females in nature, or in bolts in the laboratory, are able to produce up to 80 eggs (Heidger 1994). The females in the sandwich experiment laid much fewer eggs – possibly as a result of unfavourable conditions in the sandwich. The number of eggs is related to total length of mother tunnels to some extent. Females keep quite regular span between neighbouring eggs which results in a relatively similar outcome as in the previous paragraph. However, the number of eggs showed the relatively lower density of eggs in the variants with irradiated males. The average number of eggs per mother tunnel reached 19.57 eggs for the control group (n = 19). It was followed by 10.94 eggs in the 15 Gy irradiation experiment (n = 23) and 7.95 eggs per gallery in the 30 Gy irradiation experiment (n = 12). Females oviposited significantly less eggs in the 15 Gy (F = 5.13, d.f. = 22, P = 0.029, ANOVA) and 30 Gy (F = 5.292, d.f. = 22, P = 0.028, ANOVA) variants as compared to the control. The difference between irradiated variants was not significant (F = 1.052, d.f. = 17, P = 0.313, ANOVA). The results indicate that irradiated males are less efficient in reproduction success which has to be taken into consideration in further experiments.

The number of hatched eggs in galleries formed by 15 and 30 Gy irradiated versus non-irradiated males

The number of hatched eggs varied from variant to variant. The differences between the respective variants were higher than in the previous section, because the proportion of hatched eggs was the highest in the non-irradiated variant (70%, n = 19), lower in 15 Gy irradiation (58%, n = 23) and the lowest one in the case of 30 Gy irradiation (49%, n = 12). Thus, the differences among averages in irradiated and non-irradiated treatments were statistically significant (F = 4.986, d.f. = 17, P = 0.011, ANOVA).

Based on these results, we propose to undertake further research with doses in the range between 15 and 25 Gy. To eliminate rearing problems, logs should be prepared within one week after tree cutting. The bolts preparation has to be done shortly prior to the beginning of rearing.

Number and sex ratio of the F1 offspring were not identified in this study, because of the low number of emerged beetles in all treatments. There were some problems connected with airing the rearing substrates and retaining favourable humidity in these substrates. Fresh logs dried up quickly, the food quality was strongly modified and, as a result, the mortality of larvae was high. Thus, these experiments have to continue in the further future to estimate the parameters of F1 and F2 generations. Direct evaluation of irradiation effect to sperm of *I. typographus* males (according to Schreiber 1989), in the parental, F1 and F2 generation of the beetle, is urgently needed.

There are additional areas of research dealing with relationships between irradiated individuals and their environment. Natural insect enemies, and especially pathogens, may have adverse affect on the population of irradiated *Ips typographus* males released to suppress population of the bark beetle in nature and, thus, they can strongly modify the treatment results. As stated by Novotný and Zúbrik (2003) the patogens may kill higher proportion of irradiated individuals of gypsy moth than non-irradiated individuals and thus eliminate the effect of their release. The forest insects live in much more complicated food chain than agricultural pests where the sterile insect technique is already common. Taken in aggregate, a successful implementation of SIT in *Ips typographus* and/or other insects control may take a long time.

CONCLUSIONS

The impact of treatment on basic rearing parameters (number of galleries per log, number of mating chambers per log, number of galleries with mother tunnels, number of galleries with larvae tunnels, and number of mother tunnels) was not confirmed statistically.

Comparison of detailed rearing parameters showed statistically significant differences in the size of the mating chamber only, when both irradiated variants produced larger mating chamber.

Irradiation changed the behaviour of *Ips typographus* males, making their movements significantly faster, 15 Gy irradiated males were significantly more successful in competing for females than males irradiated with the dose of 30 Gy or those non-irradiated (control).

On the other hand, number of eggs produced by females was significantly lower in irradiated variants than in the control. Number of hatched eggs was significantly higher in the control group than in irradiated variants.

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Vliv ozáření na chování a reprodukční úspěch lýkožrouta smrkového *Ips typographus* (Coleoptera: Scolytidae)

ABSTRAKT: V roce 2006 byly realizovány experimenty s ozařováním *Ips typographus* (L., 1758) dávkami 15 a 30 Gy (kobalt 60). Byl hodnocen účinek ozáření na chování při páření a reprodukční úspěch v umělých chovech v laboratoři. Vliv variant ozáření na základní hodnocené parametry – (a) požerky na klát, (b) snubní komůrky na klát, (c) požerky s mateřskými chodbami, (d) požerky s larválními chodbami, (e) mateřské chodby – nebyl statisticky významný – (a) F = 0,609, d.f. = 4, P = 0,560; (b) F = 1,883, d.f. = 4, P = 0,194; (c) F = 1,322, d.f. = 4, P = 0,303; (d) F = 0,373, d.f. = 4, P = 0,697; (e) F = 0,519, d.f. = 4, P = 0,608, ANOVA. Porovnání dalších chovných parametrů naznačilo statisticky významné rozdíly pouze ve velikosti snubní komůrky, když samci obou ozářených variant připravili větší snubní komůrky než samci v kontrole (F = 5,113, d.f. = 4, P = 0,025, ANOVA). Ozáření změnilo chování samců; ti se pohybovali rychleji než samci z kontrolní varianty (n = 18, P = 0,001, t-test). Samci ozáření 15 Gy byli významně úspěšnější při lákání samiček než samci ozáření 30 Gy (F = 8,067, d.f. = 6, P = 0,015, ANOVA). Naopak počet vajíček kladených samicemi byl významně nižší při variantě ozářené 15 Gy (F = 5,13, d.f. = 17, P = 0,029, ANOVA) a 30 Gy (F = 5,292, d.f. = 17, P = 0,028, ANOVA) v porovnání s kontrolní variantou. Rovněž počet vylíhlých vajíček byl statisticky významně vyšší v kontrolní skupině než ve variantách ozářených 15 Gy (F = 5,415, d.f. = 17, P = 0,025, ANOVA) a 30 Gy (F = 6,916, d.f. = 17, P = 0,014, ANOVA). O získaných výsledcích se diskutuje.

Klíčová slova: *Ips typographus*; ozařování; metoda sterilního hmyzu; reprodukční úspěch

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