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The Effects of Water Extracts from Lemon Balm on Pea Leaf Weevil and Black Bean Aphid Behaviour

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ABSTRACT

The objective of this study was to determine the effects of various concentrations of water extracts prepared from the fresh or dry matter of lemon balm on *Sitona lineatus L.* and *Aphis fabae* Scop. behaviour. The assessment pertaining to the feeding intensity of beetles was carried out by measuring the surface of feeds caused by *S. lineatus*. While examining the effect of extracts on *A. fabae*, the mortality of wingless female and aphid larvae was determined. In the studies on the olfactory reaction glass olfactometer "Y-tube" and 4-armed arena olfactometer were used. The results of the experiment showed that the water extract prepared from dry matter of lemon balm with 2% concentration limited the feeding of both female and male of *S. lineatus*. The increase in the mortality of the black bean aphid females and larvae was obtained only after applying the extracts from fresh and dry matter at highest concentrations. The evident deterrent reaction of the odour substances obtained from the lemon balm plants towards the beetles of *S. lineatus*, could find application in ecological farms via introducing the plant as an accompanying crop to the main crops. The winged individuals of *A. fabae* did not react to the abovementioned factor.

Keywords: water extracts, *Melissa officinalis* L., olfactometer, biological control

INTRODUCTION

Application of chemical means of pest control is increasingly contributing to the emergence of pest resistance to the active substances contained in these compounds, resulting in their reduced effectiveness [Hansen 2008, Pimentel et al. 2009, Wawrzyniak et al. 2015]. Furthermore, these compounds pose the threat to all elements of the natural environment [Tscharntke et al. 2005]. In line with the principles of integrated plant protection, priority should be given to non-chemical methods where the use of insecticides is limited to the necessary minimum. One of the non-chemical methods of reducing the numbers of pests of cultivated plants is the use of water extracts prepared from fresh or dry parts of herb plants. The olfactory stimuli are very important in the life of insects. The odour of a host plant could be the principal factor determining their behaviour and the possibility to locate their host plant. For this reason, the substances which prevent the identification of the host plant by the pest, or have a deterrent effect on the latter are increasingly often applied in plant protection [Korczyński and Koźmiński 2007].

Lemon balm (*Melissa officinalis* L.) is a perennial herb of the mint family (Lamiaceae), used in many applications. It is used in medicine because of its antimicrobial and anti-inflammatory properties [Rostami et al. 2012], and it also functions as a natural anti-oxidant [Koksal et al. 2011, Saeb et al. 2011]. Owing to their allelopathic properties, the water extracts from the plant can be used in protection against weeds [Kato-Noguchi 2001]. It is thus justified to test the lemon balm as a natural means for the protection against pests.

The objective of the presented study was to determine the effects of various concentrations of the water extracts prepared from the fresh matter (FM) and dry matter (DM) of the lemon balm upon the feeding by pea leaf weevil (*Sitona lineatus* L.) and on the mortality of the black bean aphid (*Aphis fabae* Scop.). Furthermore, their reactions to the odour of the lemon balm were studied by the use of olfactometer.

MATERIAL AND METHODS

The experiment was conducted in the laboratory, in six replicates. The extracts from dry matter of *Melissa officinalis* L. were prepared at concentration assumed conventionally as 2%, 5% and 10% (dried plants + cold redistilled water in the ratios of 2 : 100, 5 : 100 and 10 : 100) and at the concentrations of 10%, 20% and 30% for fresh matter (fresh above-ground parts of plants + cold redistilled water in the ratios of 10 : 100, 20 : 100 and 30 : 100). The extracts were stored in the dark for the period of 24 hours, and then filtered through filter papers and immediately used to conduct the experiment. The test was performed on Petri dishes, and the substrate consisted of moist filter paper. Plant leaves (pea in case of *Sitona lineatus* L., broad bean in the case of *Aphis fabae* Scop.) were soaked for 3 seconds in adequate plant extracts and in distilled water used as control, and then dried at room temperature. In each dish, a single leaf of a plant, suitable for a specific object was placed and then pests were introduced – one adult of *S. lineatus* (male and female separately), six wingless females of *A. fabae* and ten larvae of aphid.

The assessment of the feeding intensity of beetles was carried out by measuring the surface of feeds caused by *S. lineatus* at 12 hour intervals. In addition, the values of palatability index as the ratio of the percentage area of leaves consumed in individual objects to the percentage area of leaves consumed in the control was calculated. Furthermore, an absolute deterrence index, which takes into account the relationship between the area of leaves consumed in the individual objects and the area of leaves consumed in the control, was established:

$$Bwd = [(K–T) : (K+T)] \cdot 100 \quad (1)$$

where: *Bwd* – absolute deterrence index.
K – area of leaves consumed in control [mm^2].
T – area of leaves consumed in individual objects [mm^2] [Kiełczewski 1979].

While examining the effect of *M. officinalis* extracts on *A. fabae*, the mortality of wingless female and aphid larvae was determined at 12 hour intervals.

In the studies on the olfactory reaction of the abovementioned insects, for *S. lineatus* glass olfactometer "Y-tube" was used and for *A. fabae* – 4-armed arena olfactometer, applied in multiple choice tests. There are commonly used for the evaluation of odour preferences in insects [Vet et al. 1983, Schaller and Nentwig 2000, Ukeh and Umoetok 200, Ranjith 2007]. "Y- tube" had one incoming arm and two test arms. The area of the olfactometer has the central field and four test arms. The air, cleaned in a carbon filter was forced in by a pump, and directed to each test arms. Then, the air stream was flowing through the source of odour i.e. glass container with either 30 g of fresh matter of lemon balm together with either a wet circle of filter paper (to ensure appropriate air humidity) or only wet filter paper (control) in the case of "Y- tube". In the case of four-arm arena, air was pumped into two arms through separate containers, containing lemon balm and wet filter paper, and through two more, containing only wet filter paper (control). An imago of pests was placed in the outlet of incoming arm in the case of "Y- tube" or in the central part of central field of four-arm arena and its behaviour was observed for 10 minutes, recording the number of incursions into particular test arms of the olfactometer (with or without the odor derived from lemon balm). The experiment with pest insects was performed in 12 repetitions.

The obtained results were then subjected to analysis with STATISTICA 10.0 software. The significance of differences between the means were tested by univariate analysis of variance, and the means were differentiated by Fisher's LSD test at $\alpha = 0.05$. The Student's t-test for independent groups (the grouping variable was either the presence or the lack of test plant odour influx) was used to determine the statistical significances of differences in the results obtained with the use of an olfactometer.

RESULTS AND DISCUSSION

The extracts prepared from dry matter of the lemon balm at the two highest concentrations (5% and 10%) at all dates of observations, led to a significant reduction in the feeding by males of pea leaf weevil (Table 1). Similar regularities were observed in the case of the extract from dry matter, with the concentration of 2% but only up to 96 hours following the beginning of experiment. After the experiment was concluded, the dry matter extract with the concentration of 10% resulted in the decrease in the surface area of eaten-up places in pea leaves, resulting from male feeding by nearly 30% compared with the control

Table 1. The effect of extracts from *Melissa officinalis* L. on the surface area of places eaten-up in pea leaves by females and males of *Sitona lineatus* L. [mm^2]

Object	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h	108 h	120 h
Males										
C	11.6 c*	26.1 b	45.5 b	72.9 b	105.2 bc	184.3 bc	214.2 bc	240.8 bc	267.5 bc	294.8 b
DM 2%	1.3 ab	2.8 a	5.3 a	26.4 a	46.2 a	116.2 a	129.7 a	158.9 a	206.0 ab	241.3 ab
DM 5%	0.3 a	2.5 a	2.8 a	21.4 a	45.2 a	104.9 a	125.9 a	146.6 a	189.0 a	218.1 a
DM 10 %	1.3 ab	2.8 a	7.2 a	22.0 a	45.2 a	112.4 a	131.6 a	158.3 a	183.4 a	206.6 a
FM 10%	4.7 abc	15.7 ab	36.7 ab	55.9 ab	79.8 ab	141.6 ab	148.5 ab	181.5 ab	202.8 ab	222.2 ab
ŚW 20%	9.7 bc	31.4 b	43.0 ab	61.5 b	93.9 abc	181.8 bc	211.6 b	236.8 bc	244.2 bc	248.7 ab
FM 30%	5.3 abc	22.9 ab	38.3 ab	55.3 ab	132.2 c	193.1 c	243.0 c	266.9 c	284.2 c	298.3 b
Females										
C	14.4 c	32.3 c	53.1 c	96.7 b	155.7 b	243.0 c	282.9 c	356.7 c	400.7 c	426.5 c
DM 2%	6.0 ab	13.8 ab	24.2 ab	38.9 a	68.8 a	95.1 ab	111.5 ab	121.8 ab	140.4 ab	185.0 ab
DM 5%	1.6 a	7.9 a	15.1 a	43.3 a	79.1 a	162.0 abc	183.7 bc	195.9 ab	222.6 ab	230.3 ab
DM 10 %	1.9 a	7.9 a	13.5 a	22.9 a	31.7 a	48.7 a	52.8 a	63.1 a	71.0 a	82.2 a
FM 10%	8.2 abc	24.8 bc	44.3 bc	69.1 ab	98.9 ab	177.4 bc	197.5 bc	239.3 bc	258.7 bc	269.6 bc
FM 20%	9.4 bc	22.0 abc	35.8 abc	54.6 ab	82.3 ab	121.2 abc	153.9 abc	178.7 ab	206.0 ab	217.8 ab
FM 30%	10.4 bc	19.5 abc	32.3 abc	40.5 a	49.6 a	65.3 ab	79.1 ab	120.3 ab	146.0 ab	182.3 ab

C – control, DM – dry matter, FM – fresh matter.

* Values for individual terms of observations marked by different letters are statistically different ($\alpha = 0.05$).

object. No significant effect of any of the extracts prepared from the fresh matter upon the analysed feature was noted.

The females of pea leaf weevil showed more intensive feeding than the males of the pest. After 120 hours, the area of places eaten-up by females was greater by more than 130 mm^2 than that in the case of males. Similarly as in the case of males, the dry matter extracts contributed to a major reduction in feeding by females (the differences were statistically significant at all dates of observations, except for the extract with 5% concentration after 72 and 84 hours after the inception of the experiment). The extracts from fresh matter at the highest concentrations (20 and 30%) after 96 and 48 hours, respectively, also led to significant reduction of female feeding.

In all objects, the absolute deterrence index for the females of pea leaf weevil reached positive values, which testifies to the inhibiting effect of the applied extracts towards the feeding by the studied pest (Fig. 1). In the case of males, after applying the fresh matter extract at 30% concentration, a negative value of the index was noted indicating the stimulating effect of food. The strongest deterrent effect for both males and females appeared in the dry matter extract with 10% concentration. In the case of the extracts prepared from the fresh matter, the increases in the absolute deterrence index in females were noted along the increases in concentrations, whereas a reverse relationship was found in males. In all objects, the values of the absolute deterrence index were definitely higher for females than that for males.

In males, the palatability index was the highest when the extract from the fresh matter with 30% concentration was applied whereas in females – in the extract from fresh matter but at 10% concentration (Fig. 2). In all objects, the value of the analysed index was higher in males than in females when the same kind and concentration of extract was applied. The lowest values of the palatability index for both sexes were noted after the extract with 10% concentration, prepared from the dry matter of lemon balm plants was applied.

The extract prepared from the fresh matter of the highest concentration (10%) resulted in a significant increase in the mortality of the wingless females of the black bean aphid after 48 hours from the inception of the experiment, and this status was maintained up to the end of the experiment (Table 2). After 72 hours, the extract from the fresh matter at 30% concentration also resulted in the increased mortality; however, the effect was nearly fourfold weaker that the action of the extract from dry matter at 10% concentration. The remaining extracts prepared from either dry or fresh matter had no significant effect on the analysed feature.

Similar regularities were observed in the case of the larvae of the black bean aphid. The extracts from dry and fresh matter applied in the highest

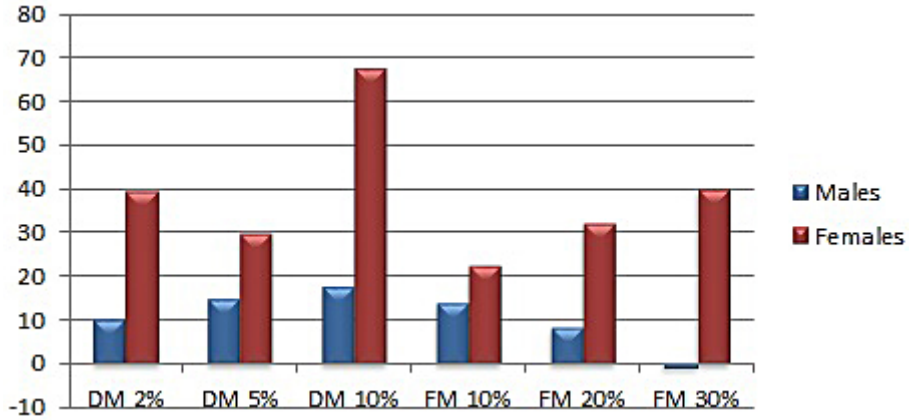

Fig. 1. Absolute deterrence index. Symbols as in Table 1

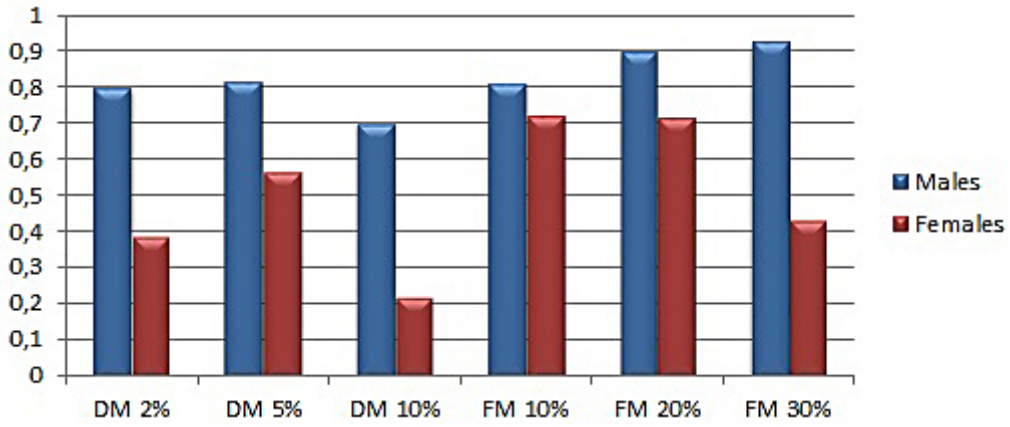

Fig. 2. Palatability index. Symbols as in Table 1

Table 2. The effect of extracts from *Melissa officinalis* L. on mortality of wingless females and larvae of *Aphis fabae* Scop. [%]

Object	12 h	24 h	36 h	48 h	60 h	72 h	84 h	96 h	108 h	120 h
Wingless females										
C	0.0 a*	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	2.8 a	2.8 a	5.6 a	5.6 a
DM 2%	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	2.8 a	8.3 ab	11.1 ab	13.9 ab	16.7 ab
DM 5%	0.0 a	0.0 a	0.0 a	2.8 a	2.8 a	2.8 a	11.1 ab	13.9 ab	19.4 ab	19.4 ab
DM 10 %	0.0 a	0.0 a	0.0 a	19.4 b	33.3 b	41.7 c	50.0 c	66.7 c	75.0 b	100.0 c
FM 10%	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	2.8 a	5.6 ab	5.6 a	11.1 ab
ŚW 20%	0.0 a	0.0 a	0.0 a	0.0 a	2.8 a	2.8 a	2.8 a	2.8 a	5.6 a	13.9 ab
FM 30%	0.0 a	0.0 a	0.0 a	2.8 a	5.6 a	13.9 b	16.7 b	19.4 b	22.2 b	25.0 b
Larvae										
C	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	1.7 a	1.7 a	6.7 a	11.7 a	27.8 a
DM 2%	0.0 a	0.0 a	1.7 ab	1.7 ab	3.3 ab	3.3 ab	6.7 ab	10.0 a	20.0 ab	28.3 ab
DM 5%	0.0 a	0.0 a	1.7 ab	3.3 ab	3.3 ab	6.7 ab	11.7 abc	18.3 ab	36.7 bc	48.3 bc
DM 10 %	0.0 a	0.0 a	5.0 b	6.7 b	8.3 b	13.3 b	26.7 c	51.7 c	73.3 d	83.3 d
FM 10%	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a	1.7 a	6.7 ab	16.7 ab	21.7 ab	30.0 ab
ŚW 20%	0.0 a	0.0 a	0.0 a	0.0 a	3.3 ab	5.0 ab	11.7 abc	20.0 ab	31.7 abc	38.3 abc
FM 30%	0.0 a	0.0 a	1.7 ab	5.0 ab	5.0 ab	6.7 ab	18.3 bc	31.7 b	43.3 c	56.7 c

Symbols as in. **Table 1**. *Values for individual terms of observations marked by different letters are statistically different ($\alpha = 0.05$).

concentrations resulted in a significant increase in the mortality of the studied pest (after 36 and 84 hours after the inception of experiment, respectively). Furthermore, the extract from dry matter with 5% concentration, in the two last dates of observations, also contributed to a significant increase among the larvae of the black bean aphid.

Among the available scientific publications, there are few studies on the effects of the water extracts prepared from the lemon balm upon the feeding by the pests of cultivated crops. Only Hiesaar et al. [2000] demonstrated in their research that the water extract from that plant resulted in an evident drop in the number of eggs laid by the females of *Trialeurodes vaporariorum* (West.). There were also some studies conducted on the effect of volatile oils obtained from the lemon balm plants, and the effect of alcohol extracts from that plant. Pavela [2004] found that the alcohol extract from the lemon balm plants showed weak repellent and deterrent effects towards the adult individuals of the Colorado potato beetle. Rafiei-Karahroodi et al. [2011] demonstrated that the volatile oil obtained from the lemon balm had a toxic effect on the larvae of *Plodia interpunctella* Hübner and contributed to the increased mortality among eggs of the pest. The aforementioned authors had also proven that with the increased concentration of volatile oil, its adverse effect towards the pest is also increased. Other authors confirmed the effectiveness of using volatile oils from the plants of Lamiaceae family in limiting the feeding by the pests of stored products [Ebadollahi 2011, Maede et al. 2011, Popović et al. 2013], *Aphis fabae* Scop. and *Brevicoryne brassicae* L. [Nottingam 1991] as well as *Frankliniella occidentalis* Pergande [Picard et al. 2012].

The studies on the effects of water extracts obtained from other herb plants upon the feeding by pea leaf weevils demonstrate that in the case of the wormwood (*Artemisia absinthium* L.), the reduction in feeding by the females of the pea leaf weevil was obtained after the application of the extract from dry matter of the plant in at least 5% concentration whereas towards the pea leaf weevil males, only the extract from dry matter with concentration of no less than 10% was effective [Rusin et al. 2016 b]. Biniaś et al. [2016 a] demonstrated that the extracts from the fennel (*Foeniculum vulgare* Mill.) seeds did not affect the feeding by the females of pea leaf weevil although they could significantly limit the feeding by the males of that pest. Against the background, the water extracts from the dry matter of lemon balm showed a stronger effect on the studied pests, as they limited the feeding of pea leaf weevils even in the lowest concentration (2%). In turn, the extracts from the fresh matter were effectively limiting the feeding of the females of the studied pest only in the highest concentration (30%).

The dry matter extracts obtained from wormwood, show an inhibiting effect on the feeding by the females of pea leaf weevils, as indicated by the positive values of the absolute deterrence index obtained in the study by Rusin et al. [2016 b]. Similar relationships were also noted in the presented experiment after applying the extract from the lemon balm, and the values of the studied index were decisively higher than those obtained after applying the extracts from the wormwood. The aforementioned authors had also demonstrated that the extracts from fresh matter can have a stimulating effect on feeding by males, which also corresponds to the results of the presented experiment regarding the fresh matter extract at 30% concentration. In the presented experiment, the highest values of the absolute deterrence index were obtained after applying the dry matter extract from lemon balm plants at 10% concentration (67 for females, and 17 for males). In the studies by Biniaś et al. [2016 a], the value of that index for pea leaf weevils, after applying the extract from the fennel seeds at the same concentration, amounted to 19 for females, and 27 for males, respectively. For males, the value of palatability index in the presented experiment was the highest after applying the extract obtained from the fresh matter with 30% concentration (more than 0.9), and in the case of females – after applying the fresh matter extract at 10% concentration (0.7). In the experiment mentioned earlier [Biniaś et al. 2016 a], the values of the index in question after applying the extract obtained from the seeds of the fennel at the same concentrations, fell into the range of 0.5–0.6, for both males and females.

The high concentrations of extracts from the dry and fresh matter of the wormwood (10% and 30%, respectively) [Rusin et al. 2016 b] and from the tarragon (*Artemisia dracunculus* L.) (10% for dry matter, and 20% and 30% for fresh matter) [Rusin et al. 2016 a], result in the increased mortality among the wingless females and larvae of black bean aphid (at 100% level for females, and nearly 50% for larvae in the case of the extracts prepared from the wormwood, and at 100% level for both females and larvae in the case of the extracts from the tarragon, after 96 hours of experiment). On the other hand, the high concentrations

of extracts prepared from the mountain savory (*Satureja montana* L.) (10% for dry matter, and 20% and 30% for fresh matter) increase the mortality of the females and larvae of the black bean aphid, up to respective 100% and 78.8% in the case of dry matter, and to more than 80% and to around 70% for fresh matter extracts, in 96 hours after the application of extract [Rusin et al. 2016 c]. Generally, also in the presented experiment, only the highest concentrations of the extracts from the lemon balm resulted in the increased mortality in the black bean aphids, but the values were lower. For the objects DM 10% and FM 30%, after 96 hours of experiment, they amounted to the respective 66.7% and 19.4% for wingless females, and 51.7% and 31.7% for larvae.

A great number of authors emphasize the fact that the olfactory stimuli can play a significant role in limiting the feeding by pests [Koschier et al. 2002; Koschier and Sedy 2003, Katerinopoulos et al. 2005]. In the presented experiment, an evident negative reaction to odour substances obtained from the fresh matter of lemon balm plants was noted in females (t=2.14, P=0.048) and males (t=3.62, P=0.002) of the pea leaf weevil (Table 3). The pests selected the arm of the olfactometer where the odour of lemon balm was pumped nearly two- to fourfold less frequent than the control arm. No significant reaction of the winged females of black bean aphids towards the odour substances obtained from *M. officinalis* (t=1.53, P=0.149) was found. In their studies, Rusin et al. [2016] also demonstrated the strong deterrent reaction of the odour substances obtained from the wormwood plants, towards the adult beetles of pea leaf weevils (males and females) but did not find such a relationship in the case of winged females of *Acyrthosiphon pisum* Harris. Similarly as in the results obtained in the presented experiment, Biniaś et al. [2016 b] did not find an evident repellent effect of odour substances derived from the common sage (*Salvia officinalis* L.) towards the black bean aphid.

CONCLUSIONS

1. The water extract prepared from dry matter of lemon balm with 2% concentration contributed to limiting the feeding of both female and male of pea leaf weevil. In the case of females, a similar relationship was observed using the fresh matter extracts with 20% and 30% concentration, however, at later dates of observations.
2. The increase in the mortality of the black bean aphid females and larvae was obtained only after applying the extracts from fresh and dry matter at highest concentrations (30% and 10%, respectively).
3. The evident deterrent effect of the odour substances obtained from the lemon balm plants towards the beetles of pea leaf weevil (both females and males), could find the application in ecological farms via introducing the plant as an accompanying crop to the main crops. The winged individuals of the black bean aphid did not react to the abovementioned factor.

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Table 3. Responses of pests to odors derived from *Melissa officinalis* L. fresh matter expressed as a number of incursions per one insect into selected areas of Y-tube olfactometer (*Sitona lineatus* L.) or four-armed arena (*Aphis fabae* Scop.).

Pest	Control	*Melissa officinalis* L.
Aphis fabae Scop. – winged females	0.75	0.38
Sitona lineatus L. – females *	0.89	0.44
Sitona lineatus L. – males *	0.88	0.22

* differences significant at $\alpha = 0.05$, in other cases differences not proven statistically.

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