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The Possibilities of Six-toothed Bark Beetle (*Pityogenes chalcographus* L.) (Coleoptera: Scolytinae) Sex Identification Based on Adults' Biometric Characteristic

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ABSTRACT

The aim of presented study was to determine a basic biometric parameters, such as weight and volume of male and female adults of the six-toothed bark beetle (*Pityogenes chalcographus* L., 1761) (Coleoptera: Scolytinae), captured to pheromone traps. It was shown that females of the six-toothed bark beetle were statistically heavier than males. Similarly, the volume of the examined insects was generally greater in the case of females, but the difference in this parameter was statistically insignificant within the weight classes distinguished for the analyzed beetles.

Key words: *Picea abies*, bark beetles, insect fresh weight, insect dry weight, insect volume weighted index, sex identification.

INTRODUCTION

The mass occurrence of cambio and xylophagous secondary pests may determine the rate of tree dieback, leading to the destruction of entire stands (Eidmann, 1992; Grodzki, 2007; Angst *et al.*, 2012). The six-toothed bark beetle (*Pityogenes chalcographus* L. 1761) (Coleoptera: Scolytinae) is, next to the European spruce bark beetle (*Ips typographus* L. 1758) (Coleoptera: Scolytinae), the most important threat to spruce stands both in central Europe and in Poland (Grégoire and Evans, 2004; Hedgren, 2004). In older stands, *P. chalcographus* accompanies *I. typographus*, inhabiting the upper parts of trees and branches, while in younger it occurs independently. However, *Pityogenes chalcographus* is an ecologically highly plastic species that under favorable weather conditions is capable of tree colonization on the entire trunk length in different spruce age classes (Grodzki, 1997). In recent years, the importance of the six-toothed bark beetle, as well as other small bark beetles significantly increases in spruce stands, in which the population of the European spruce bark beetle retrogrades (Grodzki, 2004a). Reducing the occurrence of *P. chalcographus*, as well as other bark beetle species from the group of secondary pests, mainly consists in preventive measures, such as respecting the principles of forest health through timely (before pest swarming) disposal and removal of inhabited trees (Wermelinger, 2004). Another practice is displaying trap trees or pheromone traps (Weslien *et al.*, 1989; Raty *et al.*, 1995; Faccoli and Stergulc, 2004; 2008), which mostly allows to assess the pest population density and, to a lesser extent, limit its abundance. The size of bark beetle population caught into pheromone traps is not the only information essential to the practice of forest protection against cambio- and xylophagous pests. Its sex structure, as well as biometric parameters of insects, such as: body dimensions, elytron weight or the dry weight of adults are in fact variable in time and determined by the stage of outbreak (Botterweg, 1982; Lobinger, 1996; Faccoli and Buffo, 2004; Grodzki *et al.*, 2006). Understanding the sex structure of an insect population may therefore provide valuable information for the assessment of risk to forest stands and for the designed methods of their protection using pheromone or trap trees. Detailed evaluation of the biometric parameters of insects may also be helpful in the sex identification of bark beetles, in which there is no sexual dimorphism, as for example in the European spruce bark beetle (Bednarz and Kacprzyk, 2012). Similarly, this can support the sex identification procedures in bark beetle species, whose morphological differences within opposite sexes are present, but their recognition is time consuming and requires the use of optical devices, as in the case of the six-toothed bark beetle. Given that after wintering, females of the six toothed bark beetle inhabiting trees after previous full supplemental outbreak, have a certain number of eggs or their primordia in their reproductive organs (Zumr and Soldán, 1981), a research hypothesis was put, that there can be certain differences in individual weight between both sexes. Therefore, the aim of this study was to determine the parameters of fresh and dry weight, and volume for male and female adults of the six-toothed bark beetle, caught into pheromone traps in different stages of swarming. Based on the biometric parameters the authors also raised the possibilities to distinguish *P. chalcographus* sex.

MATERIAL AND METHODS

The research was focused on the six-toothed bark beetle individuals, acquired in 2012 from Theysohn pheromone traps, which were displayed for the purposes of monitoring of this pest in the 90 year old Norway spruce stand (*Picea abies* Linnaeus, 1753; Karsten, 1881) in the Jeleśnia Forest District (the Beskid Żywiecki Mountains) at altitude 900 m a.s.l. (49º33'25.6"E, 19º20'12.6"N). The health condition of the spruce stand was reduced, as indicated by the increased dieback of trees infested by cambio- and xylophagous insects. The average volume of infested dead trees in the examined stand during the past five years remained at the level of 75.05 m^3/ha.

Traps, from which the six-toothed bark beetles were acquired, were provided with the synthetic pheromone Chalcodor®, issued by the Chemipan company (Warsaw), before the beetle swarming period (first half of April). Insects, caught in pheromone traps, were collected in four periods, corresponding to different stages of the *P. chalcographus* development, i.e. during the first generation (May 25th), sister brood swarming (July 3rd) and second generation (July 25th, August 20th). The six-toothed bark beetle individuals, collected in different periods, were placed in sealed plastic Eppendorf containers and were subjected to the cooling process in order to preserve them for further laboratory analyzes.

A group of bark beetles was randomly chosen from the trapped group of insects, and stored under uniform conditions at -20°C, after which their sex was identified. The material was analyzed until the randomly selected group of 100 individuals obtained from each trapping period. In this way, a total of 800 individuals of *P. chalcographus* adults were selected for further examinations. The sex identification of *P. chalcographus* beetles was conducted based on the differences in morphology of male and female individuals of this species, i.e. the size of teeth on elytral humerus (males are characterized by three distinct teeth, in contrast to females, in which they are much smaller) (Jarošík and Honěk, 2007). This was conducted using a stereomicroscope (Delta Optical) with a total magnification of 50x. Further laboratory analyses, i.e. measurements of fresh and dry weight, and volume were performed using the methodology proposed by Bednarz and Kacprzyk (2012), modified for the purposes of this study. The weight of analysed bark beetles individuals was determined using an ultra microbalance (UYA 2 Ultra-microbalance) produced by a Polish company Radwag. The bark beetle weight measurement was performed in triplicates with an accuracy of 0.1 µg, and the final result was the average of measurements. The acceptable error range was ±45x10^{-7} g.

In order to measure the volume of insects, due to small dimensions of the examined bark beetles, the collected male and female individuals, after measuring their fresh weight, were divided based on the created histograms (Fig. 1.) into three weight classes, assigning the insects into particular classes according to the following scheme class I: 2001x10^{-7} - 2500 x10^{-7} g, class II: 2501x x10^{-7} - 3000 x10^{-7} g, class III: 3001 x10^{-7} - 3500 x10^{-7} g. Determine the volume of the six-toothed bark beetle adults, based on placing a certain and known number of insects in the burette with a known sex, pre-determined weight class and the trapping period, subsequent scanning and measurement of the distance between the engraved mark (i.e. the fixed reference point) and the upper meniscus of the liquid. The fact that the liquid volume changed after placing the beetles in the pipette, was used to accurately determine the volume of the group of insects in a given weight class. The next stage of the study included drying the insects from each weight class with a laboratory dryer. In order to do this, each group of insects of a given sex, weight class and trapping period was placed separately in described boxes, set on the dryer's shelf. The measurement of the insects' dry weight was conducted after 48 hour drying at 105°C.

The last stage of laboratory analyzes included weighing the insects representing each weight class, sex and trapping period. This allowed to determine the dry weight of male and female individuals of the six-toothed bark beetle. In order to present the individual weight variability within the analyzed sample of male and female individuals of *P. chalcographus*, the weight-volume index, which characterized the actual weight variation within the insect sexes, was calculated for the designated weight classes, based on the measured parameters. This index was determined both for fresh and dry weight of beetles, by referring the weight values to the insect volume and by calculating the index value per each individual. The obtained data, i.e. fresh and dry weight, volume and the calculated weight volume index of the insects, were statistically analyzed.

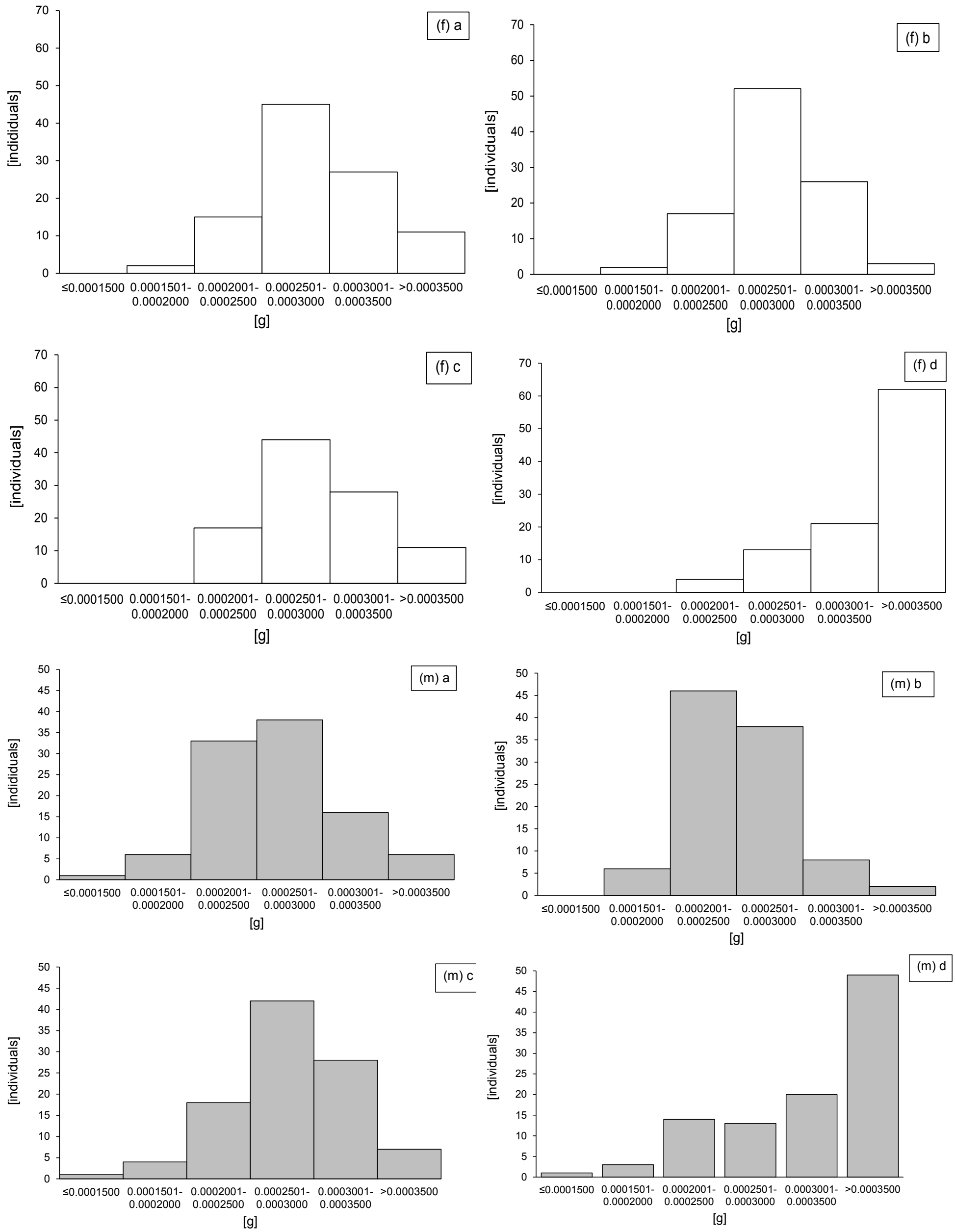

Fig. 1. Empirical distributions of fresh weight of female (f) and male (m) *Pityogenes chalcographus* (L.) adults, acquired from different trapping periods (period I - a, period II - b, period III - c, period IV - d). Distribution graphs of fresh weight in males were indicated in grey color, whereas the white color indicates fresh weight distribution in females.

The significance of differences within the means of the examined parameters between the representatives of opposite sexes, were examined using the nonparametric Mann Whitney *U test*. In order to compare the mean values of fresh weight between male and female individuals of the six toothed bark beetle, trapped in different periods of swarming, the nonparametric equivalent of one way ANOVA, i.e. the Kruskal - Wallis test, was applied. The use of nonparametric tests was preceded by the tests that verified the variable distribution type (Shapiro Wilk *W test*) and the homogeneity of variance (Levene test). The statistical significance of the results was verified at the significance level of α=0.05. All statistical calculations were performed in the Statistica PL software (v. 10.0 Statsoft Poland).

RESULTS

The analysis of fresh weight of the collected *P. chalcographus* beetles showed that its value was greater than 100µg, both for male and female individuals. Males with the smallest weight were lighter than the lightest females by over 27%, while in the case of the maximum values of fresh weight of beetles, this difference reached over 15% in favor of females (Table 1). At the same time, the examined group of bark beetles was dominated by males weighing less than 3000 $x10^{-7}$ g (32.75%) and less than 2500 $x10^{-7}$ g (27.75%), while the majority of females consisted of beetles with the fresh weight between 2501 $x10^{-7}$ g and 3000 $x10^{-7}$ g (38.50%) and between 3001 $x10^{-7}$ g and 3500 $x10^{-7}$ g (25.50%) (Fig. 1.). It was found that the mean value of fresh weight of females was higher than the mean value of male weight by 320 $x10^{-7}$ g (10 %) and this difference was statistically significant (Mann - Whitney *U test*: Z=5.294, p<0.05). Moreover, the value of this parameter was more variable in females (Table 1). Having regard to the swarming stages of female *P. chalcographus* individuals, it was proved that the individuals trapped in the period IV were characterized by the highest fresh weight, while the smallest values of fresh weight were recorded in the case of female beetles trapped in the period II. In the case of males, the difference between the greatest and the smallest value of mean fresh weight reached 1066 $x10^{-7}$ g and referred to periods II and IV, respectively (Table 2). It was shown that the differences in the fresh weight of adults between various swarming stages were statistically significant both for males and females of *P. chalcographus* (Kruskal - Wallis test, ♀♀: H (3, N=400)=103.852, p=0.0000; ♂♂: H (3, N=400)=83.128 p=0.0000). By comparing the mean values of fresh weight of females and males in each period of insect trapping, it was shown that the smallest differences in the values of the examined parameter occurred in the period III, when females proved to be heavier than males on average by 108 $x10^{-7}$ g (4.00%). On the other hand, the greatest differences in the fresh weight between males and females were recorded in the period IV, when the weight of females was greater than the weight of males on average by 644 $x10^{-7}$ g (15.00%) (Table 2). The demonstrated differences in the mean values of fresh weight between males and females in different trapping periods were, with the exception of the period III (Mann - Whitney *U test*: Z=1.357, p=0.17271) statistically significant

(Mann - Whitney *U test*, period I: Z=2.837, p=0.0046, period II: Z= 4.963, p=0.0000, period IV: Z=3.080, p=0.00207). The dry weight of female and male individuals of *P. chalcographus* assigned to three weight classes was higher in females than in males, with the greatest difference in the mean dry weight of individual female, i.e. at the level of 163 x10^{-7} g, recorded in the class I. In the case of beetles assigned to the class II, the mean value of dry weight per one individual turned out to be similar for females and males of *P. chalcographus* (Table 3). The volume measurements of *P. chalcographus* adults qualified into three weight classes showed that the mean volume of a single male exceeds the mean volume of a single female by 19.03% only in the case of beetles assigned to the weight class II. In the remaining classes, the proportion was reversed in favor of females and reached 7.68% for the class I and 26.93% for the class III (Table 3). Considering all weight classes in total, no statistically significant differences were found in the volume of beetles between the representatives of different sexes (Mann - Whitney *U test*: Z=-0.837, p =0.4025). Having regard to both parameters, i.e. the weight and volume of insects, the advantage of females over males was demonstrated in the first two weight classes. It was found that the mean value of weight and volume index for fresh weight of a single *P. chalcographus* female, assigned into weight class I, was greater by 189 x10^{-6} gcm^{-3} (27.00%) than the mean value of this index for a single male of this species in the same class. On the other hand, females from the weight class I were characterized by dry weight greater on average by 171 x10^{-6} gcm^{-3} (31.15%) than males in the same class. The difference in mean values of this index per one male and female individual assigned into the weight class II amounted to 111 x10^{-6} gcm^{-3} (15.68%) in the case of fresh weight and 100 x10^{-6} gcm^{-3} (18.90%) in the case of dry weight in favor of females (Table 4). Moreover, when considering all periods of trapping beetles and all distinguished weight classes of beetles, it was observed that the differences in mean values of the weight and volume index between males and females were statistically insignificant both for dry and fresh weight (Mann - Whitney *U test*; fresh weight: Z=-0.087, p=0.9309, dry weight: Z=0.029, p=0.9769).

Table 1. Basic descriptive statistics for the fresh weight [μg] of *Pityogenes chalcographus* adults, acquired from pheromone traps

**S.D.* - standard deviation; ***C.V.* - the coefficient of variation [%]

Gender	The value of statistics				
	Mean	Min.	Max.	*S.D.**	*C.V.***
Male	290.3 N=400	129.4	599.9	91.6	26.59
Female	321.6 N=400	172.0	799.4	84.7	30.43

Table 2. Basic descriptive statistics for the fresh weight [μg] of *Pityogenes chalcographus* adults, from different trapping periods

*I - May 25th 2012, II - July 3rd 2012, III - July 25th 2012, IV - August 22nd 2012. **Mean value of fresh weight of the six-toothed bark beetle in each trapping period was given for N=100 of each sex. ****S.D.* - standard deviation

Term of beetles catch*	Gender	The value of statistics			
		Mean**	Min.	Max.	*S.D.****
I	♂	267.6	149.3	387.5	48.9
	♀	290.7	172.0	493.3	54.4
II	♂	252.6	170.4	386.1	38.7
	♀	279.2	185.7	399.5	37.9
III	♂	281.9	145.2	385.1	48.2
	♀	292.7	208.2	410.8	43.9
IV	♂	359.2	129.4	129.4	104.2
	♀	423.6	227.0	799.4	134.1

Table 3. Mean value of dry weight [μg] and volume [mm^3] of female and male adults of *Pityogenes chalcographus*, caught in pheromone traps in the weight classes separated based on the fresh weight

*class I: 200.1 - 250.0 μg, class II: 250.1 - 300.0 μg, class III: 300.1 - 350.0 μg

Class*	Parameter			
	Dry weight	Volume	Dry weight	Volume
	♀		♂	
I	206.5	0.4682164	190.2	0.4320699
	N=81		N=142	
II	231.3	0.4366997	231.3	0.5393278
	N=173		N=140	
III	250.6	0.6981371	235.9	0.5101406
	N=83		N=68	

Table 4. Volume index for fresh and dry weight [gcm^{-3}] for each male and female of *Pityogenes chalcographus* (L.) caught in pheromone traps, in the weight classes separated based on the fresh weight

*class I: 200.1 - 250.0 μg, class II: 250.1 - 300.0 μg, class III: 300.1 - 350.0 μg

Class*	Dry weight [$x10^{-6}$]		Fresh weight [$x10^{-6}$]	
	♂	♀	♂	♀
I	378	549	511	700
II	429	529	597	708
III	462	360	631	5134

DISCUSSION

Diagnostic procedures of threat to forest stands ecological stability from cambio-xylophagos insects are base on biotechnical methods using various types of synthetic pheromone-baited traps (Zumr, 1983; Weslien and Lindelöw, 1989; Raty *et al.*, 1995; Hedgren and Schroeder, 2004). The bark beetles population abundance together with sex structure are the most important aspects of forest protection management in the prevention of secondary pests outbreaks in Norway spruce stands (Byers, 1993; Lobinger, 1996; Faccoli and Buffo, 2004; Grodzki *et al*., 2006). From the ecological and economic points of view, sex ratio is a crucial information to determine cambio-xylophages swarming phase and outbreak stage. Determination of bark beetles gender is either distinguished on external morphology (e.g. *P. chalcographus, Trypodendron* spp.) or, when it does not clearly exist, practiced on genital extraction (*Ips typographus* L.) (Annila, 1971; Botterweg, 1983; Byers, 1989). The insects weight and volume parameters could be also helpful in gender identification, what was successful confirmed for the eight-thoothed spruce bark beetle (Bednarz and Kacprzyk 2012). Using some of other basic biometric parameters, such as: body dimensions, elytron weight and the dry weight of bark beetles adults were so far the subject of studies concerning threat of Norway spruce health caused by *I. typographus* in different stages of pest outbreaks (Botterweg, 1982; Lobinger, 1996; Faccoli and Buffo, 2004; Grodzki *et al.*, 2014). Therefore, due to protection practices the reflection rise up if the biometric parameters such as adults weight and volume could be use for sex determination of other important biotic mortality agent in Norway spruce stands as *Pityogenes chalcographus*. What is more, it is also interesting if the differences in mentioned biometric parameters distinguished sex are related to different insect swarming phases. Presented in this paper study results are an attempt to answer these questions.

In order to analyse *P. chalcographus* adult's biometric characteristics the beetles collected from Theysohn pheromone baited traps localized at poor health of the Norway spruce stand in the Żywiec Beskid area. It was proved that the fresh weight of *P. chalcographus* females is greater than males by 10% and, what is more, the significance of this difference was statistically confirmed. Larger fresh weight of females is most likely, similarly as in the case of the European spruce bark beetle, related to having primordia or fully developed eggs in their abdomen (Zumr and Soldán, 1981, Bednarz and Kacprzyk 2012). However, in relation to dry weight of *P. chalcographus* (L.) adults, which was measured in three weight classes, separated based on fresh weight histograms, the obtained results were not that clear. The advantage of females over males in dry weight was relatively insignificant and was observed only in the lightest and the heaviest individuals from the analyzed sample of insects, i.e. those with fresh weight belonging to the weight class ranging from 2001 $x10^{-7}$ g to 2500 $x10^{-7}$ g (7%) and from 3001 $x10^{-7}$ g to 3500 $x10^{-7}$ g (8%). On the other hand, this regularity was not observed in the most numerous group of *P. chalcographus* individuals, i.e. those with fresh weight within the range from 2501 $x10^{-7}$ g to 3000 $x10^{-7}$ g. Similarly, the lack of results, expected based on the previous work by Bednarz and Kacprzyk (2012), concerned the volume of *P. chalcographus* beetles. The measurement of adults showed that the mean volume of each female exceeds the mean volume of each male in the first and third weight class, distinguished based on the fresh weight of bark beetles. In the case of bark beetles from the weight class between 2501 $x10^{-7}$ g and 3000 $x10^{-7}$ g, larger volume was recorded in males. The results were subjected to statistical tests which, however, did not confirm the significance of differences in the volume between opposite sexes of *P. chalcographus* adults. Consequently, the weight and volume index was determined based on biometric measurements of the examined insects. The resulting values of this index also showed no statistically significant differences between *P. chalcographus* beetles representing opposite sexes. The variability of weight and volume index, in separated weight classes of both female and male *P. chalcographus*, was on the other hand characterized by exceptionally large differences in both fresh and dry volume weight index at level ranged respectively from 18,90% (II class) to 31,15% (I class) and from 15,68% (II class) to 87,71% (III class), which could be an obstacle in explicit defining of the studied relationships. Thus, the obtained results may indicate the inability to practical use the analyzed biometric parameters in the sex identification of the six-toothed bark beetle females and males caught into Theysohn pheromone traps. The lack of significant differences between the weight and volume index may result from the method of volume measurement or, more likely, may be associated with the site of insect collection. The six-toothed bark beetle, compared to the European spruce bark beetle, in which the mentioned relationship was statistically significant (Bednarz and Kacprzyk 2012), is, in terms of its size, inconspicuous. In fact, 1cm^2 of area covered with insects can accommodate on average 11 adults of *I. typographus*, while the same area can be occupied by on average 109 adults of *P. chalcographus* (Bednarz, unpublished data). In addition to this, the sexual dimorphism that occurs in the six-toothed bark beetle, reflected by distinct, well-developed teeth on elytral humerus in male individuals, may substantially increase its absolute volume, as in females of this species teeth are either absent or poorly visible. The presence of teeth on elytral humerus in males, could have also a significant impact on the weight values and their sex related differences. Moreover, the examined bark beetles were collected from pheromone traps, therefore the analyzed material could be heterogeneous in terms of origin. Additionally, it is assumed that the period of insect remaining in pheromone traps could be varied. Therefore, it is likely that the diversity of conditions prevailing in pheromone traps had the greatest impact on the obtained results. In the case of trap trees, both the temperature and humidity of the material is characterized by smaller variation in time, while the ability of bark beetles to feed in trap trees causes that the bark beetles collected from classical traps, feeding under the bark, are physiologically more uniform. Unlike insects collected from the trap trees, bark beetles collected from artificial traps, e.g. Theysohn type, due to variable, often adverse conditions, may be characterized by different weight loss ratio. Thermal and moisture conditions, prevailing in the site of exposition, are also important for the physiological condition of insects caught into artificial pheromone traps. This is confirmed by the results of Heinrich (1981) and Prange (1996), who suggested the presence of active thermoregulation in insects based, among others, on intentional, increased water evaporation in the process of intense breathing, or as a result of direct evaporation of water from the body surface of insects. Since it is documented that females of Coleoptera (Scolytidae) have usually longer body lenght than males and, what is more have much larger fat content (Honěk, 1993; Akkuzu *et al.,* 2009), the bark beetles body size could be highly important, especially for females, because of a positive correlation between size and fecundity (Honěk, 1993). During body drying process of beetle the fat stores are not completely reduced, therefore the weight of adults may be varied by energy resources content especially in case of females. Botterweg (1982) and Gries (1985) also proved, that fat content of *Ips typographus* decreases during its dispersal and what is more the long flyers characterized higher fat content (Jactel, 1993). However in opposite, the relation between individual fat content and flight duration has been found as insignificant (Botterweg, 1982; Forsse and Solbreck, 1985; Jactel, 1993). Botterweg (1983) reported, that dry weight and lipid reserves of the *I. typographus* correlates negatively with attack density. Increasing attack density results in decreasing beetle size, weight and fat content (Thalenhorst, 1958; Botterweg, 1983; Saarenmaa, 1983; Anderbrandt *et al.*, 1985). However that statements does not explain our results, which indicate the heaviest fresh weight of *Pityogenes chalcographus* bodies in the last two trapping periods refer to the second generation of insects. Similar interesting results got Grodzki (2004b), who studied the relationship between body length of *I. typographus* in different breeding conditions. The author did not confirmed the regularity of the trees infestation density influence on bark beetle body length increase. It is likely that it was an effect of positively environmental impact and especially different population share origin in pheromone trapping. The beetles sampled from pheromone traps might not only originate from local populations since dispersal abilities of *I. typographus* is very high (Botterweg, 1982, Gries, 1985). Therefore the information coming from bark beetles mass trapping are relatively useless to be applicable for cambio-xylophas population characterize (Grodzki, 2007). Concluding, the bark beetles adults body dimension have to be considered as insufficient feature to predict population density (Sallé *et al.*, 2005).

The results obtained in this study contradict the initial hypothesis that the differences in weight of the six toothed bark beetles result from the sexes of *P. chalcographus* caught into pheromone traps in different periods of swarming. Based on the presented observations, further hypothesis shall be put that if this study is continued, it would be more accurate to obtain adults from the material of uniform physiological condition, similarly as in the study by Bednarz and Kacprzyk (2012). It would be also advisable to distinguish classes of insects, for which the volume would be measured, based on the parameter of length and width of bark beetles, instead of fresh weight, which can largely differ, as the derivative of different physiological condition of bark beetles caught in pheromone traps.

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