

ORIGINAL ARTICLE

The effect of different seeding densities of linseed (*Linum usitatissimum* L.) on flax flea beetles (Coleoptera: Chrysomelidae)

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Vol. 57, No. 2: 158–166, 2017

DOI: 10.1515/jppr-2017-0022

Received: February 9, 2017

Accepted: June 5, 2017

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Abstract

Linseed, one of the oldest cultivated crops, is again gaining in importance, mainly due to its nutritional benefits and biomedical applications. Therefore, it is expected that herbivores will also exist in greater abundance. Among them the flea beetle, *Aphthona euphorbiae* Schrank and *Longitarsus parvulus* Paykull are considered to be serious pests of flax grown for fibre and seeds in Europe. The aim of this study was to determine flax flea beetles' abundance, species richness and seasonal dynamics on linseed grown at different densities. It was expected that linseed seeding density can significantly affect flea beetle populations. The experiment was carried out in Lower Silesia, Poland, from 2011 to 2013. A genetically modified type of linseed overproducing flavonoids was used. Flea beetles and the damages they caused were determined on plants and also a sweep net was used for the collection of adult beetles. During the three years of the study 15 species of flea beetles were identified from oil flax plants, with *A. euphorbiae* and *L. parvulus* being dominant. In terms of the total catch, the tendency was for beetle numbers to decrease with increasing plant density. Flax flea beetles feeding on linseed plants, irrespective of plant density, had two peaks of abundance. The first peak was lower and occurred in June, when plants were at the blooming stage. This peak was caused by overwintering adults who colonized crops in spring. The second, higher peak of abundance was recorded in the second half of July, when plants were at the ripening stage. This peak was formed by adults of the new generation. Each year, at the higher population peak of abundance, the flea beetles were most numerous on plants grown at the lowest density. There was one period, lasting either from mid-May to the first few days of June, or from the beginning of June to mid-June, during which the number of holes and damage on plants of each treatment were highest. During the three years of the study there were several cases of significantly higher numbers of flea beetle feeding symptoms on plants grown at the lowest density as compared to the medium and highest densities.

Key words: abundance, flax flea beetles, linseed, seeding density effect, species richness

Introduction

Linseed (*Linum usitatissimum* Linnaeus, 1753), one of the oldest cultivated crops, continues to be widely grown for oil, fibre, and food (Jhala and Hall 2010; Heller *et al.* 2015). Linseed oil has commercial value as a component of adhesives, paints and varnishes, plasticizers, inks, and linoleum. It is a precursor of nylon

and composite materials (Shim *et al.* 2014; Zuk *et al.* 2015). The raw material of this oil is also used for the production of biodiesel fuels (Demirbas 2009). Because of linseed oil's protective properties, it is recommended in the treatment of gastrointestinal tract diseases (e.g. chronic peptic ulcers, chronic inflammation of

mucous membranes) and respiratory tract diseases. The dietary properties of flax oil are also very important. It acts positively on peristalsis and metabolism, and prevents skin diseases. Flaxseed oil lowers blood cholesterol and glucose levels (Rodriguez-Leyva *et al.* 2013). Linseed lignans inhibit cell proliferation and growth, making them potential anticancer agents (Alphonse and Aluko 2015). Flaxseed is added to animal feed to improve animal reproductive performance and health (Turner *et al.* 2014).

Two species of flax flea beetles, *Aphthona euphorbiae* (Schrank, 1781) and *Longitarsus parvulus* (Paykull, 1799) are considered to be serious, worldwide pests of flax grown for fibre and seeds (Heller *et al.* 2015). These two species occur together, but *A. euphorbiae* thrives within a wider temperature and humidity range than *L. parvulus*. *Aphthona euphorbiae* prefers warm, dry weather which can be fatal to the pre-adult stages of *L. parvulus*. Conversely, *L. parvulus* prefers cool, moist conditions and is often the dominant species in cool, wet summers (Żurańska 1965a,b; Wise and Soroka 2003). As many as 14 species of flea beetles have been found on flax (Żurańska 1965b). Most of them are relatively rare and are not known to cause noticeable crop damage.

The aim of this study was to determine flax flea beetle abundance, species richness and seasonal dynamics on linseed grown at different densities. It was expected that linseed seeding density can influence flax flea beetle populations.

Materials and Methods

The experiment was conducted at the Experimental Research Station in Pawłowice, near Wrocław, Lower Silesia, Poland (51°1737'N, 17°1176'E) during three growing seasons (2011–2013). A genetically modified type of *L. usitatissimum* (cv. Linola) overproducing flavonoids (Zuk *et al.* 2011) was used. Plants were grown on 15 m² plots (10 × 1.5 m, row spacing 30 cm) of sandy soil. A 0.3 m-wide space between the experimental plots was maintained mechanically as bare soil, and the plots were weeded regularly by hand. The seeds were sown at three different densities per 1 m²: the

lowest – 400 seeds (marked as 1), the medium – 600 seeds (2) and the highest – 800 seeds (3). The experiment was designed as a split-plot with four replicates for each sowing density. After linseed emergence and again before harvest the number of plants per m² was counted.

The number of flea beetles and the symptoms of their damage (small shot-holes on leaves and damage on stems) were recorded on 50 whole, consecutive plants in the middle row of each plot during the flax growing season. A total of six (2011–2012) or seven (2013) observations of the presence of flea beetles on plants were made during the research seasons, and respectively, 10 (2011), 11 (2012) and nine (2013) times the damage was evaluated. Every second week adults were also collected with a sweep net (38 cm in diameter). A sample consisted of 10 180°-sweeps taken in the middle of each plot on each sampling date. Samples were placed individually in cardboard containers with a paper towel saturated with 95% ethyl acetate. Samples were taken to the laboratory, where the insects were hand separated from plant material and placed in vials containing 75% ethyl alcohol. Samples were later sorted, and specimens were counted and identified. Chrysomelidae species names were adopted from Borowiec *et al.* (2011).

The numbers of flea beetles and the symptoms of their damage found in different treatments of the experiment were compared for each date separately using analysis of variance (ANOVA) followed by Tukey's HSD (post-hoc). Statistical significance was evaluated at $p \leq 0.05$. For statistical analysis software Statistica, version 12 PL, was chosen.

Results and Discussion

Plant density

As shown in Table 1, higher seed sowing density caused, as expected, higher plant density. The mean emergence capacity over three years ranged from 47.4% to 56.8%, and increased with the decreasing number of sown seeds. The highest percentage of plant reduction during the growing season (33.5%) occurred in the

Table 1. Changes in linseed plant density during the growing season, Pawłowice, Poland, means from 2011–2013

Number of seeds sown per 1 m ²	Mean number of plants per 1 m ²		Emergence capacity [%]	Reduction during growing season [%]
	after emergence	before harvest		
400	227	163	56.8	28.2
600	299	205	49.8	31.4
800	379	252	47.4	33.5

Table 2. Total number of flea beetles and damage observed on linseed plants

Sowing seed density	2011		2012		2013		2011–2013	
	beetles	damage	beetles	damage	beetles	damage	beetles	damage
400	52	498	22	4,092 a*	354	17,060 a	428	21,650
600	63	412	18	3,564 b	345	14,457 b	426	18,433
800	45	389	27	2,978 c	284	13,896 b	356	17,263
Total	160	1,299	67	1,0634	983	45,413	1,210	57,346

*means followed by different letters within columns are significantly different (ANOVA, $p \leq 0.05$)

treatment with the highest seed sowing density. Sowing 400, 600 and 800 seeds per m^2 resulted in 163, 205 and 252 plants before harvest, respectively. Many factors can influence the germination of linseed cultivars, e.g. weather conditions, temperature, seed colour, linolenic acid concentration and chilling of flax seeds (Kurt 2010; Jacobsz and van der Merwe 2012; Heller *et al.* 2015). Generally, the germination capacity of linseed is low. In trials conducted in France only half of the sown seeds germinated (Flenet *et al.* 2006). According to Zajac *et al.* (2012) the highest yield of linseed was achieved when 400 to 600 seeds per $1 m^2$ were sown.

Abundance of flea beetles and the damage they caused – direct observations on plants

In 2011 and 2012 the number of flea beetles observed directly on the linseed plants during the whole growing season was very low (Table 2). There was a greater abundance of these insects only in 2013. No significant differences in the total number of beetles between treatments were observed. During two of the three years of this study (2012, 2013) significantly more damage was done by flea beetles on linseed grown at the lowest density than at the medium and the highest densities. A similar tendency was also observed in 2011. Additionally, in 2012 the amount of damage at medium seeding density was significantly higher than at the highest density.

Abundance and species richness – sweep net collection

In 2011, six collections using a sweep net were done. A total of 3,116 flea beetles belonging to five species were identified in the collected material from all the treatments (Table 3). No significant differences in the number of recorded insects were found ($F = 0.095$, $df = 2$, $p \leq 0.909$), but there was a tendency for the number of flea beetles to decrease with increasing plant density. In each treatment *A. euphorbiae* was the dominant species. This species made up from 91.9% (2) to 93.9% (1) of all identified flea beetles. The second most numerous species was *L. parvulus*, comprising 6.9%

of the insects from all the treatments (1–5.9%, 2–7.6%, 3–7.4%). Only a few specimens were found of the three other flea beetle species identified in the entomological material.

In 2012, in six-sweep net collections, a total of 1,475 flea beetles belonging to 13 species, were identified (Table 3). As in the previous year, no significant differences in the number of caught insects were found between treatments ($F = 0.779$, $df = 2$, $p \leq 0.463$), but again there was a tendency for the numbers of flea beetles to decrease with increasing plant density. In each treatment the most numerous species was *A. euphorbiae*, making up from 70.5% (2) to 75.3% (3) of all collected beetles. *Longitarsus parvulus* was also abundant this season. The percentage of this species in the total number of flea beetles recorded in each treatment was similar and fluctuated from 21.6% (3) to 25.7% (1). *Phyllotreta vittula* constituted 2% of all flea beetles caught that year. The remaining species occurred as single specimens in the collected material and not in each treatment.

In 2013, seven collections using a sweep net were done. A total of 7,039 flea beetles belonging to seven species were identified (Table 3). This was the highest number of flea beetles identified during the three years of the study. Plant density had no effect on the total number of recorded insects in the studied treatments ($F = 0.0123$, $df = 2$, $p \leq 0.982$). As in the previous years, *A. euphorbiae* dominated, making up 92.0–93.1% of all identified flea beetles. The second most numerous species was *L. parvulus*, comprising only 7.2% of all the insects from all the treatments (1–7.8%, 2–7.4%, 3–6.7%). Other identified species occurred sporadically in the collected material.

In the Interactive Agricultural Ecological Atlas of Russia and Neighbouring Countries (Afonin *et al.* 2008) three species of flea beetles are mentioned as specialists: *A. euphorbiae*, *A. flaviceps* and *L. parvulus*. During the three years of our study, 15 species of flea beetles occurring on linseed plots were identified. Each year, *A. euphorbiae* was the dominant species, making up more than 70% (2012) or even more than 90% (2011, 2013) of all collected beetles. The second most numerous species, *L. parvulus*, was numerous only

Table 3. Species richness of flea beetles collected by sweep net sampling of linseed plants grown at different densities near Pawłowice, Poland, in 2011–2013

Species	2011						Total	[%]
	400*		600		800			
	N**	[%]	N	[%]	N	[%]		
<i>Aphthona euphorbiae</i> (Schrank, 1781)	1,090	93.9	925	91.9	877	92.3	2,892	92.8
<i>Longitarsus parvulus</i> (Paykull, 1799)	68	5.9	76	7.6	70	7.4	214	6.9
<i>Phyllotreta vittula</i> (Redtenbacher, 1849)	1	0.1	3	0.3	1	0.1	5	0.1
<i>Phyllotreta undulata</i> (Kutschera, 1860)	1	0.1	2	0.2	1	0.1	4	0.1
<i>Phyllotreta striolata</i> (Illiger, 1803)	–	–	–	–	1	0.1	1	0.1
Total	1,160	100	1,006	100	950	100	3,116	100
Number of species	4		4		5		5	
2012								
<i>Aphthona euphorbiae</i> (Schrank, 1781)	402	70.9	336	70.5	324	75.3	1,062	71.9
<i>Longitarsus parvulus</i> (Paykull, 1799)	146	25.7	117	24.6	93	21.6	356	24.0
<i>Phyllotreta vittula</i> (Redtenbacher, 1849)	7	1.2	13	2.7	9	2.1	29	2.0
<i>Phyllotreta astrachanica</i> Lopatin, 1977	3	0.5	3	0.6	1	0.2	7	0.5
<i>Phyllotreta undulata</i> (Kutschera, 1860)	3	0.5	4	0.8	–	–	7	0.5
<i>Phyllotreta cruciferae</i> (Goeze, 1777)	2	0.4	1	0.2	–	–	3	0.2
<i>Chaetocnema aridula</i> (Gyllenhal, 1827)	1	0.2	1	0.2	1	0.2	3	0.2
<i>Chaetocnema picipes</i> Stephens, 1831	1	0.2	1	0.2	1	0.2	3	0.2
<i>Longitarsus curtus</i> (Allard, 1861)	–	–	–	–	1	0.2	1	0.1
<i>Longitarsus melanocephalus</i> (De Geer, 1775)	–	–	–	–	1	0.2	1	0.1
<i>Longitarsus anchusae</i> (Paykull, 1799)	–	–	1	0.2	–	–	1	0.1
<i>Chaetocnema concinna</i> (Marsham, 1802)	1	0.2	–	–	–	–	1	0.1
<i>Phyllotreta nigripes</i> (Fabricius, 1775)	1	0.2	–	–	–	–	1	0.1
Total	567	100	477	100.0	431	100	1,475	100
Number of species	10		9		8		13	
2013								
<i>Aphthona euphorbiae</i> (Schrank, 1781)	2,209	92.0	2,129	92.4	2,174	93.1	6,512	92.1
<i>Longitarsus parvulus</i> (Paykull, 1799)	188	7.8	171	7.4	157	6.7	516	7.2
<i>Phyllotreta vittula</i> (Redtenbacher, 1849)	3	0.3	1	0.1	1	0.1	5	0.2
<i>Phyllotreta undulata</i> (Kutschera, 1860)	–	–	1	0.1	1	0.1	2	0.2
<i>Chaetocnema aridula</i> (Gyllenhal, 1827)	1	0.1	–	–	–	–	1	0.1
<i>Phyllotreta atra</i> (Fabricius, 1775)	1	0.1	–	–	–	–	1	0.1
<i>Phyllotreta striolata</i> (Illiger, 1803)	–	–	2	0.1	–	–	2	0.1
Total	2,402	100	2,304	100	2,333	100	7,039	100
Number of species	5		5		4		7	
Total number (2011–2013)	4,129		3,787		3,714		11,630	
Total reduction in reference to 400 seeds · m ⁻²	100		91.7		89.9		–	

*sowing seed density; **number of flea beetles

in 2012 (more than 20% of all collected flea beetles). The third flax flea beetle listed in the Atlas, *A. flaviceps*, which has not been identified in Poland (Borowiec *et al.* 2011), was not found in our collections. Other species were relatively rare in our collected material, and most of them do not feed on *Linum* (Afonin *et al.*

2008). *Aphthona euphorbiae* and *L. parvulus* are considered worldwide to be serious pests of flax grown for fibre and seeds (Heller *et al.* 2015). Unfortunately, little information concerning the exact proportion between numbers of these two species in the linseed crop can be found. In the Warmia–Masuria province,

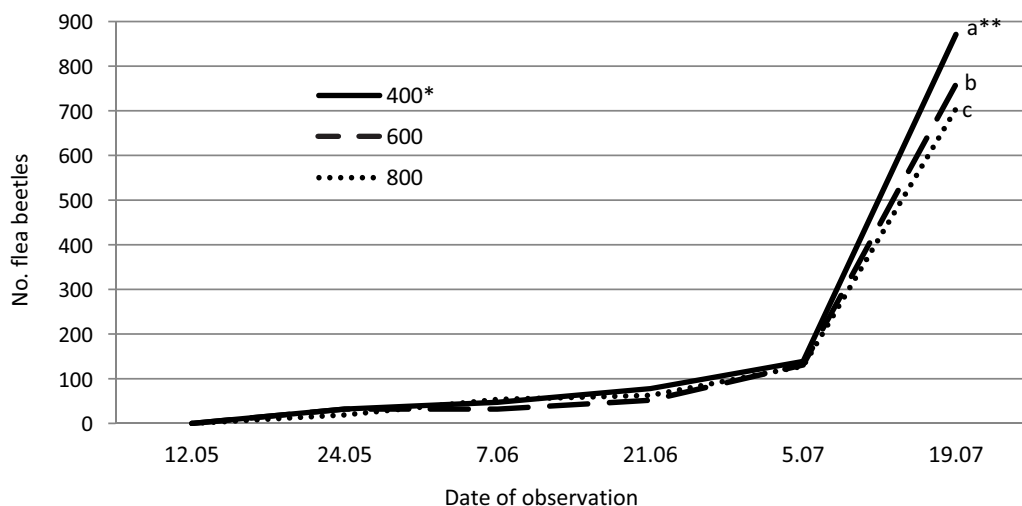
Poland, Żurańska (1965b) found *A. euphorbiae* to make up about 80% and *L. parvulus* about 20% of all flea beetles on flax plants in this region in 1955–1956. In our research *A. euphorbiae* was also much more numerous than *L. parvulus*. In the total number of flea beetles in each treatment, there was a tendency for the numbers of beetles to decrease with increasing plant density. Over the three years, the total reduction in flea beetle numbers was 8.3% at 600 seeds · m⁻² and 10.1% at 800 seeds · m⁻² when compared to 400 seeds · m⁻² (Table 3).

Seasonal changes

In our trials the number of flea beetles observed directly on the linseed plants was very low in 2011 and 2012.

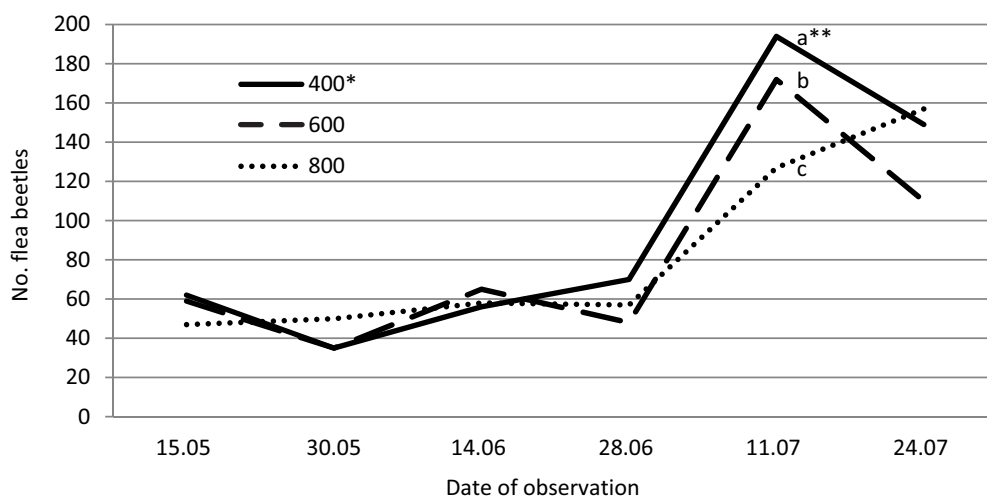
A greater abundance of these insects was found only in 2013. Because of the low number of beetles visible directly on plants, the seasonal changes are presented on the basis of the results of sweep net collection. Sampling with a sweep net is the most effective method of flea beetle collection in linseed when the crop emerges from the soil (Wise and Soroka 2003).

In 2011, single beetles were caught during the first net collection done at the end of May when plants had reached BBCH stage 30 (Fig. 1). The first overwintering beetles were visible on plants ten days earlier. In June, when plants started to bloom, the number of feeding flea beetles slowly increased and reached more than a hundred individuals in each treatment at the beginning of July. The maximum numbers of



*sowing seed density; **means followed by different letters for particular dates are significantly different (ANOVA, $p \leq 0.05$)

Fig. 1. The seasonal dynamics of flea beetles collected by sweep net sampling on linseed sown at different densities, near Pawłowice, Poland, 2011



*sowing seed density; **means followed by different letters for particular dates are significantly different (ANOVA, $p \leq 0.05$)

Fig. 2. The seasonal dynamics of flea beetles collected by sweep net sampling on linseed sown at different densities, near Pawłowice, Poland, 2012

these insects occurred in the last collection on 19 July (BBCH 83), when the new generation beetles emerged. Significantly more beetles were caught at low seeding density (871) than in the other two treatments (760 and 705). Additionally, the number of flea beetles at medium seed density was significantly higher than at high seed density.

In 2012, the first collection was done in mid-May (Fig. 2). A month later the first lower population peak was noted (BBCH 65–69). This peak was caused by overwintering adults, which colonized crops in spring. Flea beetle numbers increased during the second half of June, and reached a second peak in mid-July, when plants were at BBCH stage 83. In this case the peak was formed by adults of a new generation. Significant differences in the numbers of beetles at the second maximum peak of their population were found. Significantly more insects occurred on plants grown at the lowest density (194) than at the medium (172) or highest densities (127).

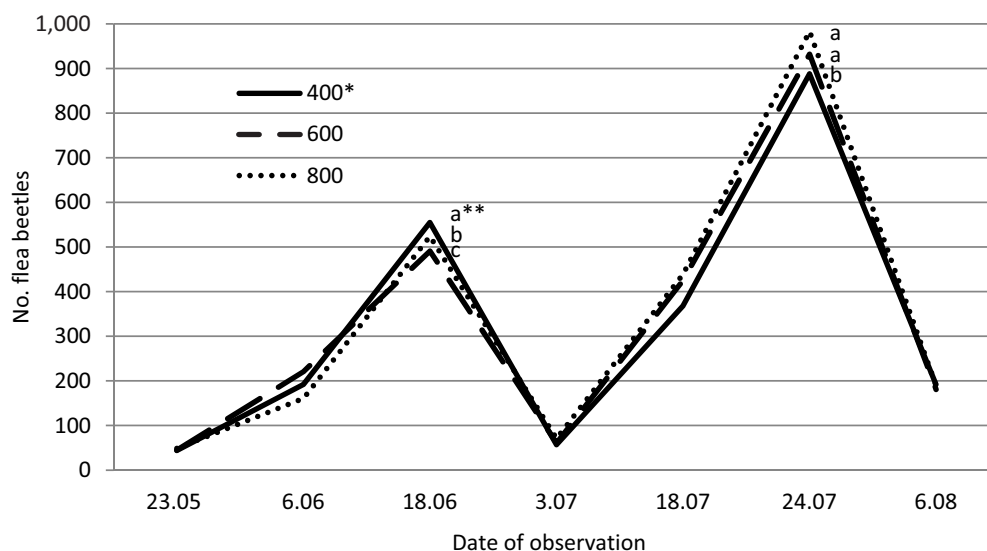
During this season flea beetles were collected on flax oil plants till the end of July (BBCH 85). In 2013, the first flea beetles were collected at the end of May, when the plants had reached BBCH stage 30 (Fig. 3). Their numbers increased during the first half of June, and reached the first peak in mid-June (BBCH 60–61). At this maximum peak, significantly more beetles were caught at low seed density than at higher densities. Additionally, at high density more beetles were found than in treatment 2. The second peak of flea beetles in 2013 was recorded at the end of July on BBCH 83. At that time, significantly more beetles were caught on plants grown on the lowest and medium densities than at the highest density (935, 932 and 804, respectively).

During this season beetles were collected on linseed till the beginning of August (BBCH 89). Similarly, two peaks of flea beetle abundance on flax were reported by Żurańska (1965b). In our experiments (Lower Silesia) the first and second maximum peaks occurred almost at the same time as in the Warmia–Masuria province, Poland (Żurańska 1965b).

Feeding damage

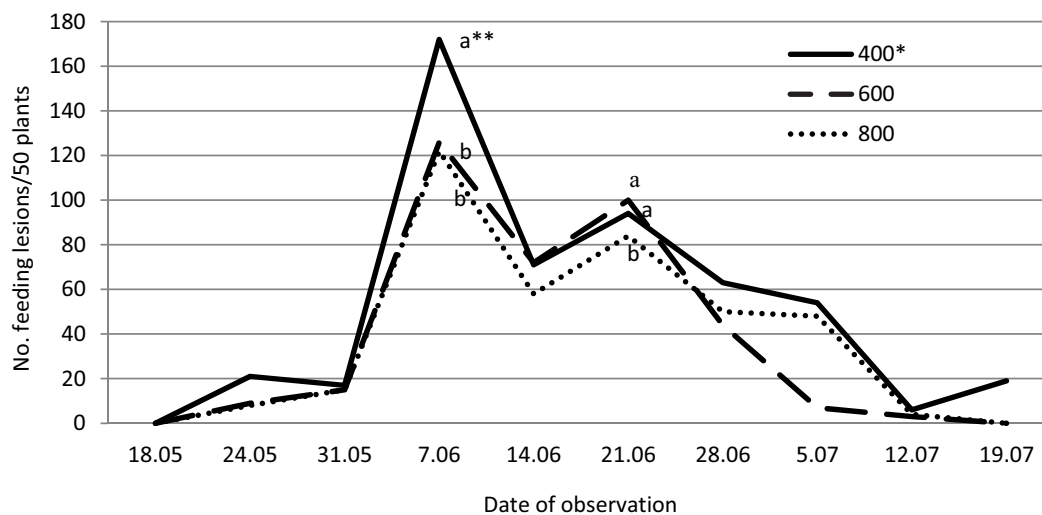
Adult flea beetles feed on the leaves and stems of linseed. Each year the number of feeding symptoms caused by beetles (small shot-holes on leaves and lesions on stems) differed between the three treatments. In 2011, the first shot-holes were observed on young plants (BBCH 19) at the end of May (Fig. 4). The highest number of holes in each treatment was observed in mid-June, when linseed was at BBCH growth stage 60–65. Later in the season the number of feeding symptoms slowly decreased. Although the adult insects were most numerous at the end of the linseed growing season (new generation), there were few holes and lesions at that time. Plants were at the ripening stage, and insects did not feed on them. In this year, in two observations out of nine, significantly more shot-holes were found in the plants growing at the lowest density as compared to the highest density.

In 2012, the first symptoms of the beetles feeding were visible on young plants (BBCH 18–19) at the beginning of May, earlier than in the previous year (Fig. 5). One period, mid-May, was distinguished by the fact that the number of holes in each treatment was highest. In this period plants were at BBCH stage 30–60. Fewer feeding symptoms were observed till mid-July.



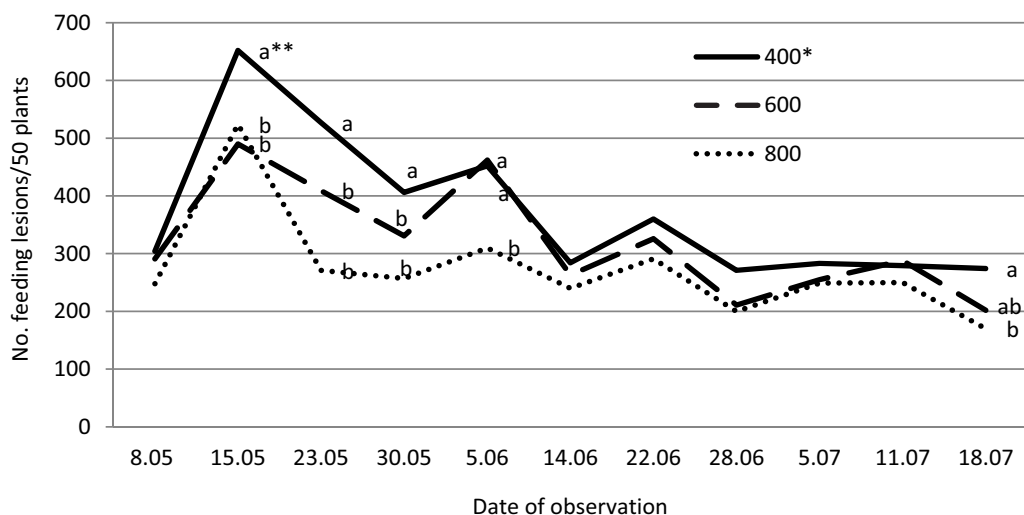
*sowing seed density; **means followed by different letters for particular dates are significantly different (ANOVA, $p \leq 0.05$)

Fig. 3. The seasonal dynamics of flea beetles collected by sweep net sampling on linseed sown at different densities, near Pawłowice, Poland, 2013



*sowing seed density; **means followed by different letters for particular dates are significantly different (ANOVA, $p \leq 0.05$)

Fig. 4. Number of shot-holes and lesions caused by flea beetles on leaves and stems of linseed plants sown at different densities, near Pawłowice, Poland, 2011



*sowing seed density; **means followed by different letters for particular dates are significantly different (ANOVA, $p \leq 0.05$)

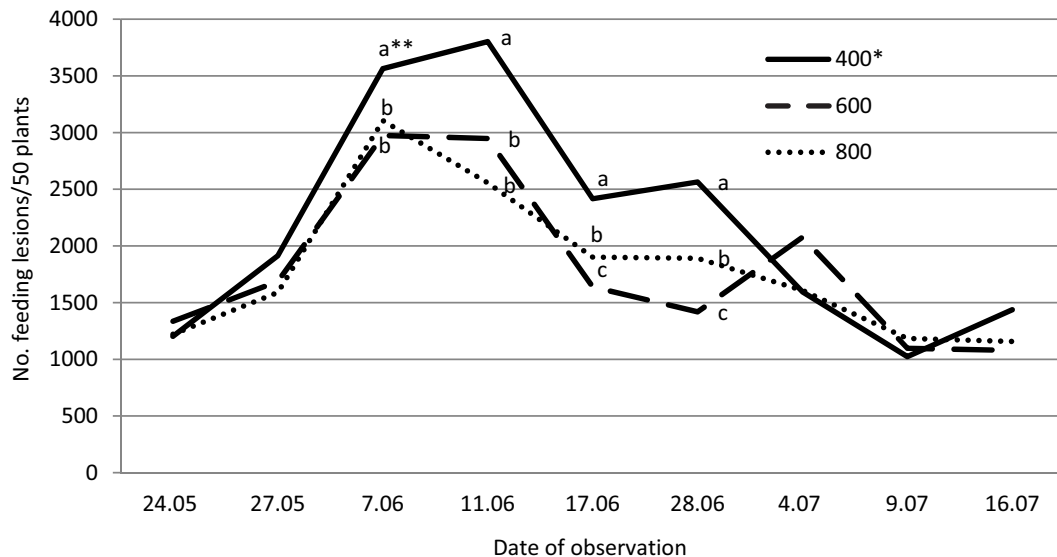
Fig. 5. Number of shot-holes and lesions caused by flea beetles on leaves and stems of linseed plants sown at different densities, near Pawłowice, Poland, 2012

In this season, on five out of 11 observation dates, significantly more shot-holes were observed on plants at low seed density (400 seeds per 1 m²) than on plants at high seed density (800 seeds per 1 m²). Additionally, in three out of 11 observations more holes were found on plants at low seed density than at medium.

In 2013, the first feeding symptoms caused by the overwintering adult flea beetles were noted on linseed plants at BBCH stage 11–13 in the latter part of May (Fig. 6). At the end of May the number of feeding holes increased significantly, with the greatest maximum in each treatment occurring at the beginning of June. A high number of feeding symptoms was observed till mid-June on plants of BBCH stage 30–65. In July plants started to mature, and the feeding intensity of

new generation beetles was much lower. On four observation dates out of nine significantly more shot-holes and lesions were found on plants growing at the lowest density (1) as compared to the medium (2) and the highest densities (3).

Seed sowing density, and hence plant density, can have variable effects on different pest species. Most experimental results show that the density of herbivores per plant decrease with increasing density of host plants (Neumann and Ulber 2006; Rodriguez *et al.* 2012; Halpern *et al.* 2014). Only a few experiments supported the opposite effect (Ralph 1977; Fischer and Ulber 2006), and some experiments did not detect consistent results (Neumann and Ulber 2006; Cherry *et al.* 2013). In the presented trials the tendency was for



*sowing seed density; **means followed by different letters for particular dates are significantly different (ANOVA, $p \leq 0.05$)

Fig. 6. Number of shot-holes and lesions caused by flea beetles on leaves and stems of linseed plants sown at different densities, near Pawłowice, Poland, 2013

flea beetle numbers to increase with decreasing plant density. There were also several cases of significantly more flea beetle feeding symptoms on plants grown at the lowest density. This phenomenon could be partially explained by a pattern called resource dilution effects (Otway *et al.* 2005) or resource diffusion effects (Yamamura 1999), in which the herbivore densities are lower in resource-dense patches.

Conclusions

1. During the three years of the study 15 species of flea beetles occurring on linseed were identified. *Aphthona euphorbiae* was the dominant species in each treatment. *Longitarsus parvulus*, the second most numerous species, made up only a small percentage of all flea beetles caught. Other identified species were relatively rare and very often occurred only on plants of single treatments. In terms of the total catch, the tendency was for beetle numbers to decrease with increasing plant density.
2. Flea beetles feeding on linseed plants, irrespective of plant density, had two peaks of abundance. The first, which was lower, occurred in June, when plants were at the blooming stage. This peak was caused by overwintering adults, which colonized crops in spring. The second, higher peak of abundance, was recorded in the second half of July, when plants were at the ripening stage. In this case the peak was formed by adults of a new generation. Each year, at the higher population peak of abundance, the flea beetles were most numerous on plants grown at the lowest density.

3. There was one period during which the number of holes and lesions on plants of each treatment was highest. This period lasted from mid-May to the first few days of June (2012), or from the beginning of June to mid-June (2011, 2013). During this period the overwintering adult beetles fed on linseed at BBCH stage 30–65. During the three years of the study there were several cases of significantly higher numbers of flea beetle feeding symptoms on plants grown at the lowest density as compared to the medium and highest densities.

Acknowledgements

Research was carried out with the financial support of the National Centre for Research and Development, Poland, under project No. 12017110/2011. We are also grateful to Joanna Magiera-Dulewicz from the Department of Plant Protection for her input with sample collection and laboratory work.

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