University of South Bohemia in České Budějovice

Faculty of Science

Sister broods in the spruce bark beetle, *Ips typographus* (L.)

Master thesis

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Annotation:

The aim of this thesis was to elucidate the establishment of sister broods in the spruce bark beetle, *Ips typographus* (L.), at three localities of different elevation in Sumava Mountains. Fecundity, sex ratio in the offspring, regeneration feeding, development of reproductive organs, re-emergence rate and other ecophysiological topics were studied as well.

Content

1   Introduction

The spruce bark beetle, (*Ips typographus* L.), is a serious pest of spruce monocultures in Eurasia (Doležal & Sehnal, 2007) that causes considerable economic and environmental damage throughout its entire distribution area. Yearly volume of timber felled in the Czech Republic due to bark beetle infestations reached more than 650,000 m^3 in 2012 (Knížek & Modlinger, 2013). In the European scale, the cost of protective measures and losses on commercial value of wood products represent billions of euros every year.

Ips typographus is considered a secondary pest that prefers weakened and dying trees over 60 years of age (Zahradník & Knížek, 2007) and starts the process of wood and bark decomposition. Spruce bark beetle is a typical r-strategist, which is able to exponentially multiply under suitable weather conditions and ambient food source available after windthrows and other natural calamities (Wermelinger 2004). High population densities lead to outbreaks, during which bark beetles attack also living healthy trees, regardless of age (Wermelinger, 2004; Zahradník & Knížek, 2007), overcome their defense mechanisms and become a primary pest that causes tree mortality on large areas (Kausrud et al., 2012). Most aspects of spruce bark beetle life cycle are well known and reviewed by numerous literature (Zumr, 1995; Wermelinger, 2004).

In the spring, overwintered males of the spruce bark beetle attack suitable host trees, bore a mating chamber and start producing aggregation pheromones. In Central Europe, the timing of their emergence (i.e., spring swarming) depends on temperature/elevation. In lowlands, overwintered adults usually start to emerge at the end of April, although rainy weather may cause a delay until the beginning of June. In elevations above 800 m a.s.l., swarming begins up to more than one month later compared to lowlands (Skuhravý, 2002). Swarming is strongly affected by temperature. According to Baier (2007) bark beetles started to swarm approximately 60 degree days (DD) from the beginning of April and the first infested trees were recorded after 140 DD. DD were calculated from daily means of bark temperatures from which lower developmental threshold of 8.3 °C was subtracted. Lower temperature threshold for flight activity was 16,5 °C and temperature optimum was between 21 °C and 26 °C (Wermelinger, 2004; Baier, 2007) in the population from Kalkapen Alps, Austria. One male mates with one to three females (Zahradník & Knížek, 2007). Fertilized females bore maternal galleries parallel with the trunk axis and lay eggs aside. One female lays approximately 50 eggs per ovarian cycle (Zumr & Soldán, 1981; Skuhravý, 2002).

Three reproductive cycles were well documented in the spruce bark beetle females by Zumr & Soldán (1981), who stated that the average number of oviposited eggs did not differ between the ovarian cycles. Oviposition period lasts up to 10 days (Skuhravý, 2002) and females oviposit in temperatures ranging from 12 °C to 33 °C (Wermelinger, 1999). Hatched larvae bore larval galleries vertical to maternal tunnels. *Ips typographus* larvae go through three larval instars and the last instar larvae create pupation chambers, where metamorphosis from pupae to adults occurs. Development of one generation in the temperature conditions of Central Europe lasts 6 to 10 weeks and is exclusively temperature dependent until the shortening day length in the fall prolongs the development of larvae (Doležal & Sehnal, 2007). Immature stages of the spruce bark beetle continuously develop at temperatures ranging between 15 °C and 30 °C. Within this range under laboratory conditions, the 1st instar larvae hatched in 3 – 12 days, larval stage lasted 11 – 30 days and pupae developed 2 - 11 days (Wermelinger & Seifert, 1998). According to Annila (1969), the lower developmental threshold was 5 °C. However, 45 % of immature stages continued to develop regardless of this low temperature. When recorded in the field in southern Finland, 1st instar larvae started to hatch in 5 – 29 days. Under laboratory conditions, the average time of embryonic development was 12 days at 14 °C and 3.5 days at 24 °C. Larval development at 14 °C lasted 31 days. Higher constant temperature of 24°C considerably shortened developmental time and the larvae pupated in 9 days. Pupal development at constant temperatures of 24 °C and 14 °C lasted 4 and 13 days, respectively (Annila, 1969).

The onset of summer swarming depends exclusively on completion of development in the filial generation. According to Baier (2007), the thermal sum required for development from egg to adult was 557 DD. Similar results were obtained both in the field and under laboratory conditions. Summer swarming in Central Europe is usually asynchronous with bark beetles emerging since early to mid-July at lower elevations and south facing slopes. At higher elevations and north facing slopes summer swarming starts at the end of July and even later in the mountains (Zahradník & Knížek, 2007).

A phenomenon that is common to *Ips typographus* and several other species of Scolytinae subfamily is called sister broods or re-emergence. Re-emergence means that parental females finish oviposition, re-emerge and replenish their energy reserves by so called regeneration feeding. Depending on the phloem quality, regeneration feeding may occur prior to re-emergence in the original host tree or in the newly infested tree. Once energetic reserves and ovaries are replenished, females continue egg laying, without a need of additional mating (Martinek, 1956; Anderbrant & Löfqvist, 1988). The most probable

advantage of re-emergence is seeking new material for regeneration feeding and egg laying, reduction of intraspecific competition and suitable habitat for development of offspring. On the other hand, re-swarming increases the risk of dying before infesting a new host tree. Kirkendall (1983) proposed three hypothesis, why sister broods occur:

1) The "bankruptcy" hypothesis – egg production and oviposition is nutrient demanding and re-emergence allows restoring the reserves in a fresher material.

2) The "greener pastures" hypothesis – the risk of death during re-swarming is outweighed by an advantage of oviposition and development of offspring in a fresh material.

3) The "overcrowding" hypothesis – due to high density, the new progeny is endangered by older larvae (intraspecific cannibalism) and therefore re-emergence to new material is a preferred choice.

In Central Europe, re-emergence of sister brood females and emergence of filial beetles commonly overlap. Timing of sister brood re-emergence depends predominantly on temperature. When spring weather is cold and rainy, spring swarming is delayed and if unfavorable conditions prevail, the re-emergence of sister brood adults is delayed too. Such adverse conditions reduce the number of sister broods. On the contrary, if the weather in the spring is favorable, spring swarming occurs at the end of April / beginning of May and the number of sister broods is higher. Furthermore, number of sister broods is influenced by elevation (Martinek, 1961). According to Martinek (1956, 1957, 1961), second sister brood does not occur above 700 m a.s.l. Over 60 % of females establish the first sister brood and 20 – 35 % re-emerge for the second time (Martinek, 1961). According to Annila (1969) and Anderbrant & Löfqvist (1988), reproductive potential of spruce bark beetle females is not exhausted during a single ovarian cycle, and thus the number of females establishing sister broods is rather restrained by weather or by the lack of suitable host trees then by their reproductive capacity. Under laboratory conditions and exceptionally high infestation densities (8 females/dm^2) the females re-swarmed earlier than the ones in the logs with low infestation density (2 females/dm^2). One third of females were allowed to repeatedly mate with males after emergence. However, repeated mating did not increase the number of eggs laid, when compared to non-mated females. Furthermore, the size of egg-batches did not depend on infestation density and was not affected by the first brood size (Anderbrant & Löfqvist, 1988).

Spruce bark beetles overwinter as adults in the state of imaginal diapause. Diapause is induced by shortening day-lenght and low night temperatures of late summer. Critical

photoperiod that induces diapause in 50% of adult bark beetles of Central European population is 14.7 hours. In the nature, such day-length occurs in mid-August (Doležal & Sehnal, 2007). Recent literature has defined several distinct phases of diapause that can be recognized in the spruce bark beetle as well. In general, diapause has three main phases, pre-diapause, diapause and post-diapause. Pre-diapause is characterized by continuation of direct development, but diapause inducing stimuli are being perceived and trigger numerous behavioral and physiological changes. Once the stimuli reach critical level, diapause is fully induced. Diapause phase itself can be divided to three sub-phases. During initiation phase direct development is arrested and metabolic rate slowly decreases, even though adults may continue feeding and even emerge and seek suitable winter habitat. Alternative metabolic pathways are activated and diapause intensity increases. During maintenance phase development is ceased, metabolic rate is low and diapause is maintained by external factors (photoperiod). Unknown processes lead to slow decrease in diapause intensity and increased sensitivity to factors that terminate diapause. Termination processes are not very well understood but with changing environmental conditions diapause intensity decreases to minimum and diapause ends under conditions that support resumption of direct development (Košťál, 2006). As in many insect species of temperate zone, diapause in *Ips typographus* ends in December. At that time adverse environmental conditions still inhibit direct development and diapause is followed by so called post-diapause quiescence (Košťál, 2006, Doležal & Sehnal, 2007). Metabolic rate and development during post-diapause quiescence are arrested by exogenous conditions and resumed as soon as favorable environmental conditions prevail (Košťál, 2006). Hand in hand with diapause goes the ability to survive sub-zero temperatures either by being freezing tolerant or by freeze avoidance. Such strategies were evolved by many overwintering insects and other ectothermic animals. The mechanisms of overwintering survival were extensively studied by Košťál et al. (2011), who found out that spruce bark beetles supercool their body fluids, and thus avoid formation of ice crystals. Cold tolerance in the spruce bark beetle varies during the year. In experiments of Košťál et al. (2011), the field sampled beetles were kept at temperatures 5, -5 and -10 °C. The level of cold tolerance more than doubled from August (beetles survived 2 to 6 weeks) to March, when adults survived 11 - 13 weeks lasting exposure to low temperatures. Survival of beetles kept at 5 °C remained low during the experiment, which was probably due to higher metabolic rate and inability to replenish energy reserves as the adults were kept without food. Supercooling point (SCP), i.e. temperature in which body water freezes, also increases during autumn and winter. SCP in the adults collected in August and September

4

reaches –12 °C. During winter months, it drops to -22 °C and later increases to values around -6 and – 9 °C in the spring. Ability to survive harsh temperatures is fostered by accumulation of cryoprotective substances. The main cryoprotectants in *Ips typographus* are trehalose and glucose. Their concentration gradually increases during autumn and winter and accumulation is mediated by a temperature drop below certain threshold. The exact temperature is not yet known (Košťál et al., 2011). Same overwintering strategy was evolved by a closely related six-toothed spruce bark beetle *Pityogenes chalcographus*. The beetles overwinter as adults and the accumulation of lipid reserves in August suggests that diapause begins at the end of summer. Similarly to *Ips typographus*, SCP of *Pityogenes chalcographus* adults collected in August was -13 °C, and then slowly decreased to -15 °C in November and later to the minimum of -26 °C in January. Accumulation of glycogen and trehalose and their transformation to glycerol is linked with the decrease in SCP. In January, the concentration of glycogen was the highest and *Pityogenes chalcographus* adults in the experiment survived 14 days at -5 °C completely enveloped in ice (Košťál et al., 2014). In contrary, *Ips typographus* individuals were not able to survive such conditions and mortality rapidly increased (Košťál et al., 2011). In the spring, glycerol was gradually transformed back to glycogen and trehalose and SCP returned back to -12 °C (adults collected in April) (Košťál et al., 2014).

2   Materials and Methods

2.1   Study sites

Experiments were carried out at three localities in the Šumava Mountains that differed in elevation. Study sites were located at altitudes 600, 800 and 1100 m a.s.l. and all sites were chosen to be situated on southern slopes, with spruce stands of similar age structure (80 - 100 years old). All localities consisted of dense forest and clearcut patches with sunlit stand edges attractive for bark beetles. Local populations of *Ips typographus* were of the same abundance and according to legislative terminology in gradation phase. The site at 600 m a.s.l. was located 1.5 km north-west from Vlachovo Březí (49°4′27.399″N; 13°55′38.643″E) and *I. typographus* completed two to three filial generations per vegetation season, so that this locality was considered polyvoltine. At 800 m a.s.l. (500 m south from Škarez (49°1′49.231″N; 13°53′4.448″E)) emergence and development of second filial generation strongly depended on weather conditions. Therefore, site Škarez was considered partially bivoltine. Only one filial generation per vegetation season completed development to adults and emerged at locality with the highest altitude (1100 m a.s.l., 7 km south-west from Kubova Huť (48°58′9.218″N; 13°42′3.504″E)).

All three study sites were located in state owned managed commercial forests. Protective measures against bark beetles included trap trees, poisoned trap trees and pheromone traps. Both pheromone traps and trap trees were used to monitor population dynamics of the spruce bark beetle. Several series of trap trees were applied during vegetation season. First series were felled in spring, another three to four series followed depending on population dynamics and abundance of the spruce bark beetle. Pheromone traps were placed at least 10 meters from stand edges and there were not more than 2 to 3 traps per site. Theysohn type pheromone traps with FeSex Typo dispensers (Ubik Karel, Prague, Czech Republic) were used in all study years.

Air temperature on sunlit stand edges and in the shade was continuously measured with Cometter dataloggers (Comet Systems Ltd., Rožnov pod Radhoštěm, Czech Republic) in 30 minutes intervals at all sites.

2.2 Experiments

Experiments were first conducted in 2011 and repeated in 2012. Third replicate was done in 2013 to assess the sex ratio in the filial adults of sister-brood generation. Two cages were placed at each site at the beginning of vegetation season. One was placed on the sunlit stand edge and the other one was positioned inside the forest canopy to be fully shaded. All cages were of the same size (1 x 1 x 1 m) and construction. The base consisted of waterproof hardboard, frame was made of battens (4 x 5 cm) and the walls were covered with polypropylene net. Upper part of the cage was hinged and adjusted by lockable clips. Double layer of window seal was glued between the lid and the frame to prevent escape of bark beetles.

12 logs (each 50 - 100 cm long and approx. 25 cm in diameter) were cut from infested trap trees at all localities at the end of spring swarming. All logs were cut from the same tree to have similar infestation density and thickness of phloem. 6 infested logs were paired with 6 logs cut from freshly felled uninfested trees (approx. 60 cm long and approx. 25 cm in diameter) and altogether placed into cages (see above). Reemergence of spruce bark beetles was checked weekly or twice a week during exceptionally warm periods. New boreholes found on the surface of uninfested logs were marked with colored sprays. Development of filial generation in the logs that were cut from trap trees was regularly checked. The logs were removed as soon as the occurrence of 3rd instar larvae and pupae was recorded and replaced by freshly cut uninfested logs.

Logs removed from the cages were marked; transported to Institute of Entomology and debarked to record the following parameters:
1. Total area of phloem
2. Number of boreholes – date of infestation was assigned to each borehole/gallery according to the color mark
3. Number and length of maternal galleries
4. Number and length of regeneration feeding galleries
5. Number of eggs laid
6. Number of hatched larvae

Non-emerged parental beetles were collected and frozen for later dissection to assess the sex of the adults and status of ovaries in females. Ovaries were photographed using light microscope Leica S6D (Leica Microsystems Inc., Wetzlar, Germany) with camera Canon EOS 500D (Canos Inc., Japan).

Daily averages were derived from temperature records and used to calculate sums of degreedays (DD) needed for completion of key events in bark-beetle life cycle. DD were calculated as a subtraction of lower developmental threshold (8.3 °C (Baier et al., 2007)) from average daily temperatures. Values below zero were not included in the calculation of DD sums.

2.3   Statistical analysis

STATISTICA v. 10 software (StatSoft Inc., Tulsa, OK, USA) and the method of Principal Component Analysis (PCA) was used to analyze the data. Generalized linear model (Wald statistic method) was used to analyze the sex ratio in the offspring of sister-brood females.

3 Results

3.1 Vegetation season 2011

3.1.1 600 m a.s.l.

Overwintered bark beetles started to swarm massively in the first week of May. No boreholes were recorded in subsequent weeks on the surface of the trap trees. Thus, logs from the trap trees were cut and placed into the cages on May 26. At that time, the infestation density was 1.7 boreholes/dm^2.

In the sunlit cage, 100 % of sister brood adults re-swarmed during the first June decade. According to temperature measurements, re-swarming began 364 degree days (DD) (35 days) after the spring swarming. 11 % of females emerged in the first June week and 89 % in the second week. The second re-emergence started 344 DD (28 days) after the first sister brood and only 19 % of females infested the fresh logs. Bark beetles were re-emerging for four weeks with the highest intensity (41 % of total) in the first week of July. Very few females (2 %) re-swarmed in the following week, when the activity was interrupted by rainy weather. The rest of adults (36 %, resp. 21 %) re-swarmed in the third and fourth week. Occurrence of not numerous (15 %) adults of the third sister brood was recorded only at the beginning of August, 346 DD (28 days) after the previous swarming (**Fig.1, 2**).

A delay of one week was recorded in the shaded cage, where the first sister brood females emerged at the beginning of the second June decade and only 45 % of the total number re-swarmed. The swarming began 472 DD (42 days) after the spring swarming. Second re-swarming in the shade corresponded to the one in the sunlit cage only the sum of degree days was considerably lower - 280 DD (21 days). Similarly, only 8 % of the adults established sister broods in the fresh logs. The re-swarming lasted four weeks and was interrupted in the second week of July when only 3% of the females re-emerged. No clear peak of the re-swarming was recorded with approx. one third of females emerging in the first, third and fourth week of July. Third sister brood occurred at the beginning of August, 381 DD (28 days) after the previous re-emergence, and only 22 % of females re-emerged (**Fig.1, 3**).

3.1.2　800 m a.s.l.

Spring swarming started in the first May decade when most of the overwintered beetles emerged. Logs were cut and placed into the cages on May 26. At that time, the infestation density was 1.5 boreholes/dm^2.

Re-swarming at 800 m a.s.l. corresponded to the locality at 600 m a.s.l. First boreholes in the sunlit cage were recorded in the first week of June, 263 DD (35 days) after the spring swarming. Very few adults emerged at that time and the majority of bark beetles re-swarmed the following week. In total, 78 % of parental adults established the first sister brood. The second sister brood followed after 234 DD (28 days) at the beginning of July and continued for four weeks. The number of newly recorded boreholes almost did not differ among weeks with an exception of the second July week, when only 3 % of bark beetles re-emerged due to rainy weather. 17 % of bark beetles re-swarmed in total. Higher elevation caused a two week delay in the occurrence of the third sister brood. All females re-swarmed during the second August decade, 409 DD (42 days) after the previous brood and only 16 % of adults re-emerged (**Fig.1, 2**).

The absence of solar irradiation caused a delay of one week, so that all bark beetles re-emerged during the second week of June (169 DD (42 days) after the spring swarming). In total, 54 % of all bark beetles re-swarmed. Adults of the second sister brood started to swarm after 118 DD (21 days), in the first week of July, and almost half of the bark beetles migrated to uninfested logs before rainy weather disrupted their activity in the second July week, when no new boreholes were recorded. Swarming continued in the third and fourth week of July, when the rest of bark beetles re-emerged. 10 % of beetles established the second sister brood. The timing and the percentage of females establishing the third sister brood corresponded to the observations made in the sunlit cage. Very few females (13 %) migrated to fresh logs and established third sister brood 275 DD (42 days) after the previous swarming (**Fig.1, 3**).

3.1.3　1100 m a.s.l.

Bark beetles started to emerge in the third week of May, when the majority of beetles swarmed. Logs from infested trap trees were cut and placed into the cages on May 26. The infestation density was 1.3 boreholes/dm^2.

Re-swarming at 1100 m a.s.l. substantially differed from those at lower localities. In the sunlit cage, the re-emergence began in the first week of July, 337 DD (49 days) after trap trees were infested. At that time the activity culminated and more than half of the beetles emerged. Boreholes in fresh logs were recorded continuously for four weeks except the second July week when rainy weather disrupted bark beetle activity. In total, 41 % of the adults established the first sister brood. The second sister brood was recorded in the third week of August (after 323 DD (42 days)), lasted only one week and total of 15 % of bark beetles established new brood (**Fig.1, 2**).

In the shaded cage, bark beetles started to re-swarm in the first July decade (after 275 DD (49 days)), when the majority of adults emerged. Sister brood in uninfested logs was established by approximately one fifth of bark beetles. There were no differences in the second sister brood establishment between the sunlit and shaded cage. Bark beetles started to emerge after 266 DD (42 days) and relatively high percentage (90 %) of females established the brood. The occurrence of the third sister brood was recorded neither in sunlit nor in shaded cage (**Fig.1, 3**).

3.2   Vegetation season 2012

3.2.1   600 m a.s.l.

Overwintered bark beetles started to emerge in the first week of May and the spring swarming culminated one week later. Logs from infested trap trees were cut and placed into the cages on May 15. The infestation density was 2.3 boreholes/dm^2.

In the sunlit cage, boreholes in the fresh logs were first recorded in the third week of May after 240 DD (22 days). Re-swarming lasted four weeks and peaked at the beginning; the activity in the following weeks was negligible. In total, only 35 % of adults re-emerged and established sister brood in uninfested logs. The second sister brood was established in the second week of July (after 285 DD (18 days), when 11 % of the females re-swarmed. Third re-emergence was not recorded (**Fig.1, 2**).

The timing of the first re-swarming in the shaded cage did not substantially differ from the above mentioned sunlit cage. Swarming started in the third week of May (after 262 DD (22 days), lasted four weeks and the majority of adults re-swarmed in the first two weeks. Re-swarming in the shade was less numerous and only 7 % females emerged. Second re-swarming started one week earlier than in the sunlit cage (after 186 DD (11 days) and was

equally distributed to the entire two weeks period. The percentage of re-swarming bark beetles increased to 21 %. Third re-emergence was recorded in the second week of August (after 517 DD (38 days)). However, none of 8 % of females that re-swarmed laid any eggs and only regeneration feeding galleries were found in the phloem of infested logs (**Fig.1, 3**).

3.2.2   800 m a.s.l.

Spring swarming started in the first week of May, when the majority of bark beetles emerged. Logs were cut from the trap trees and placed into the cages on May 15. At that time, the infestation density was 1.8 boreholes/dm^2.

In the sunlit cage, females of the first sister brood started to re-swarm in the third week of May, 127 DD (22 days) after the spring swarming. Re-swarming culminated in the following week, when almost three quarters of adults infested the fresh logs. In the following two weeks the occurrence of new boreholes was sparse. In total 43 % of bark beetles established the first sister brood. The second sister brood boreholes were first recorded in the last June week (after 260 DD (35 days)), lasted three weeks and the proportion of emerging adults was equally distributed. In total, 36 % of adults re-swarmed. Females of the third sister brood started to re-swarm in the second week of August, 428 DD (42 days) after the previous swarming. The majority of adults re-emerged in the first week. Only 6 % of the bark beetles left the original logs and re-swarmed (**Fig.1, 2**).

The first re-swarming in the shaded cage was delayed by one week compared to the one in the sunlit cage. It started 134 DD (29 days) after the infestation of trap trees. Re-emergence of sister brood beetles lasted three weeks and more than three quarters of females infested the fresh logs in the first week. In the following two weeks, numbers of re-emerging bark beetles gradually declined. In total 45 % of adults re-swarmed. The second sister brood corresponded to the one in the sunlit cage. Re-swarming started in the last week of June, 167 DD (28 days) after the previous swarming, lasted three weeks and proportion of re-emerging adults was evenly distributed. In total, 38 % of bark beetles established sister broods in the phloem of fresh logs. Only one female re-swarmed for the third time (after 347 DD (42 days)) in the second week of August. However, the female did not lay any eggs and only regeneration feeding gallery was found in the phloem (**Fig.1, 3**).

3.2.3 1100 m a.s.l.

The first signs of infestation on the surface of the trap trees were recorded in the first week of May, when the swarming culminated. The logs were cut from infested trap trees and placed into the cages on May 15. The density of boreholes was 1.7 /dm^2.

In the sunlit cage, the first bark beetles started to emerge in the third week of May, after 126 DD (22 days) from the spring swarming. The activity lasted four weeks and the majority of bark beetles re-emerged in the first two weeks. In total, 74 % of adults established the first sister brood. Second re-swarming was less numerous. The emergence started in the first week of July, after 319 DD (42 days) and only 11 % of females infested the fresh logs. The third re-emergence started in the second August week (after 228 DD (28 days)). In total, 17 % of the females re-bred (**Fig.1, 2**).
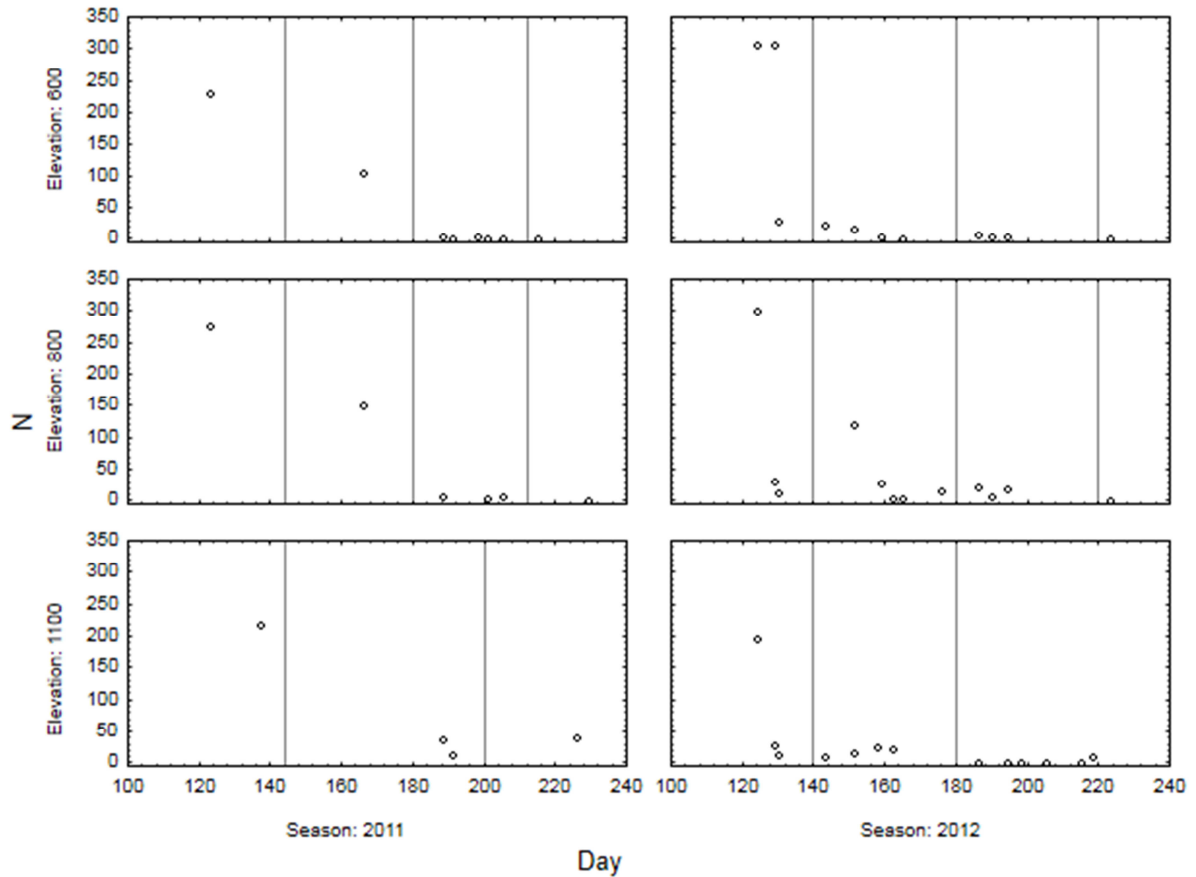
Timing of the first sister brood in the shaded cage corresponded to the one in the sunlit cage. It was less numerous; only 30 % of the bark beetles re-swarmed. Most females re-swarmed in the third week of June. Emergence started 103 DD (22 days) after the infestation of trap trees. The second sister brood started in the first week of July (after 284 DD (42 days)) and lasted six weeks. The majority of bark beetles re-swarmed in the last week. In total only 14 % of adults re-emerged. The third sister brood was not recorded (**Fig.1, 3**).
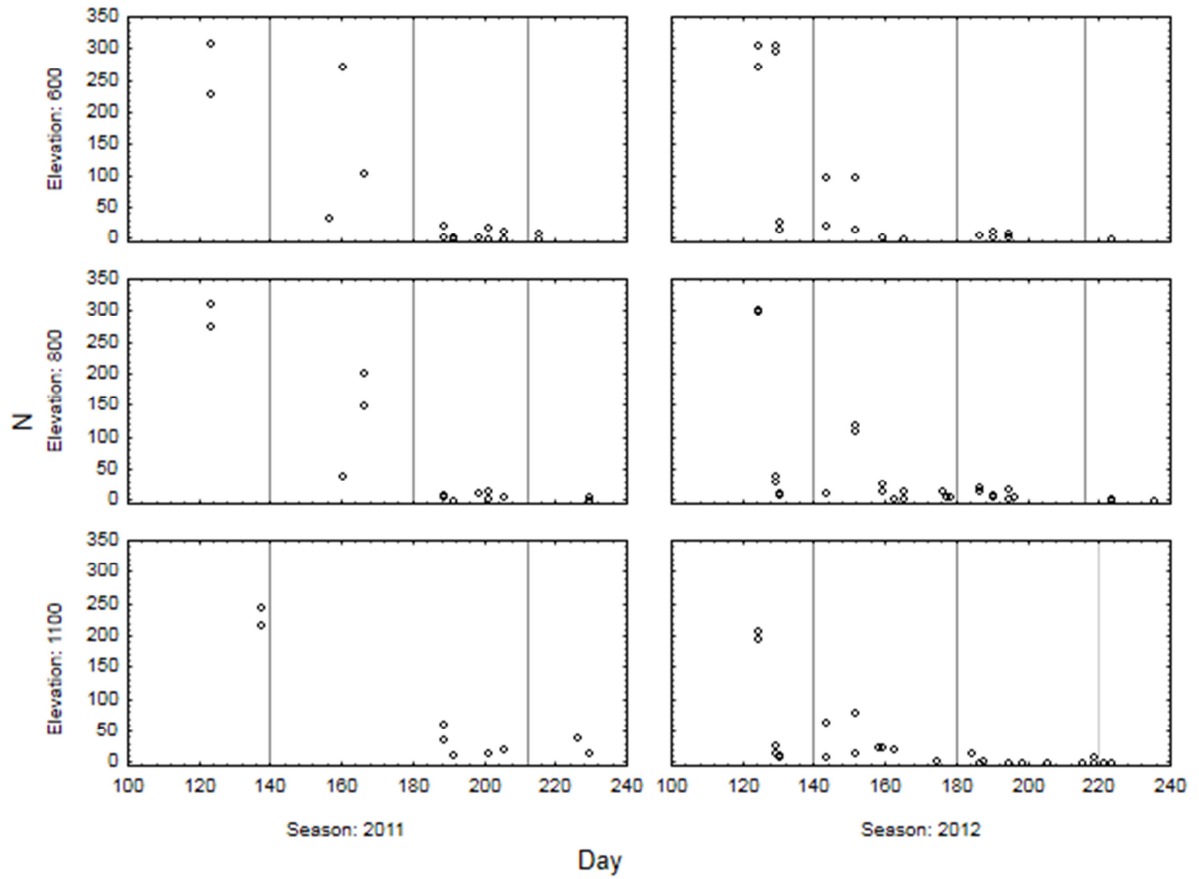
Fig. 1 – Timing of sister brood re-emergence in vegetation seasons 2011 and 2012. Data from shaded and sunlit cages were pooled. Horizontal axis shows time (days from the beginning of the year), vertical axis displays the number of re-emerged females. Grey lines indicate the end of re-swarming.
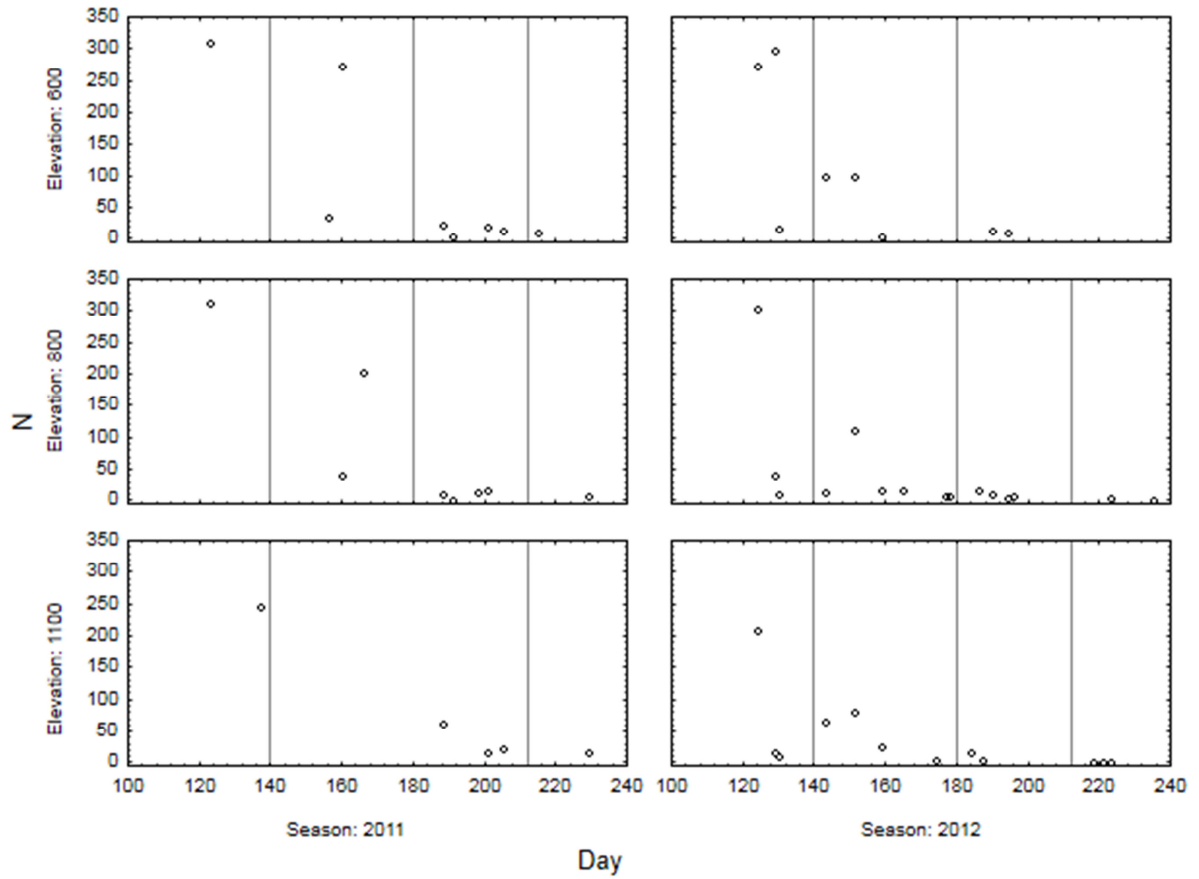
Fig. 2 – Timing of sister brood re-emergence in vegetation seasons 2011 and 2012. Horizontal axis shows time (days from the beginning of the year), verticalaxis displays the number of females that re-emerged in the sunlit cages. Grey lines indicate the end of re-swarming.

Fig. 3 – Timing of sister brood re-emergence in vegetation seasons 2011 and 2012. Horizontal axis shows time (days from the beginning of the year), vertical axis displays the number of females that re-emerged in the shaded cages. Grey lines indicate the end of re-swarming.

3.3 Oviposition - vegetation season 2011

Number of eggs laid by a single female was clearly visible as incisions on edges of maternal galleries. Recording the number of incisions enabled a comparison with number of larval galleries with pupal chambers i.e. the percentage of progeny that successfully completed development to adults. Females of the spring swarming oviposited in average 20 to 30 eggs and the recorded maximum was 45 eggs. Number of eggs laid in the second ovarian cycle (the first sister brood) decreased to 10 – 20, with a maximum of 53 eggs. Then the average size of egg batches increased to 20 – 30 in the third and fourth ovarian cycle. Maximal number of eggs per female was 39 in the second sister brood and 55 in the third brood (**Fig. 4, 5**).

16

3.4 Oviposition - vegetation season 2012

In 2012, the fecundity of females did not differ in the first three sister broods. The size of batches ranged from 30 to 40 eggs. Maximal number of eggs oviposited by a single female during the spring swarming was 40, then it increased to 43 in the first sister brood and almost doubled to 72 eggs in the second brood. During the third re-swarming, most females laid only up to 10 eggs, the recorded maximum was 37 (**Fig. 4, 5**).
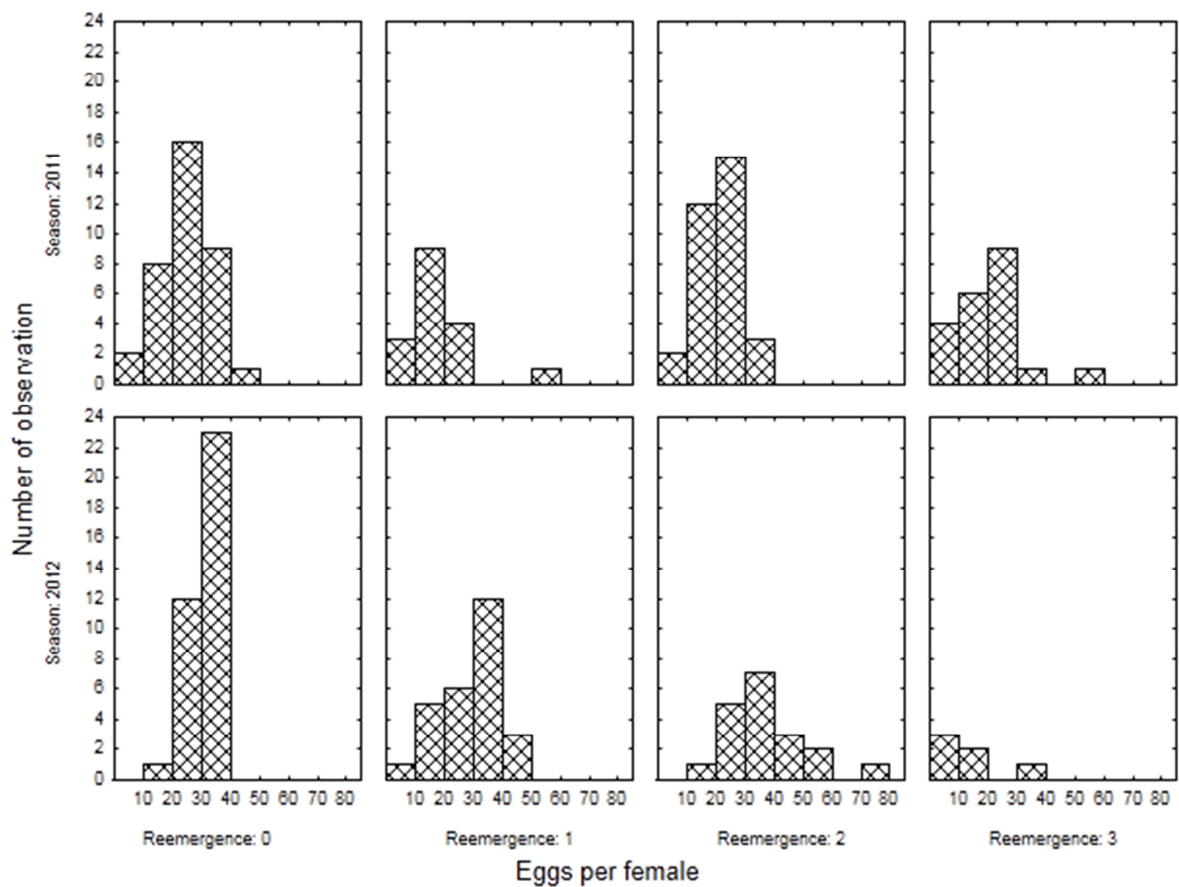

Fig. 4 – Histogram shows the frequencies of egg batches by a single female during individual re-swarmings in 2011 and 2012. Horizontal axis shows number of eggs and re-emergence (Reemergence 0 is spring swarming). Vertical axis displays the frequency of observation.
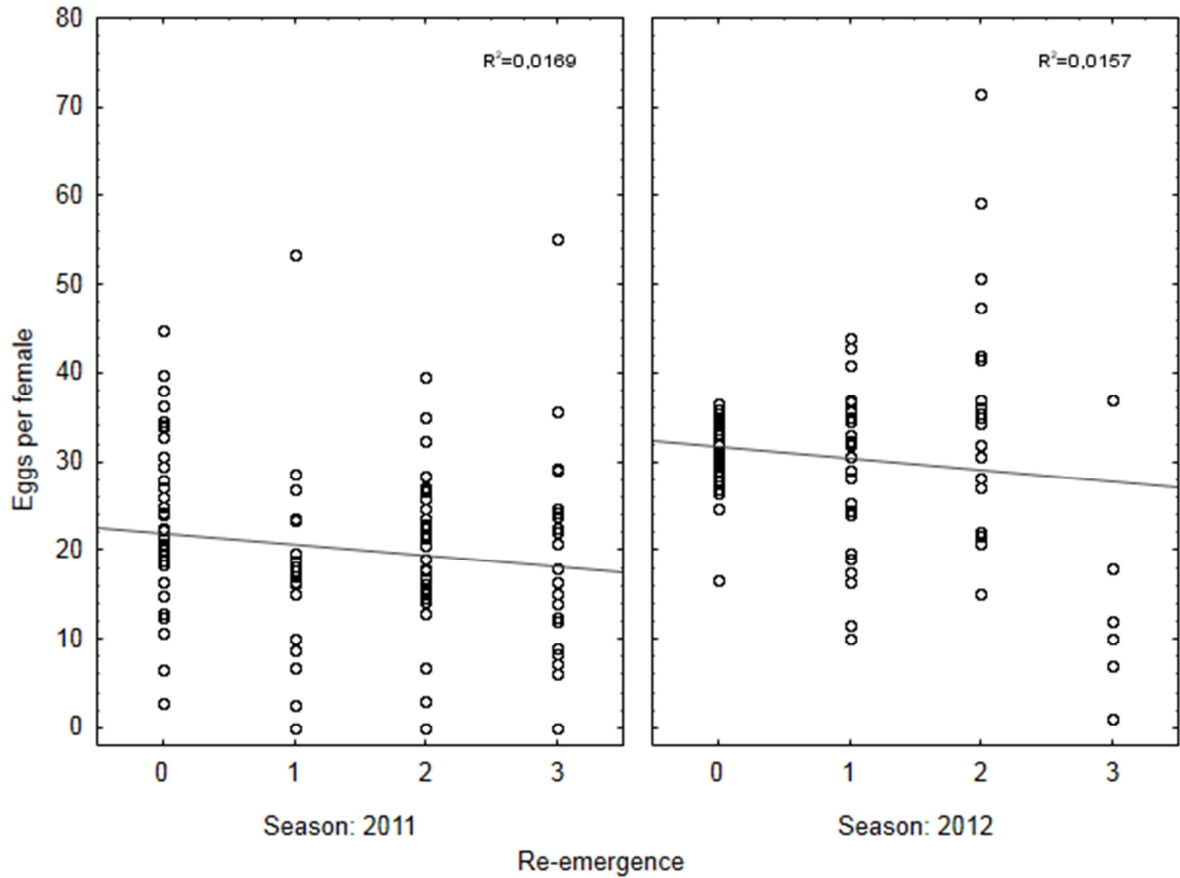
Fig. 5 – Number of eggs laid by a single female per ovarian cycle in vegetation seasons 2011 and 2012. Each circle represents one female. Horizontal axis shows the number of re-emergence; vertical axis displays the number of eggs laid by individual females. Regression curves were fitted to the data and the coefficients of determination R^2 are displayed in the upper right corner of each graph.

3.5 Sex ratio in the offspring of re-emerged females

Statistical tests proved no dependence of sex on the position of the egg in the maternal gallery (Wald. stat., p = 0.2615; Wald. Stat = 1.261, df = 1) (**Fig. 6**).
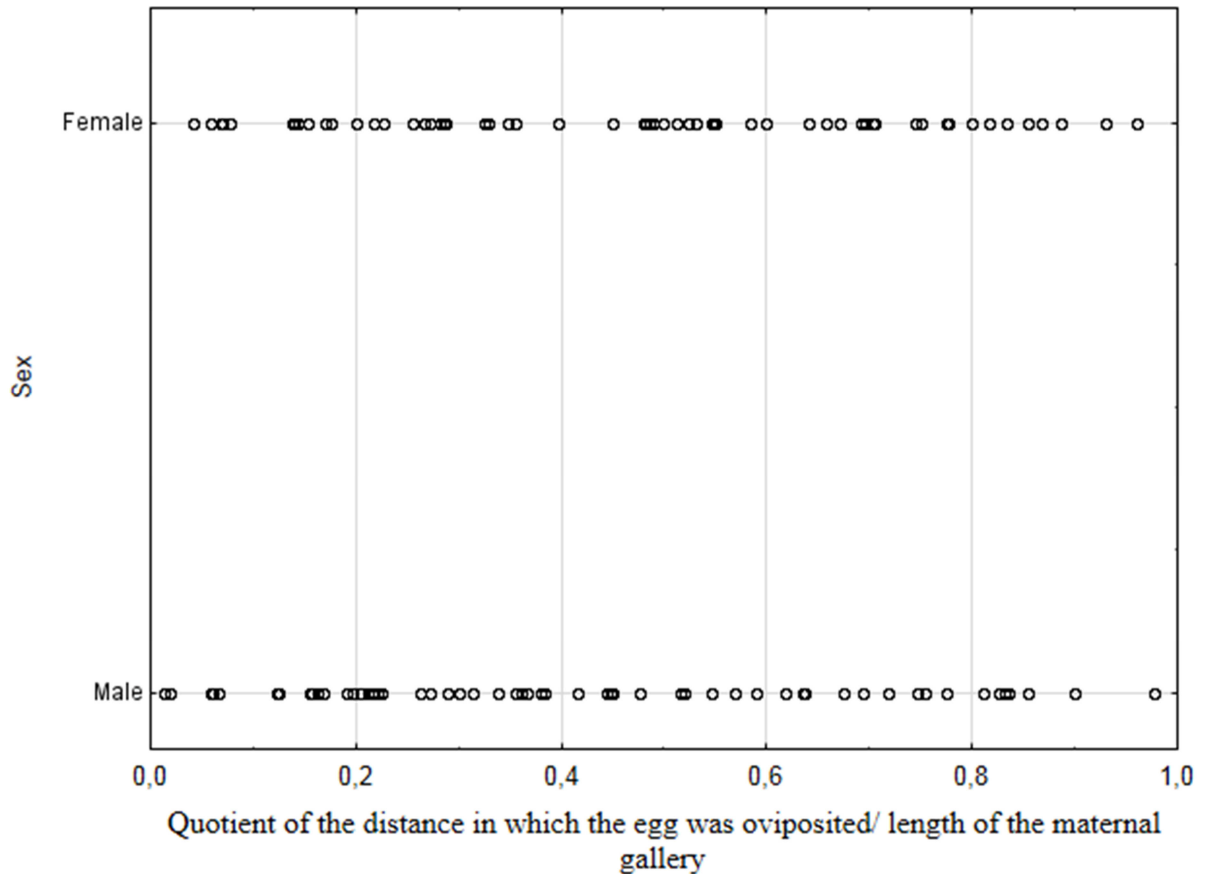
Fig. 6 – Sex ratio recorded in the offspring of sister-brood females. X axis displays the position of the egg in the gallery (quotient of the distance in which the egg was oviposited and the length of the maternal gallery). Y axis displays the sex of the bark beetles. One circle represents one filial adult.

3.6 Statistical analysis - vegetation season 2011

Data were statistically evaluated using Principal component analysis (PCA). The results indicate that sister broods (factors **Reemergence 1, Reemergence 2, Reemergence 3**) are independent from the number of laid eggs (factor **Eggs**) and number (factor **Galleries**) and length (factor **Maternal galleries**) of maternal galleries (**Fig. 7**).
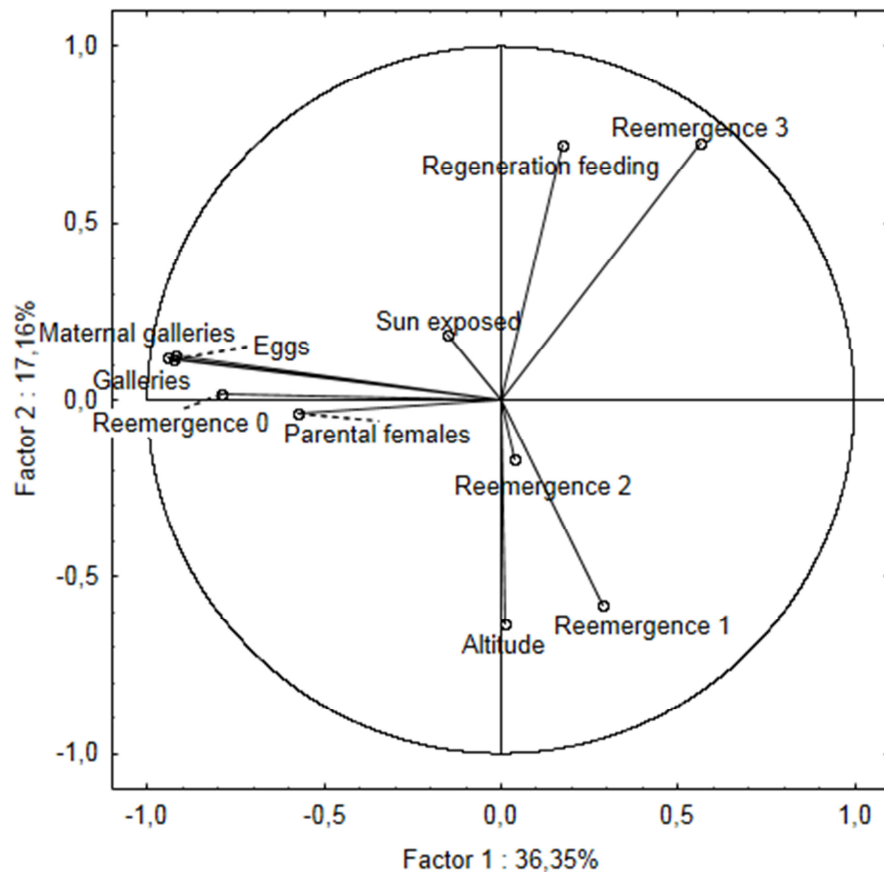
Fig. 7 – Principal component analysis of vegetation season 2011. Assessed factors were: **Eggs** – total number of eggs laid; **Galleries** – total number of maternal galleries; **Maternal galleries** – total length of maternal galleries; **Reemergence 0** – spring swarming; **Remergence 1 – 3** – sister broods, **Regeneration feeding** – total length of regeneration feeding galleries, **Altitude**; **Sun exposed** – location of cage – sunlit = 1, shaded = 0; **Parental females** – total number of females.

## 3.7 Statistical analysis - vegetation season 2012

Results of PCA for the vegetation season 2012 correspond to those of 2011. The only exception is a positive correlation between factors **Regeneration feeding**, length of maternal galleries (factor **Maternal galleries**) and number of eggs laid (factor **Eggs**) (**Fig. 8**).
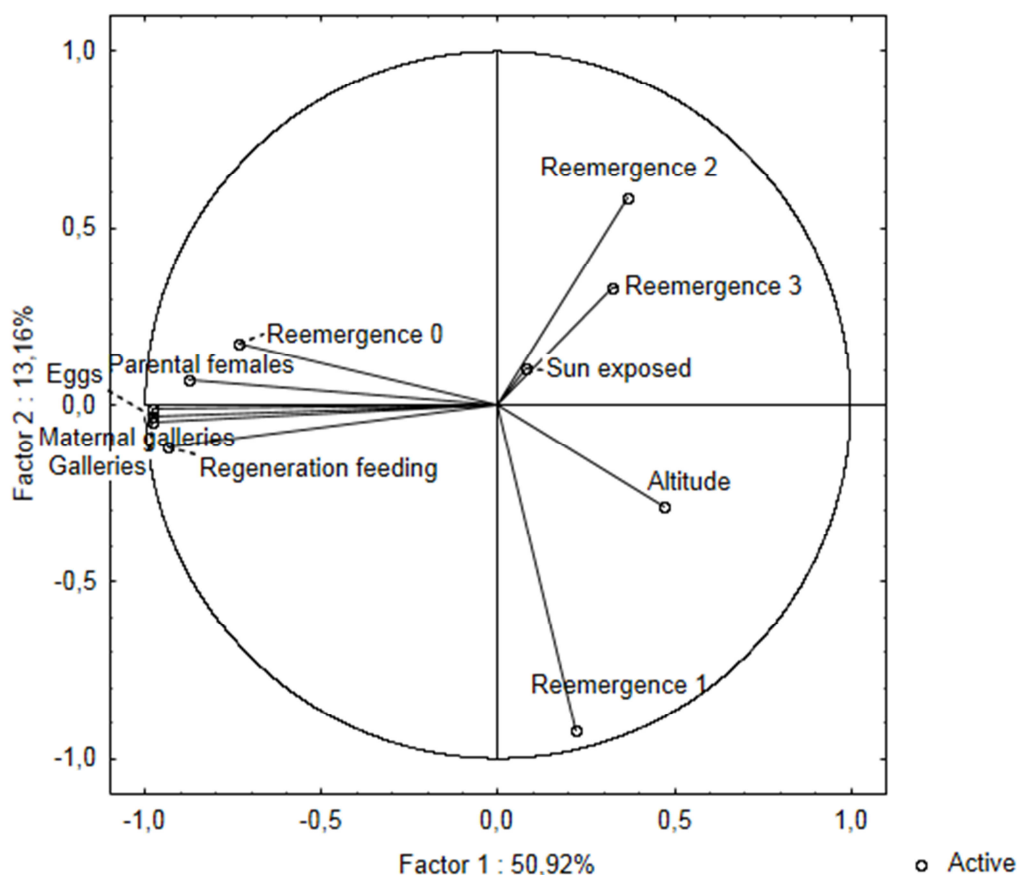
Fig. 8 – Principal component analysis of vegetation season 2012. Assessed factors were: **Eggs** – total number of eggs laid; **Galleries** – total number of maternal galleries; **Maternal galleries** – total length of maternal galleries; **Reemergence 0** – spring swarming; **Remergence 1 – 3** – sister broods; **Regeneration feeding** – total length of regeneration feeding galleries, **Altitude**; **Sun exposed** – location of cage – sunlit = 1, shaded = 0; **Parental females** – total number of females.

## 3.8 Statistical analysis - vegetation seasons 2011 and 2012 together

PCA analysis of both vegetation seasons (**Fig. 9**) confirmed the results of individual analysis. Maternal gallery length is almost invariable and infestation density is totally independent (**Fig. 10**). However, number of re-emerged females strongly depends on infestation density (**Fig. 11**). Solar irradiation is not significant. In the contradiction with vegetation season 2011, regeneration feeding galleries are positively correlated with number of eggs laid. Further analysis shows that bark beetles undergo regeneration feeding in both old and new logs, which depends exclusively on the quality of phloem (**Fig, 12, 13**).
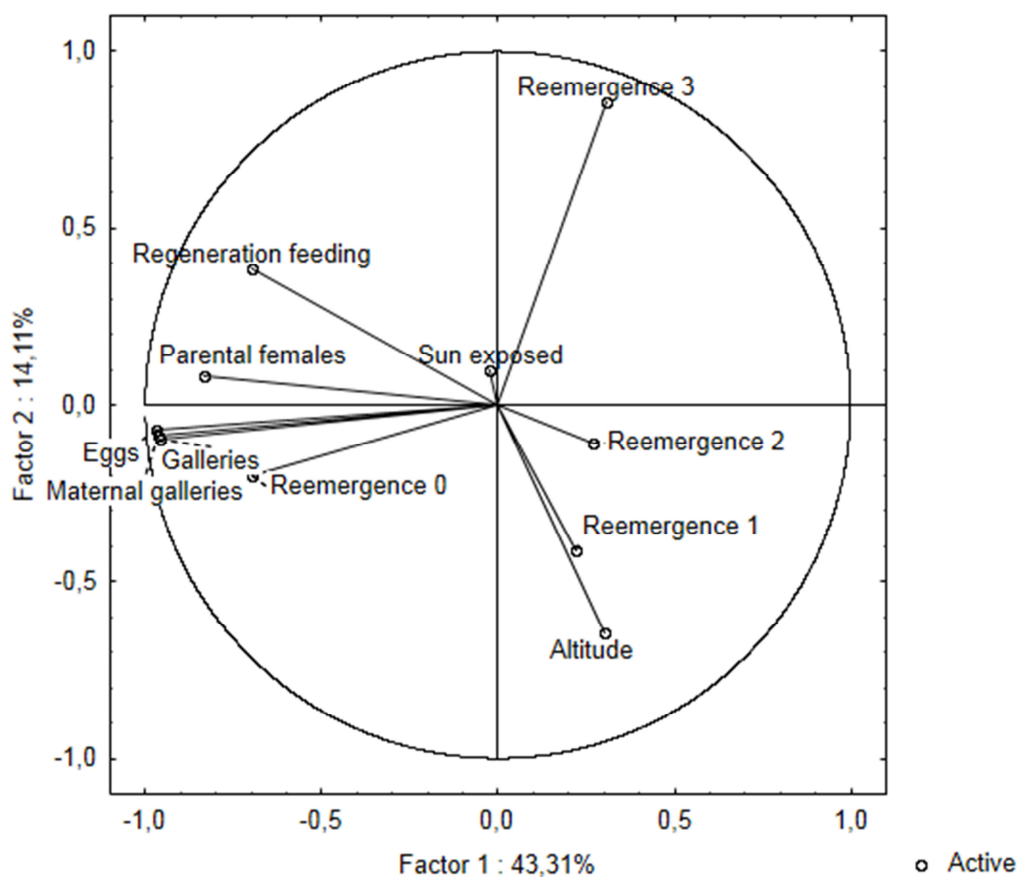
Fig. 9 – Principal component analysis for vegetation seasons 2011 and 2012. Assessed factors were: **Eggs** – total number of eggs laid; **Galleries** – total number of maternal galleries; **Maternal galleries** – total length of maternal galleries; **Reemergence 0** – spring swarming; **Remergence 1 – 3** – sister broods; **Regeneration feeding** – total length of regeneration feeding galleries, **Altitude**; **Sun exposed** – location of cage – sunlit = 1, shaded = 0; **Parental females** – total number of females.
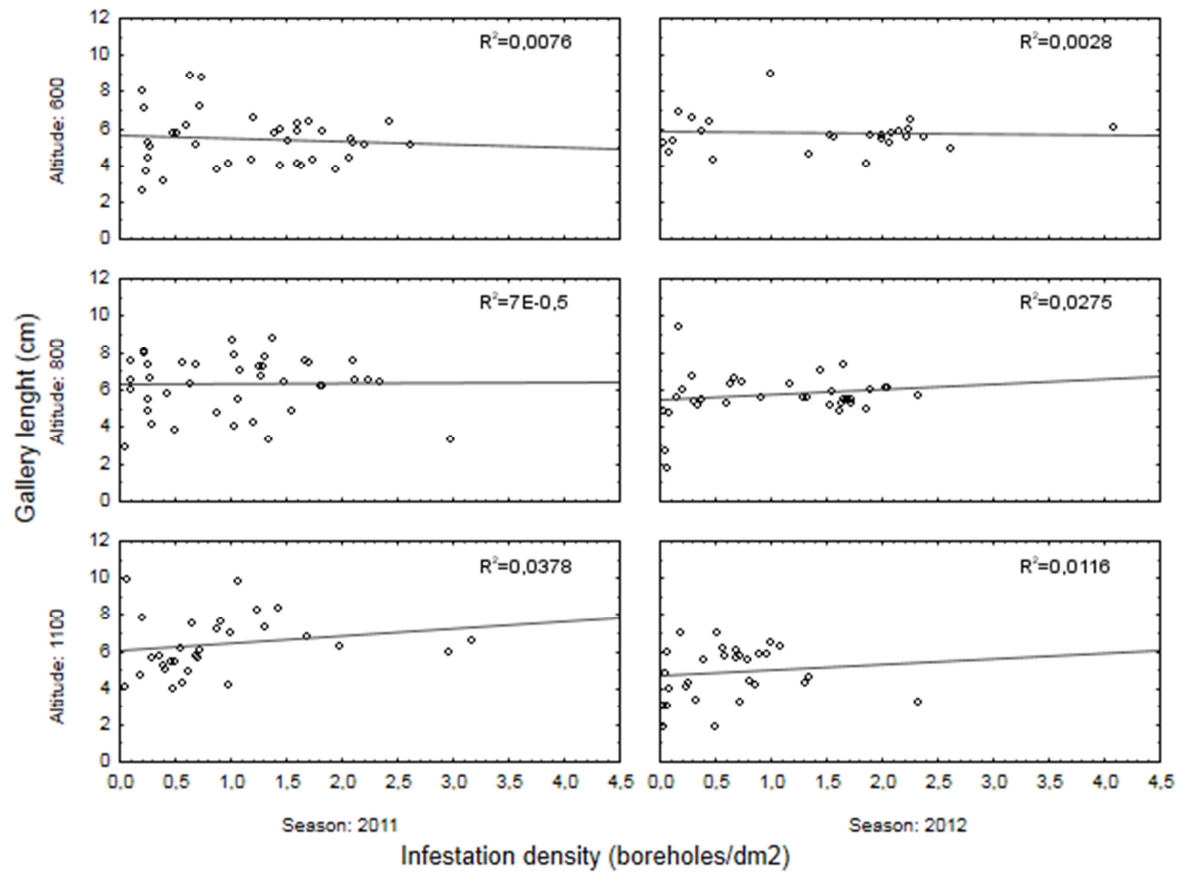
Fig. 10 – Dependence of maternal gallery length on infestation density. Horizontal axis shows infestation density (boreholes/dm^2), Vertical axis displays maternal gallery length (cm). One point marks one maternal gallery. Regression curves were fitted to the data and the coefficients of determination R^2 are displayed in the upper right corner of each graph.
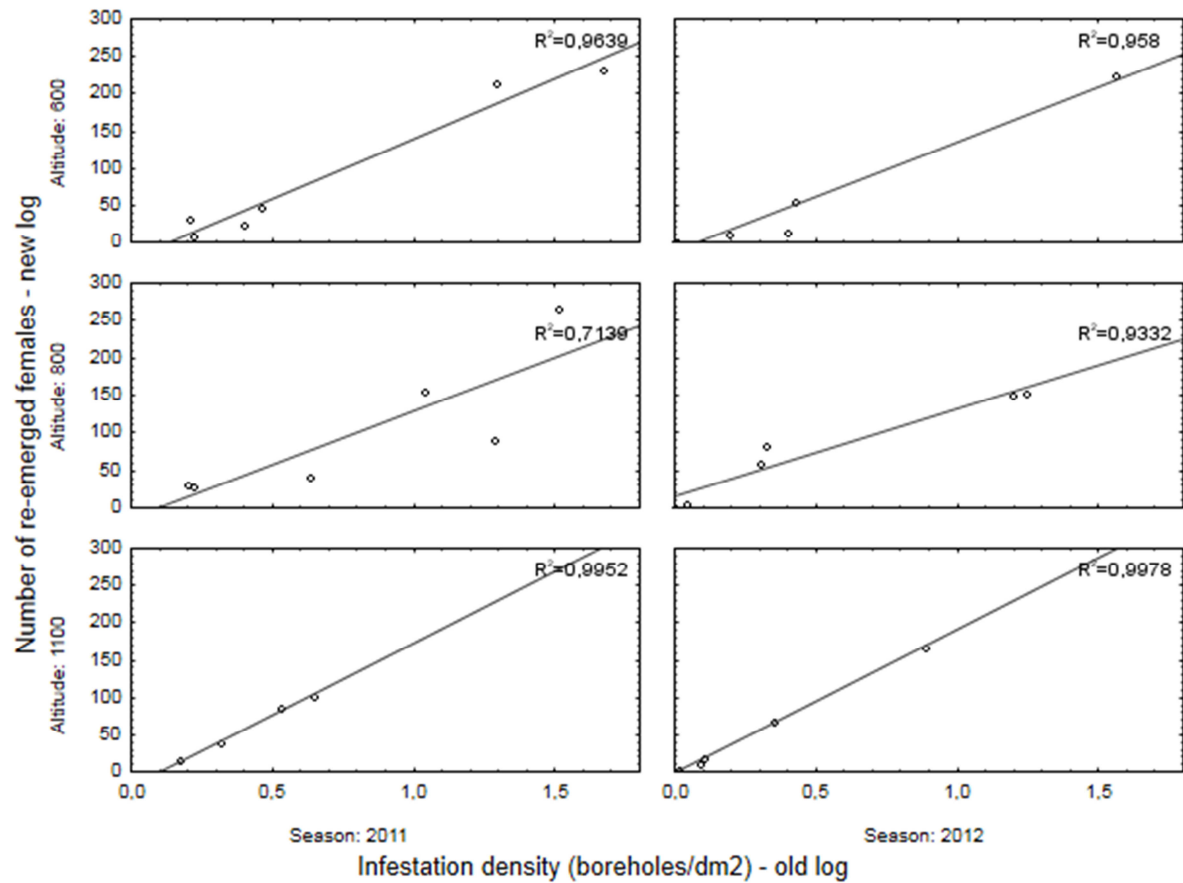
Fig. 11 – Dependence of number of re-emerged females on infestation density in the old log. Horizontal axis shows infestation density (boreholes/dm^2), Vertical axis displays number of re-emerged females. One point marks sunlit and shaded cages pooled and one series of logs. Regression curves were fitted to the data and the coefficients of determination R^2 are displayed in the upper right corner of each graph.
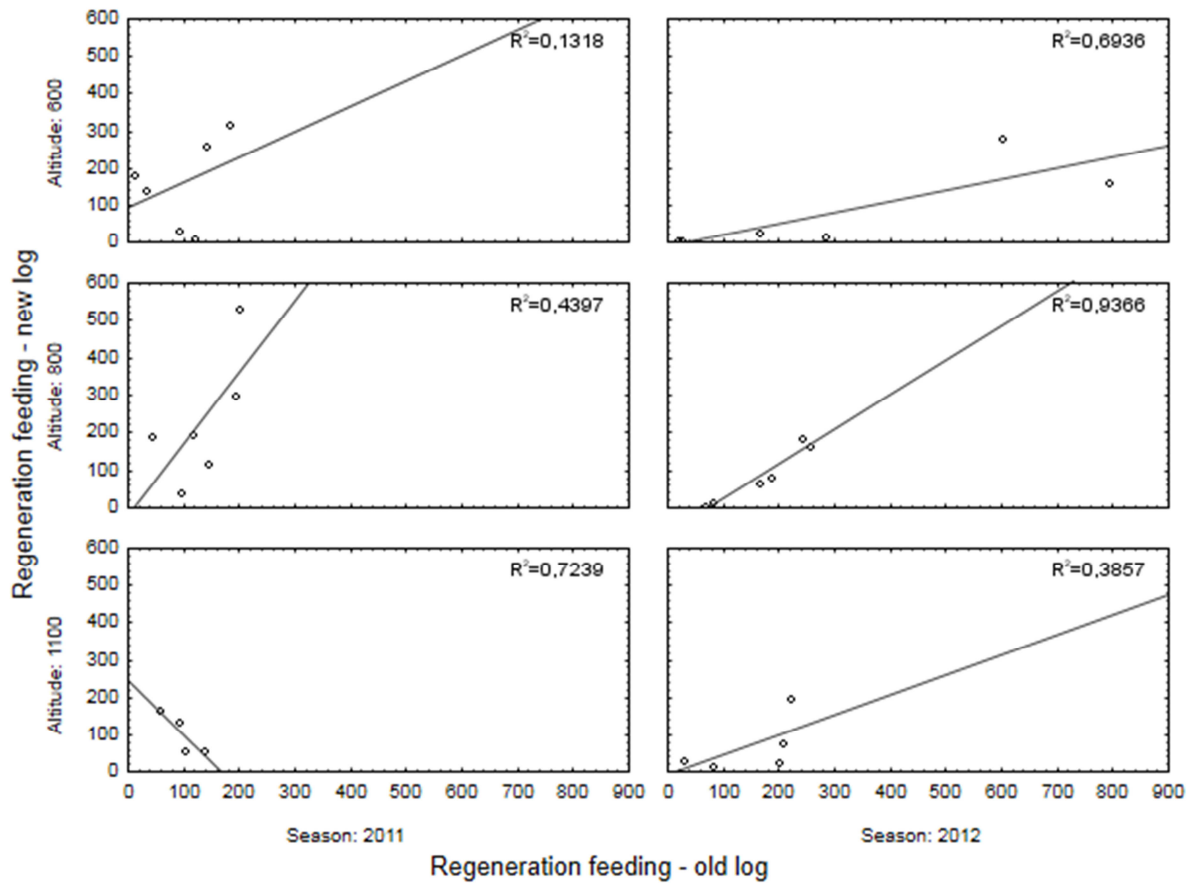
Fig. 12 – Total length of regeneration feeding galleries in the old and new logs. Horizontal axis shows total length of regeneration feeding galleries in the old logs, Vertical axis displays total length of regeneration feeding galleries in the new logs. One point marks sunlit and shaded cages pooled and one series of logs. Regression curves were fitted to the data and the coefficients of determination R^2 are displayed in the upper right corner of each graph.
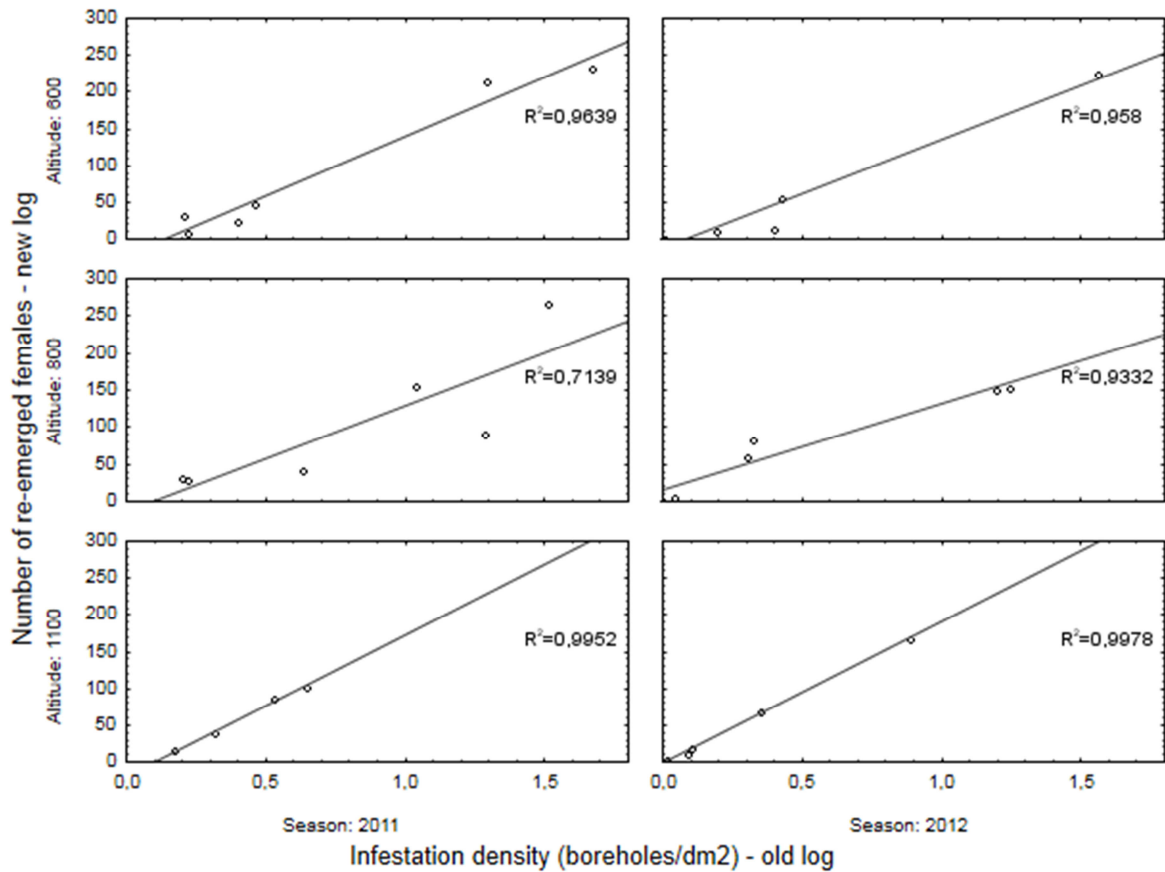
Fig. 13 – Total number of regeneration feeding galleries in the old and new logs. Horizontal axis shows total number of regeneration feeding galleries in the old logs, Vertical axis displays total number of regeneration feeding galleries in the new logs. One point marks sunlit and shaded cages pooled and one series of logs. Regression curves were fitted to the data and the coefficients of determination R^2 are displayed in the upper right corner of each graph.

4 Discussion

In 2011, up to 100 % of the females established the first sister brood at 600 and 800 m a.s.l. This observation corresponds with the studies of Martinek (1956, 1961), who found out that 91 % of the females re-swarmed at 550 m a.s.l. and up to 74 % at 750 m a.s.l. Re-emergence at 1100 m a.s.l. was considerably lower with only 41 % of re-swarming females. This percentage is considerably lower than 65 % - 70 % recorded by Martinek (1957) at 1050 m a.s.l. Lower percentage of re-swarming females in present experiment might be caused either by higher elevation, different weather conditions or by nutritional value and quality of the phloem. The number of re-emerging females was generally lower in the shade; the minimum was 18 % at 1100 m a.s.l. Reduced number of re-swarming females in the shade might be due to lack of solar irradiation as the cages were placed inside the forest canopy. In contrary, Martinek (1956, 1957, and 1961) placed the cages in the sunlit or only partially shaded places. In the vegetation season 2012, the number of females establishing the first sister brood was lower than in the previous year. At 600 and 800 m a.s.l. only 35 – 45 % of adults re-swarmed. On the contrary, 74 % of the females established the first sister brood in the sun at 1100 m a.s.l., which is in compliance with the results of Martinek (1957).

The course of the second sister brood at 600 m a.s.l. also corresponds to previously published results. Up to 19 % of the females established the second sister brood in present experiment which concurs to 13 % at 550 m a.s.l and 20 – 35 % at 500 m a.s.l. (Martinek, 1956, 1961). At 800 and 1100 m a.s.l., 17 %, respectively 15 % of the females re-emerged in the sunlit cage and 90 % in the shade. Higher percentage of females that re-emerged in the shade is probably linked to low proportion of bark beetles that established the first sister brood. The observation of the second re-swarming at higher elevations is in the contradiction with Martinek (1957, 1961), who did not record its establishment at elevations above 700 m a.s.l. However, the results of 2011 were confirmed also in the following vegetation season, when up to 38 % of the females re-swarmed at 800 m a.s.l. and 11 - 14 % at 1100 m a.s.l. The percentage of adults that established the second sister brood at 600 m a.s.l. remained constant.

The third sister brood was recorded in both vegetation seasons at 600 and 800 m a.s.l. The only exception was caused by entomopathogenic fungi that killed all the adults in the sunlit cage in 2012 at 600 m a.s.l and therefore no re-swarming occurred. In 2012, we recorded the third sister brood even at 1100 m a.s.l. in the sunlit cage. The occurrence of the third sister brood is not mentioned in the relevant literature. However, according to

Wermelinger & Seifert (1999) the importance of sister broods for the population size increases with altitude.

The occurrence of more than one sister brood at higher elevations and the third sister brood at all localities in 2012 cannot be explained by a single factor. Bark beetles were regularly supplied with fresh logs, so that suitable food source was always available. According to Annila (1969), limiting factors for sister brood establishment are appropriate weather conditions and sufficient amount of suitable host trees for regeneration feeding and oviposition. In the laboratory experiment of Zumr & Soldán (1981), the females of *Ips typographus* went through three ovarian cycles interlaced with periods of regeneration feeding. The number of sister broods is positively correlated with infestation density and the onset of swarming is strictly density dependent (Anderbrant & Löfqvist 1988). Last but not least, climate change may play a role in the entire process. The weather conditions considerably changed since 1950s, when Martinek's experiments were conducted. Monthly temperature averages increased by 5 – 10 °C. Analysis of temperature recordings proved that this increase is caused exclusively by higher daily minima and not by a change in the maximum temperatures (see **Fig. 1S – 6S**, **Supplementum**). Temperature fluctuations recorded inside the canopy (i.e. shaded location) by Martinek (1956, 1957) in 1954 and 1956 are added for comparison (see **Fig 7S – 9S**, **Supplementum**). As the life cycle of the spruce bark beetle is predominantly temperature dependent, higher temperatures may have caused faster development and thus increased number of sister broods (Faccoli, 2009).

The females of the third sister brood in 2012 did not lay any eggs. Only a few exceptions with very limited size of egg batches were found in the entire experiment. Similar phenomenon was described by Annila (1969), who observed only exceptional oviposition at the end of vegetation season, when imaginal diapause is being induced (Doležal and Sehnal, 2007).

Number of oviposited eggs was higher in 2012, probably due to higher quality of phloem. Overall number of eggs in batches varied from 20 to 40 and did not differ between ovarian cycles with the exception of the third sister brood in vegetation season 2012, when very few eggs were laid. It also corresponds with the studies of Martinek (1956, 1957, and 1961) in which average numbers of oviposited eggs were 13 – 46. Moreover, he did not record any differences between the ovarian cycles. Similar conclusions were made by Annila (1969), with an average number of eggs varying from 20 to 60, and Anderbrant & Löfqvist (1988), who also did not record any differences in the size of egg batches when comparing regular swarming to sister broods. Moreover, the number of oviposited eggs did not vary

among the different infestation densities. Statistical analysis of present data also supports this hypothesis. According to Martinek (1957) and Anderbrant & Löfqvist (1988) the number of eggs laid depends on the intensity of regeneration feeding. In the study of Martinek (1957), sufficient length of regeneration feeding gallery needed for complete restoration of ovaries was at least 1.3 cm. Statistical analysis of vegetation season 2012 and both vegetation seasons together confirmed this hypothesis. However, in 2011, the intensity of regeneration feeding was completely independent. Infestation density is a key factor influencing the number of established sister broods. According to our results, the relation between these two factors is almost linear and the number of re-swarming females increases with infestation density. Similar conclusions were made by Annila (1969) and Anderbrant & Löfqvist (1988).

5 Conclusions

The percentage of females that established one or more sister broods was relatively high in both study years. Therefore, it can be concluded that timely removal of infested trap trees may prevent the emergence of sister brood females, and thus serious damage caused to the surrounding stands by re-swarming bark beetles.

Compared to the Martinek´s studies from 1950s, an increase in the number of sister broods by one or two was recorded. In lower elevations, the occurrence of three sister broods instead of two in 1950s was recorded. Similarly, in higher elevations two sister broods were recorded instead of one mentioned by Martinek (1957, 1961). Increased number of sister broods demonstrates a positive effect of changing climate, particularly an increase in temperature averages and its effects on insect population dynamics.

Hand in hand with the percentage of re-swarming females goes the number of eggs they oviposited per one ovarian cycle. The results clearly demonstrate that the size of egg batches remains constant and sister broods are thus of the same economic importance as regular broods. Moreover, fertility of the females did not change even if they did not mate, which was demonstrated by an absence of mating chambers in the galleries. The first two sister broods may therefore considerably contribute to the local population density. The importance of the third sister brood was much lower, mainly due to the fact that bark beetles responded to the shortening day-length and diapause induction ceased reproduction.

Sex ratio in the offspring of re-emerged females did not differ from the one recorded in regular broods, which also implies the importance of sister broods that pose the same risk as regular generations.

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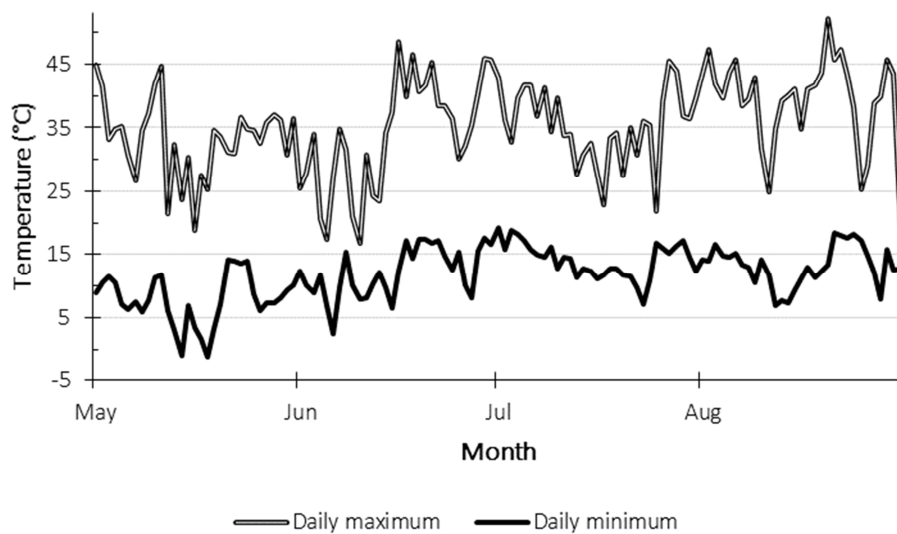**Zumr, V.** (1995): Lýkožrout smrkový – biologie, prevence a metody boje. Matice lesnická, Písek, 132 p.

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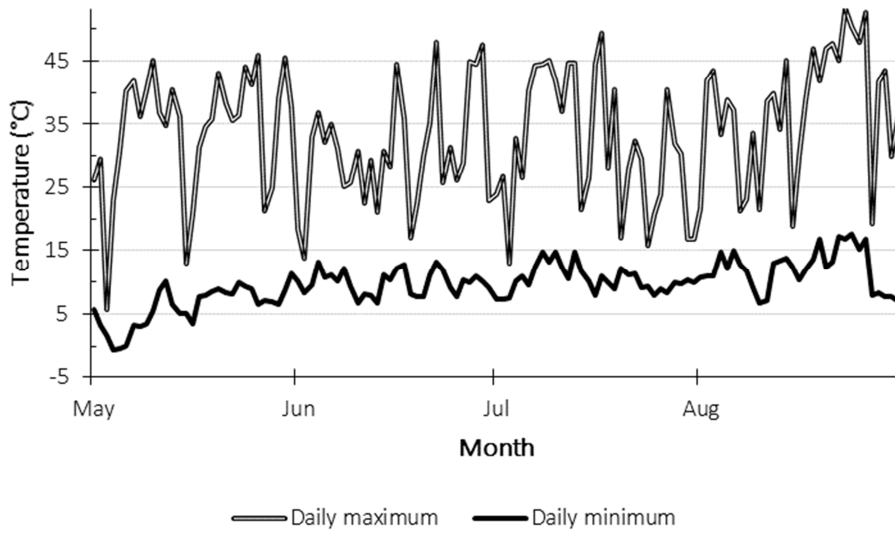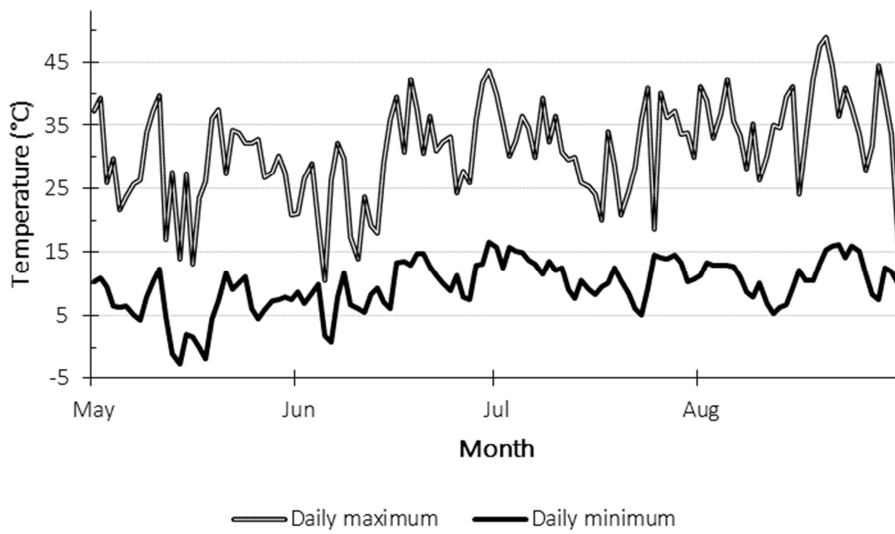
7 Supplementum
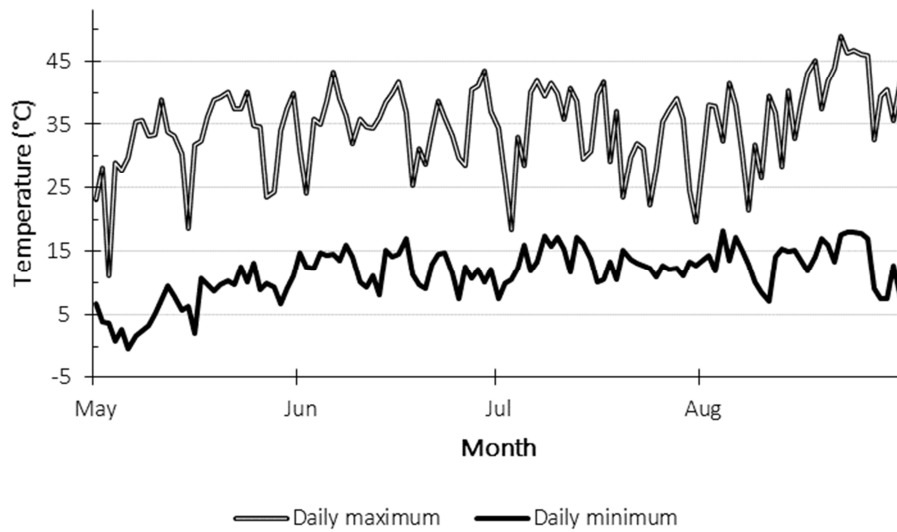
Fig 1S – Daily minimum and maximum temperatures at 600 m a.s.l. in vegetation season 2011.
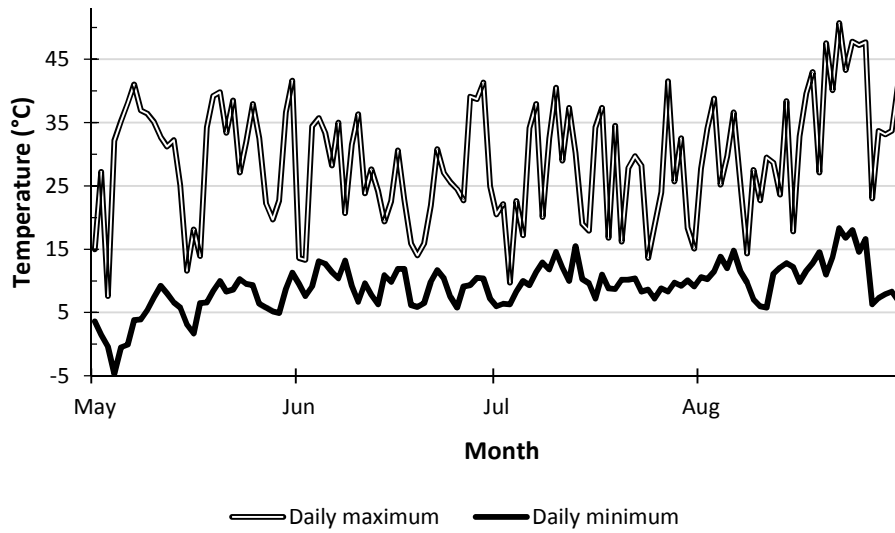

Fig 2S – Daily minimum and maximum temperatures at 600 m a.s.l. in vegetation season 2012.
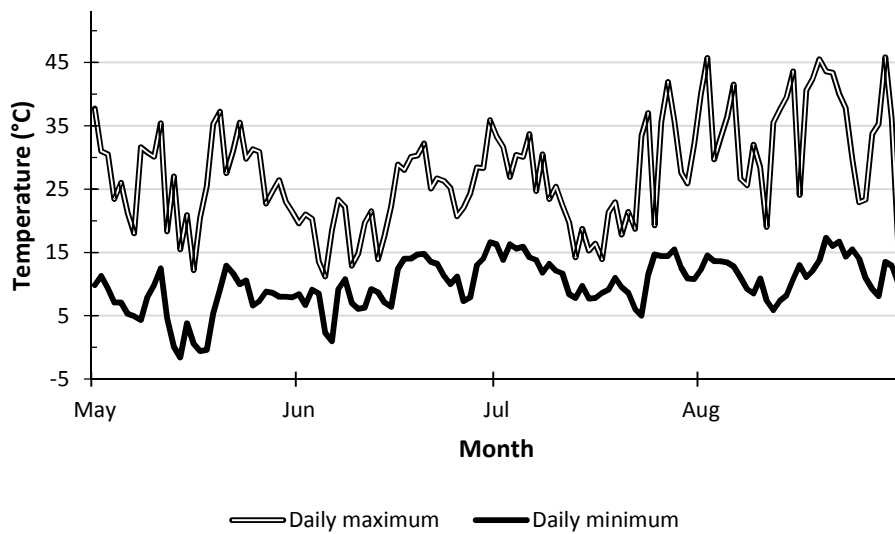
Fig 3S – Daily minimum and maximum temperatures at 800 m a.s.l. in vegetation season 2011.
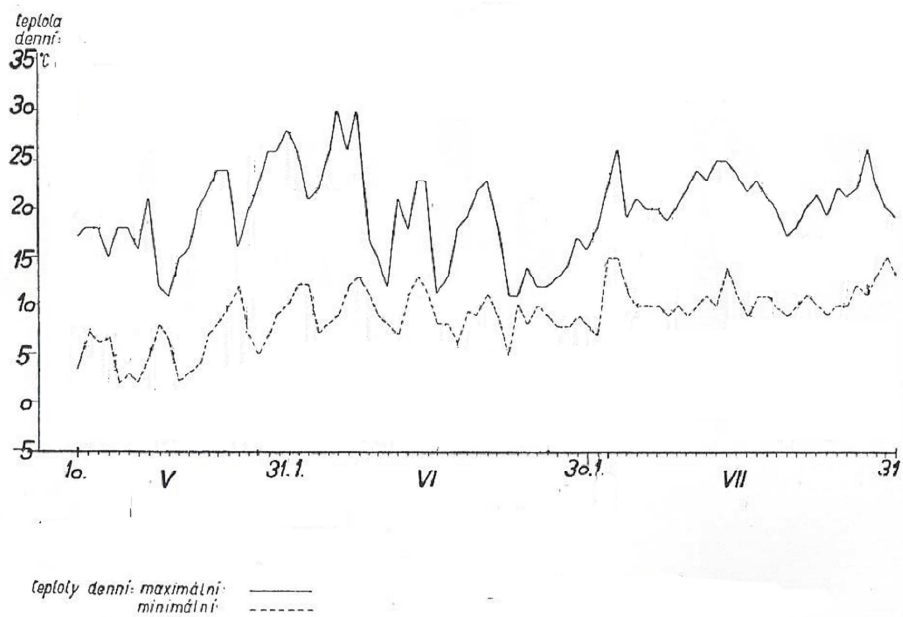

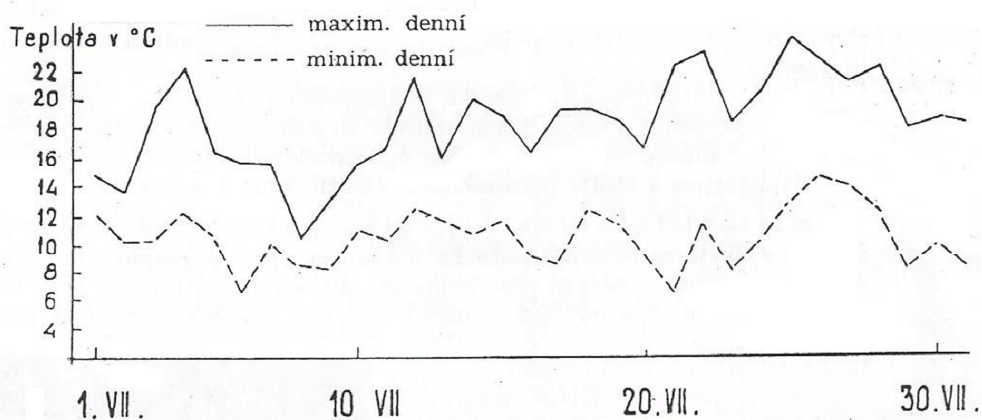**Fig 4S** – Daily minimum and maximum temperatures at 800 m a.s.l. in vegetation season 2012.

Fig 5S – Daily minimum and maximum temperatures at 1100 m a.s.l. in vegetation season 2011.
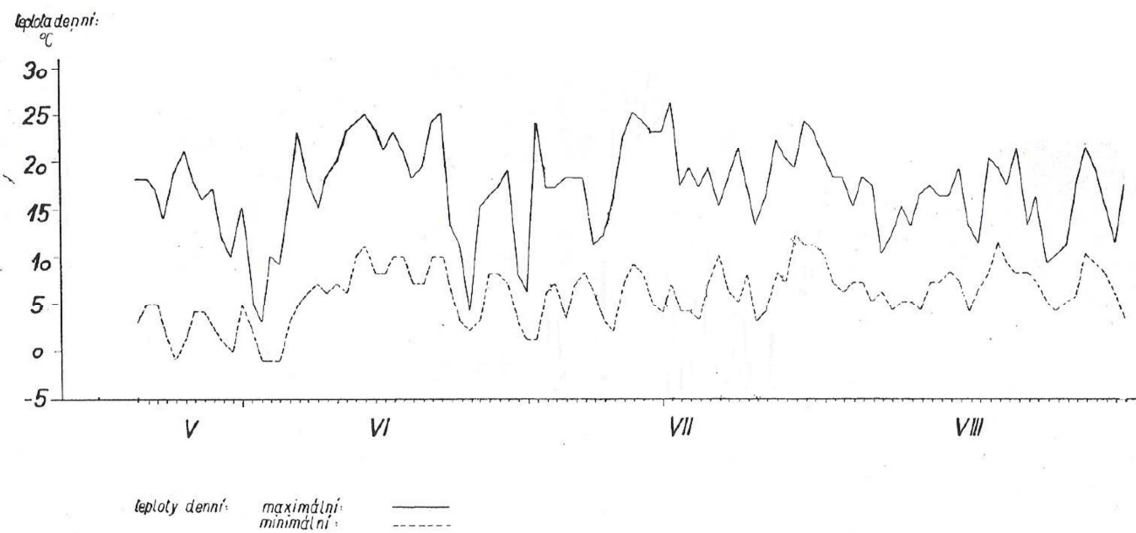

Fig 6S – Daily minimum and maximum temperatures at 1100 m a.s.l. in vegetation season 2012.

Fig 7S – Daily minimum and maximum temperatures at 280 m a.s.l. in vegetation season 1956. Recorded by Martinek (1957).

Fig 8S – Daily minimum and maximum temperatures at 550 m a.s.l. in July 1954. Recorded by Martinek (1956).

Fig 9S – Daily minimum and maximum temperatures at 1050 m a.s.l. in vegetation season 1956. Recorded by Martinek (1957).