

# TESTING THE ABILITY OF *IPS* *TYPOGRAPHUS* L. FEMALES TO DEVELOP NEW MATERNAL GALLERIES IN LAB CONDITIONS

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**Abstract:** *We report the preliminary results regarding the capacity of Ips typographus females to develop new maternal galleries in controlled conditions of temperature, humidity and 24L:0D and 16L:8D photoperiod. Despite the fact that the number of entries was higher on the logs permanently exposed to light, other parameters, such as the success in the development of new maternal galleries, the length of the maternal galleries and the density of the maternal galleries with eggs, were higher in the case of 16L:8D photoperiodic regime, but no significant differences were found.*

**Key words:** *IPS typographus, photoperiodic regime, maternal galleries, density.*

## 1. Introduction

The spruce bark beetle *Ips typographus* L. (Coleoptera: Curculionidae) is one of the most important pest of the spruce stands in Europe [6].

An important aspect that influences the flight behaviour of bark beetles, are the light conditions. Lobinger & Skatulla [13] show that even during sunny periods of only a few minutes, significantly more beetles were flying than during overcast periods.

The first observations upon the photoperiodic regime in the case of *Ips typographus* were carried out by Byers [4], who found that there was a difference of 30 minutes between the outgoing moment of the females and the outgoing moment of

the males of the new generation, in laboratory conditions, at a temperature of 25°C and 20L:4D photoperiod.

According to Paynter et al. [14] adults require a short time to distinguish the appropriate trees, about 3 minutes in case of males and 4 minutes in case of females. Usually, the females, attracted by the aggregation pheromone, enter the nuptial chamber less than a day after its construction and after that, less than 5 hours are required for the colonization with two females [7].

However, according to Anderbrant & Lofquist [2], in the absence of males, females perform alone the entry holes under the bark.

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The most important factors that influence females to resume the flight are weather conditions and fecundity. The fertility can be influenced by the phase of gradation, the age of the females, the quantity of eggs deposited in the first laying. Frequently, females exit and produce a second or even a third laying and we don't know if they need to mate again [2] or if they have to mate at regular intervals [5]. For the bark beetles in general and for *Ips typographus* in particular, sister brood contributes significantly to increasing the population [1], [9].

The ability of *Ips typographus* females to develop the new maternal galleries, is conditioned by the achievement of a threshold of at least 21.5°C [3].

As a consequence, we face the following question: is the ability of *Ips typographus* females to develop new maternal galleries influenced by the photoperiod?

## 2. Material and methods

We tested in the laboratory, the capacity of *Ips typographus* females to develop new maternal galleries, in controlled conditions of temperature, humidity and photoperiodic regime.

A series of field and laboratory preparatory work was required, namely: achievement of ten sections of spruce (40 cm of length) from five diameter classes (between 12 cm and 16 cm), two of every one; to avoid the problems related to the variability of trees, the logs were made out of a single spruce [12], [15], [16]; the logs were kept in the laboratory for some other 10 days, at a constant temperature of 18°C, for aging and further limiting the leakage of resin [16]; the log ends were waxed, so as to slow down dehydrations [10], [16]; the logs were introduced in mesh bags provided at one end with a Velcro-type closure system; the females of *Ips typographus* were collected by debarking some trees located in Râsnov and in Poiana

Brasov forests; we collected females that were at the beginning of the laying and in particular those that had been mated, but had not started yet to lay eggs.

On May 28<sup>th</sup> 2010, 16:00, one log and 30 females were placed in each mesh bag, and ten such bags were put inside two climatic chambers for which some parameters were set. Five bags with logs numbered from 1 to 5, were introduced in the first climatic chamber at 20°C, 60% r.h., and 24:0 h L:D (light:dark) photoperiod and other five bags with logs numbered from 6 to 10 were introduced in the second climatic chamber at 20°C, 60% r.h., and 16:8 h L:D (light:dark) photoperiod. In an attempt to imitate the natural conditions as closely as possible, the light was scheduled between 6:00 AM and 22:00 PM, and the dark between 22:00 PM and 6:00 AM, respectively. Starting with May 29<sup>th</sup>, 16:00, we verified the logs every day: inspecting the entry holes and marking them with different colour markers, counting the beetles; and, after inspecting their condition, we reintroduced the active ones in bags and removed those that did not show signs of activity.

On June 18<sup>th</sup>, we ended the experiment and then we analyzed the logs: each log was debarked and the attacks were copied on nylon films with the same dimensions as the logs; next, we measured the length of the maternal galleries (egg galleries), the larval galleries and we counted pupas and imago adults.

*Data analysis.* The differences between various parameters of the female activity (the number of entrances, the success in the development of new maternal galleries, the length of the maternal galleries, the density of the maternal galleries) in the case of two tested photoperiod (24L:0D and 16L:8D) were analysed by Student's t-Tests, after verification of normality with Shapiro-Wilk test and homogeneity with Hartley test. When the homogeneity was

not confirmed, we used the nonparametric Kruskal-Wallis test. In these analyses, the logs were considered as repetitions. All statistical analyses were made using XLSTAT-Pro 2015 software.

### 3. Results and discussions

#### 3.1. The influence of the log size

The logs used in the experiment could influence the boring of the entrance holes through the size and the substrate quality.

Logs from a single tree were used to minimize the differences in the nutritive value of the bark. Also, the analyses of the variance revealed no significant

differences between logs, from the point of view of the log size ( $P = 0.946$ ).

#### 3.2. Dynamics of the entries of *Ips typographus* females under the log bark

All the logs used in the experiment had entries. 111 entry holes were bored (Table 1). Studying the behaviour of the *Ips typographus* adults, Paynter et al. [14] found that from a total of 118 adults, only 33% entered under the bark and the others left the logs (31%), fell off the logs (31%) or were killed by predators (1%).

Table 1

*The total number of entries, active and inactive females*

Regime	Total	Entries	Inactive females	Active females
24L:0D	no.	59	115	112
	%	39.3	76.7	74.7
16L:8D	no.	52	115	70
	%	34.7	76.7	46.7

Consequently, the entrance percentage of 37% obtained by us was slightly higher and this might be due to the fact that we used only mated females and they were unable to fly elsewhere.

The test duration was of 21 days in the case of the 24L:0D photoperiod and of 20 days in the case of the 16L:8D photoperiod, but starting with the 14<sup>th</sup> day no activities were counted outside the logs.

In both climatic chambers, most of the entries were performed in the first 4 days, (88.1% in the case of the 24L:0D photoperiod and 84.6% in the case of the 16L:8D photoperiod); after that, the activity of the females obviously decreased outside the logs.

The entries into the logs, in the second climatic chamber, were performed during a longer period of time than in the case of the first climatic chamber (3 days longer). It was surprising that the period of 8 hours,

considered to be a required rest, was exploited by the females permanently exposed to light, in search of laying sites.

No significant differences were found between the numbers of entries of the *Ips typographus* females under the log bark, in the case of the two photoperiodic regimes that we tested. The results for day 1 and day 2 were obtained by Student's t-Tests; and for day 3 and day 4, we used the nonparametric Kruskal-Wallis test (Figure 1).

Thus, the females in our test found places for laying, in over 72 hours, in the case of the photoperiodic regime of 24L:0D, which indicates a high degree of activity of the females. In contrast with Paynter et al. [14] where the females were free to leave the logs, the females from our experiment could not do this; instead they reduced all their functions and entered rest, as dictated by the development conditions.

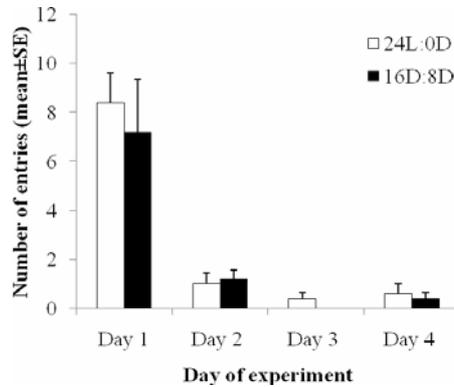


Fig. 1. Dynamics of the entries of *Ips typographus* females under the bark of logs in the 24L:0D and 16L:8D photoperiodic regime

The amount of inactive females was the same in the case of both climatic chambers (Table 1). The proportion of active females in the case of the photoperiod regime of 16L:8D seems to be lower than in the case of the photoperiod regime of 24L:0D (Table 1), but the statistical differences between the numbers of active females are not significant (Figure 2) in the case of the two photoperiodic regimes that we tested (Student's t-Tests for day 1, day 7 and day 8, and Kruskal-Wallis test for the other days).

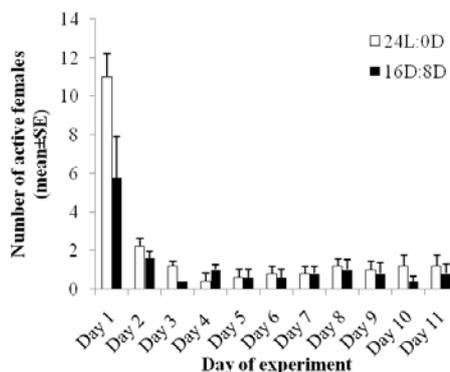


Fig. 2. Variation of the number of active females in the 24L:0D and 16L:8D photoperiodic regime

Although we cannot consider the inactivity of the females in our experiment as an entry into diapause, we could still consider that the blockage in the reproductive function of the fertilized females was forced by the development conditions inside the climatic chambers. Doležal & Sehn [8] believe that at a temperature of 20°C and a photoperiodic regime of 12L:12D, the adults stop reproducing, but the females continue laying eggs at low temperatures [17]. Instead, they note that the *Ips typographus* adults transferred from forest into lab, at a photoperiodic regime of 16L:8D and at the same temperature of 20°C, initiate reproduction.

The correlation between the number of entries of *Ips typographus* females and the bark area is evident (Figure 3). This is mainly due to the reduction in the attacks on the logs with small diameters and to the increase in the attacks on the logs with large diameters; this situation, previously found out by Zolubas et al. [18], being evident in the logs kept under normal photoperiodic regime of 16L:8D.

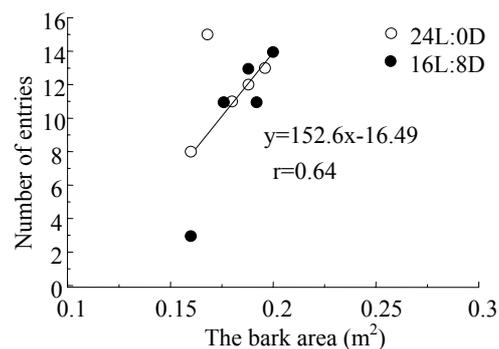


Fig. 3. Correlation between the number of entries of *Ips typographus* females and the bark area

### 3.3. The success of the development of new maternal galleries

From a total of 111 maternal galleries, 33 had eggs, leading to a success in the development of new maternal galleries, of 29.7% (Table 2).

Although the gnawing was carried out under the bark, theoretically in a place

without light, the effect of the exposure to light materialized in a higher success of the colonization when the photoperiod regime drew closer to the natural (16L:8D). I.e., 34.6% of the females had their entrance into the logs, in the photoperiodic regime of 16L:8D, changed into new maternal galleries, as compared to 25.4% in the case of permanent exposure to light.

Table 2  
The success of colonization

Photoperiod	The log	Number of entries	Number of maternal galleries with eggs	The success of colonization (%)
24L:0D	1	8	1	12.5
	2	15	4	26.7
	3	11	2	18.2
	4	12	6	50
	5	13	2	15.4
16L:8D	6	3	0	0
	7	10	2	20
	8	13	3	23.1
	9	12	6	54.5
	10	14	7	50
<b>Total</b>		<b>111</b>	<b>33</b>	<b>29.7</b>

In both climatic chambers, the success of the attacks was higher on larger diameter logs, related to the preference of *Ips typographus* beetles for larger diameters and thick bark [18]. The lack of successful attacks on the log no. 6 can be explained by the fact that it was part of the lowest category of diameters (12 cm) with thin bark, close to the minimum of 2.5 mm which adults of *Ips typographus* attack [11].

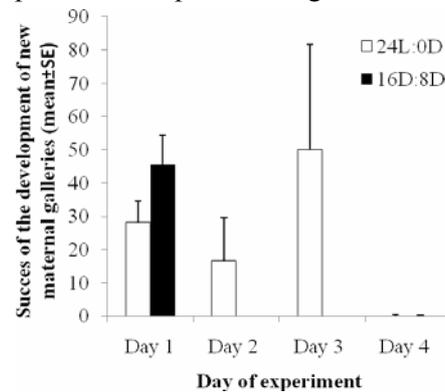


Fig. 4. The success of the development of new maternal galleries in the 24L:0D and 16L:8D photoperiodic regime

Obviously, the effect of the light regime that we chose, namely 24L:0D in the case of the log no. 1, forced a strong activity of the females in search of opportune places for laying eggs, compared to the photoperiodic regime of 16L:8D, in the case of log no.6 with the same diameter, where the number of entries is lower, reflecting the fact that the females chose to enter inactivity (diapauses) quickly, rather than deplete their energy doing wrong gnawing on a substrate inappropriate for further development of the larvae. A possible explanation for this could be the light stimulus forcing the beetles permanently exposed to light to be more potent, compared to those exposed to the light:dark regime, where turning light off corresponds to nightfall in the natural environment and to the diapauses of bark beetles.

As in the case of the dynamics of entries, the most successful attacks were found in the first 72 hours, in the case of the females permanently exposed to light; although some activity was also recorded afterwards. The females were forced by light and temperature to look for oviposition sites even if they had found the inappropriate substrate. In the case of the females exposed to diurnal normal light regime, turning light off determines the females go through an easier transition to inactivity, when they do not find the substrates suitable for laying eggs, and they can simply continue exploring the logs the next day, once with the beginning of their exposure to light. An evidence of the aforementioned is the 11-day period in which successful attacks were registered.

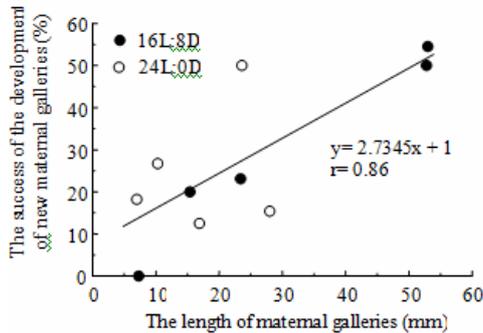


Fig. 5. Correlation between the success in the development of new maternal galleries and the length of the maternal galleries

Having failed to achieve the critical levels of density in the case of the 10 logs, the longest maternal galleries could have developed on those who had the highest success of the attack, probably because females found the best conditions for development under their bark. This correlation between the success in the development of new maternal galleries and the length of the maternal galleries was stronger in the case of the experiment conducted under a normal photoperiod of

16L:8D (Figure 5). At the same time, the attempts to colonize logs were more numerous in the case of long-term exposure to light, but the success of the colonization was higher in the case of bark beetles kept under normal day:light regime.

### 3.4. Competition between females

In our experiment, we did not use males and the presence of nuptial chambers was unlikely.

However, in some cases, more females used the same entrance hole. This happened especially in the case of higher densities and the largest diameter of logs.

The maternal galleries carried out under permanent light had an average length of 17.2 mm, almost half the average length of 30.4 mm of the maternal galleries recorded for the light-dark (16L:8D) photoperiodic regime (Figure 6), but there were not found significant differences between the length of the maternal galleries in the case of the two photoperiodic regimes that were tested (Student's t-Tests).

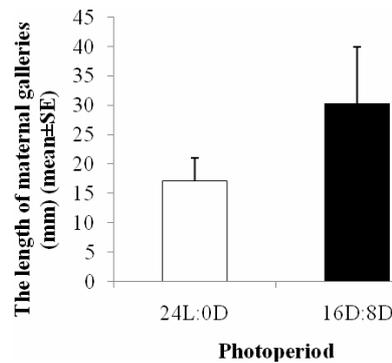


Fig. 6. The length of maternal galleries in the 24L:0D and 16L:8D photoperiodic regime

In the experiment with the normal photoperiodic regime of 16L:8D, the density of the maternal galleries, was of 19, higher than the one of the 16 maternal galleries, recorded in the case of the logs

permanently exposed to light (Figure 7). By applying the Student's t-Tests, no significant differences were found between the densities calculated as number of maternal galleries with eggs per sq of bark. In both photoperiodic regimes, the longest maternal galleries were recorded on the logs with larger diameters and lower densities (Figure 8).

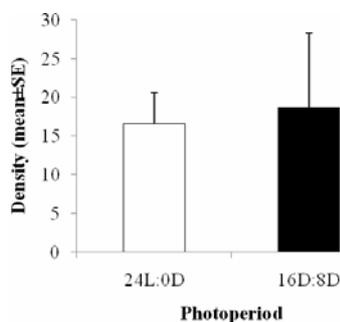


Fig. 7. Density of maternal galleries in the 24L:0D and 16L:8D photoperiodic regime

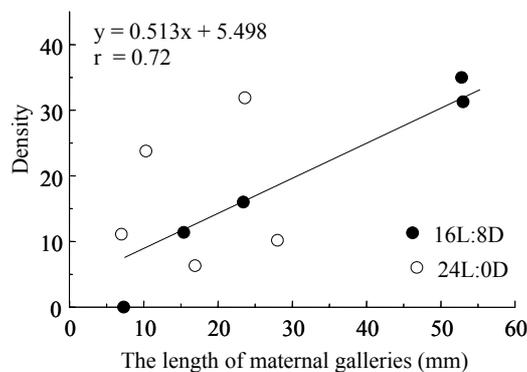


Fig. 8. Correlation between the density of the maternal galleries and their length

#### 4. Conclusions

111 entry holes were bored and the entrance percentage was 37%, 39.3% for the 24L:0D photoperiod and 34.7% for the 16L:8D photoperiod. From a total of 111 entries, 33 were led to maternal galleries with eggs, so the success in the

development of new maternal galleries was 29.7% (34.6% of the females had their entrance into logs, in the photoperiodic regime of 16L:8D, changed into new maternal galleries, as compared to 25.4% in the case of permanent exposure to light). Despite the fact that the number of entries under the bark of logs permanently exposed to light was higher than in the case of light: dark photoperiod, other parameters were higher in the case of the 16L:8D photoperiodic regime. Thus, we concluded that the photoperiodic regime affected the activity of the females outside the logs. Some differences were found in the activities under the log bark, which consisted in different development degrees of the new generation, but no significant differences were established between the success in the development of new maternal galleries, the length of the maternal galleries and the density of the maternal galleries with eggs for the two tested photoperiods.

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